Statistical Analysis Plan

A phase IIa, multicenter, double blind, placebo controlled, randomized clinical trial to assess safety, efficacy and pharmacokinetics of Rifaximin Delayed-Release (Rifaximin-EIR) in patients with moderate-to-severe papulopustular rosacea

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Indication	:	Moderate-to-severe papulopustular rosacea

Signature Page

The signatures below indicate that these individuals have reviewed this project-specific Statistical Analysis Plan and consent to this document as governing the tasks outlined within. The signatures below also indicate that the processes and quality standards set forth by this Statistical Analysis Plan are approved for use in this study.

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Document History

Version #	Issue Date	Author	Revisions
1.0	02 Feb 2023	PPD	Finalized document
2.0	07 Mar 2023		Revised Section 5.7 describing subgroup analyses Revised Section 6.6 describing the list of prohibited concomitant medications from V1 to V4 Revised Section 6.7 and Section 6.8 describing compliance and exposure Revised Section 10 describing changes respect to the Study Protocol
3.0	10 Mar 2023		Revised Section 6.8 describing exposure

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1 Abbreviations and Definitions

Abbreviations	Definitions
Σ	Sum
AE	Adverse Event
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CI	Confidence Interval
cm	Centimeter
CMH	Cochran Mantel-Haenszel
CRF	Case Report Form
CS	Clinically Significant
CSR	Clinical Study Report
DLQI	Dermatology Life Quality Index
eCRF	Electronic Case Report Form
EIR	Extended Intestinal Release
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
H_2	Hydrogen
HPLC-MS/MS	High-Performance Liquid Chromatograph Triple Quadrupole Mass Spectrometer
IBS-SSS	Irritable Bowel Syndrome - Symptom Severity Scale
ICH	International Conference on Harmonisation
IGA	Investigator's Global Assessment
IQR	Interquartile Range
IRT	Interactive Response Technology
kg	Kilogram
L-BT	Lactulose Breath Test
LOCF	Last Observation Carried Forward

LS	Least Squares
m	Meter
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mmHg	Millimeters of Mercury
PK	Pharmacokinetic
PP	Per Protocol Analysis
PT	Preferred Term
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TID	Three Times Daily
TLFs	Tables, Listings, and Figures
US	United States
V1	Screening Visit
V2	Randomization/Baseline Visit
V3	End of Treatment Visit
V4	End of Study Visit
VAS	Visual Analogue Scale
WHO-DD	World Health Organization Drug Dictionary

2 Introduction

This statistical analysis plan (SAP) describes the planned statistical analysis and reporting of the clinical study protocol titled "A phase IIa, multicenter, double blind, placebo controlled, randomized clinical trial to assess safety, efficacy and pharmacokinetics of Rifaximin Delayed-Release (Rifaximin-EIR) in patients with moderate-to-severe papulopustular rosacea" version 2.0, dated on March 25, 2022.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials [1]. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association [2] and the Royal Statistical Society [3], for statistical practice.

The purposes of this SAP are to:

- Outline the types of analyses and presentations of data that will form the basis for drawing conclusions to the study objectives and hypotheses outlined in the protocol
- Explain in detail how the data will be handled and analyzed, adhering to commonly accepted standards and practices for Good Statistical Practice

All analyses described in this plan are considered *a priori* analyses in that they have been defined prior to locking the study database and unblinding. The planned analyses identified in this SAP will be included in clinical study reports (CSRs) and may be included in regulatory submissions, or future manuscripts. Also, post-hoc exploratory analyses not identified in this SAP may be performed to further examine study data. Any post-hoc or unplanned exploratory analyses performed will be clearly identified as such in the final CSR.

The following documents were reviewed in preparation of this SAP:

- Protocol version 2.0 issued on March 25, 2022:
  - RE-ROS2002-2021_Study_Protocol_Version_2.0_25Mar2022 (signed by Sponsor) .pdf
- Electronic Case Report Forms (eCRF):
  - eCOS_UniqueCRFs_RE-ROS2002-2021_2021-12-06_15_18_37.pdf
- ICH Guidance on Statistical Principles for Clinical Trials (E9)

The reader of this SAP is encouraged to also read the clinical study protocol and other identified documents for details on the planned conduct of this study. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analyses.

Any changes to the protocol or eCRF may necessitate updates to the SAP. In case of deviations from this SAP, explanations will be provided in the CSR for minor updates and in an SAP amendment for major updates.

3 Study Objectives and Endpoints

3.1 Study Objectives

1. To evaluate the safety and the efficacy of two different doses of Rifaximin-EIR 250 mg tablets (250 mg TID and 500 mg TID), administered for 30 days in subjects with moderate-to-severe papulopustular rosacea
2. To evaluate the pharmacokinetics of these two doses of Rifaximin-EIR in a sub-group of subjects

3.2 Study Endpoints

3.2.1 Co-primary efficacy endpoints

The following co-primary efficacy endpoints will be clinically assessed by the Investigator:

1. Change from baseline in number of rosacea inflammatory lesions (papules and pustules) at the end of treatment (Day 30)
2. Percent of subjects showing treatment success, defined as an IGA score of 0 (clear) or 1 (almost clear), with at least a 2-grade improvement from baseline to the end of treatment (Day 30).

Success of the study will be declared in any of the active treatment groups if both the co-primary efficacy endpoints will be satisfied (note: the two items may not necessarily occur in the same patient).

Table 1. Investigator's Global Assessment (IGA) Score

Grade	Description	Amount and size of inflammatory lesions present
0	Clear	None
1	Almost Clear	Very few, small papules /pustules
2	Mild	Few small papules/pustules
3	Moderate	Several small or large papules/pustules
4	Severe	Numerous small and/or large papules/pustules

3.2.2 Secondary Efficacy Endpoints

1. Mean change from baseline in number of inflammatory lesions (papules and pustules) at end of study (Day 60).
2. Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at end of treatment (Day 30) and/or end of study (Day 60).
3. Percent of participants with IGA score of 0 (clear) at end of treatment (Day 30) and/or end of study (Day 60).
4. Change from baseline in the following rosacea additional features at end of treatment (Day 30) and/or end of study (Day 60):
    - pain, burning/stinging and itching (measured using a 0-10 cm Visual Analogue Scale (VAS));
    - telangiectasia (absent=0, mild=1, moderate=2, severe=3);
    - ocular manifestations (absent=0, mild=1, moderate=2, severe=3);
    - phymatous changes (absent=0, mild=1, moderate=2, severe=3).
5. Change from baseline in facial non-transient erythema (absent=0, mild=1, moderate=2, severe=3) at end of treatment (Day 30) and/or end of study (Day 60).
6. Change from baseline in abdominal pain score at end of treatment (Day 30) and/or end of study (Day 60).
7. Change from baseline in abdominal distension score at end of treatment (Day 30) and/or end of study (Day 60).

8. Change from baseline in bowel habit satisfaction score at end of treatment (Day 30) and/or end of study (Day 60).
9. Change from baseline in global severity of abdominal symptoms score at end of treatment (Day 30) and/or end of study (Day 60).
10. Percent of participants showing treatment success according to a Modified IGA scale (i.e. score of 0 or 1) including erythema but excluding rosacea inflammatory lesions at end of treatment (Day 30) and/or end of study (Day 60).
11. Percent of participant showing treatment success according to a Modified IGA scale (i.e. score of 0 or 1) including erythema and rosacea inflammatory lesions at end of treatment (Day 30) and end of study (Day 60).

3.2.3 Pharmacokinetic Assessments

Pharmacokinetics of the single dose (i.e. at Day 1 after the first dose administration) and the repeated doses (i.e. at Day 30 after the last dose) will be assessed on the PK sub-study population.

PK parameters to be calculated/estimated according to a non-compartmental approach (NCA) are detailed in Section 9.

3.2.4 Safety Assessments

Safety assessments will include:

- Complete physical examination
- Vital signs (including heart rate, blood pressure, and body temperature) and height, weight and BMI
- Routine laboratory parameters (haematology, biochemistry, urinalysis)
- Adverse events (AEs)
- Withdraw of subjects from study due to adverse events

4 Study Overview

4.1 General Study Design and Plan

Subjects will be offered participation to the PK sub-study. If they agree, and have a suitable PK profile, they will be randomized according to the relevant stratum; otherwise, they will enter the main study.

Subjects will be dispensed blinded investigational product according to the randomization schedule. Investigational product will be dispensed at the Day 1 (Randomization) visit; at this visit, each eligible subject will receive a patient's kit containing two "treatment bottles", covering the treatment necessary for 30 days of study. All tablets are identical in appearance and the strengths are placebo and 250 mg. Subjects will be instructed to take 2 tablets of investigational product three times daily (TID) by mouth, in order to maintain study blinding. For the 250 mg TID dose groups this comprises one active and one placebo tablet. For the 500 mg TID dose group, this comprises two 250 mg tablets. For the placebo dose group, this comprises two placebo tablets. TID doses of the assigned treatment will be administered from Day 1 to Day 29. On Day 30 just the first daily dose will be administered.

There will be 3 study drug treatment arms:

- Two tablets of Rifaximin-EIR 250 mg formulation TID (1500 mg daily) for 29 days; on Day 30 subjects will receive two tablets of Rifaximin-EIR 250 mg in single administration (500 mg).
- One tablet of Rifaximin-EIR 250 mg formulation TID (750 mg daily) + one tablet of placebo TID for 29 days; on Day 30 subjects will receive one tablet of Rifaximin-EIR 250 mg and one tablet of placebo in single administration (250 mg).
- Two tablets of placebo TID for 29 days; on Day 30 subjects will receive two tablets of placebo in single administration.

For subjects included in the PK sub-study the first drug intake must be performed at the investigational site in occasion of the randomization visit (Day 1), immediately after the first blood draw (for PK analysis) and preferably early in the morning (approximately 8:00 a.m.). Also, at Day 30 subjects participating in the PK sub-study will stay overnight at the center to complete the PK procedures.

4.2 Inclusion-Exclusion Criteria

Inclusion and exclusion criteria can be found in Section 9.2 and 9.3 of the study protocol.

4.3 Subject Randomization

Randomization will be carried out using an interactive response technology (IRT) system, with central randomization following a 1:1:1 ratio between the 3 treatment arms, based on a block randomization method. Randomization will be stratified by the following factor:

- L-BT status $H_2 \leq 10$ ppm
- L-BT status $H_2 > 10$ ppm

4.4 Schedule of Assessments

4.4.1 Study Schedule A: Social Media Recruitment Path

Table 2: Schedule of Assessments for Social Media recruitment path only

	Remote Data Recording (via study website)	Central Reader Validation	Phone contact for scheduling clinic visit
Visits	**V0**		
Days		Within 2 working days from V0	Within 1 week after C. Reader validation
Online pre-screening consent	X		
Contact details, demographics, health related behaviour	X		
Medical and surgery history questionnaire	X		
Concomitant medications questionnaire	X		
Face images upload	X		
Preliminary compliance with inclusion/exclusion criteria		X	X
Study description to the subject			X
Decision recording			X
Appointment set-up			X

4.4.2 Study Schedule B: Social Media and On-site Recruitment Paths

Table 3: Schedule of Assessments for Social Media and On-site recruitment paths

	Screening Period	Baseline/ Randomization Visit	End of Treatment Visit	End of Study Visit	Early Termination Visit	Unscheduled Visit
	V1	**V2**	**V3**	**V4**	**ETV**	**UV**
Days	-30 to -1	1	30 (±1)	60 (±3)	From 1 to 60	From 1 to 60

Written informed consent	X					
Demographics collection and health-related behaviour assessment	X					
Relevant rosacea-associated medical history	X					
Relevant non-rosacea-associated medical history	X					
Previous and Concomitant medications	X	X	X	X	X	X
Adverse events collection	X	X	X	X	X	X
Vital signs (heart rate, blood pressure, body temperature)	X	X	X	X	X	X
Weight, Height* and BMI		X	X		X	
Complete Physical Examination	X	X	X	X	X	X
Rosacea-associated facial inflammatory lesion count (papules and pustules)	X	X	X	X	X	X4
Five-points Investigator's Global Assessment (IGA) of Rosacea Severity	X	X	X	X	X	X4
Evaluation of erythema, telangiectasia, ocular manifestations and phymatous changes	X	X	X	X	X	X4
Visual Analogue Scale (VAS) for pain, burning/stinging and itching	X	X	X	X	X	X4
Abdominal symptoms questionnaire (IBS-SSS)1		→	→	→	→	
Dermatology Life Quality Index Questionnaire1		→	→	→	→	
Clinical chemistry, haematology, urinalysis	X		X	X	X	X4
Contraception recommendations and/or adherence^		X				
Serum pregnancy test^	X					
Urine pregnancy test^		X	X	X	X	X4
Lactulose Breath test	X		X		X	X4
Inclusion/exclusion criteria	X	X				

Randomization		X				
Blood sampling for PK analysis^^		X2	X3			
Overnight stay^^			X			
Drug dispensing		X				
Drug accountability			X		X	

*Height will be recorded at V1 only;
^Women of child-bearing potential only;
^^ Only in subjects participating in the PK sub-study;
[1]To be collected every 10 days, starting from V2 up to V4, and recording patient's responses through anelectronic diary (ePRO).
[2]Sampling to be performed right before and after the first morning dose of Rifaximin-EIR at V2 (Day 1) at thefollowing time points: immediately pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post-dosing.
[3]Sampling to be performed right before and after the morning dose of Rifaximin-EIR on day 30 at the followingtime points: immediately pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6 and 8, 12, 22, 24, 30 hours post-dosing (singledose at Day 30). The sampling started at V3 (Day 30) and will end after overnight stay, as appropriate.
4 If judged necessary by the Investigator

4.5 Sample Size

The sample size estimation is based on the co-primary efficacy endpoints, i.e. mean change from baseline in number of inflammatory lesions and percent of patients showing treatment success. CCI

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The total sample size (153) was adjusted to 171 patients (57 in each treatment group) considering an expected dropout rate of about 10% (using Freedman's formula n'= 100*n/(100-x), where x is the expected dropout rate).

The initially planned sample size has been increased to a total of 201 patients (67 in each treatment group) to mitigate the impact on the statistical power of the treatment kit misallocation that occurred for 15 patients out of the initial 30 patients randomized. A cap of 33 subjects per arm having a negative L-BT result at screening (i.e., a test showing an increase ≤10 ppm of H2 by 90 minutes compared to time 0) is established.

Sample size for the sub-study on PK is not based on formal statistical assumptions. Initially, a cap of 12 PK subjects per study arm was established. This is considered a good value for this kind of study. In any case, in order to have an adequate sample size, the study requires at least 6 PK subjects per study arm. During the course of the study, the cap has been increased to 19 due to the failure to comply with the previous limit.

5 General Considerations

5.1 Presentation

Individual patient data obtained from electronic case report forms (eCRFs), central laboratories, local laboratories, external sources, and derived data will be presented in data listings by patient. All data listings that contain an evaluation date will contain a relative study day.

All outputs will be incorporated into Microsoft Word rich text format (.rtf) files, sorted and labeled according to ICH recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, efficacy, safety, rifaximin plasma concentrations and pharmacokinetic parameters. Tabulations will be organized by using column for each treatment group, and rows for each parameter and visit (or time) where appropriate.

For categorical variables, summary tabulations of the number and percentage of patients within each category (with an "unknown" category for missing data) of the parameter will be presented. Denominators for percentage calculations will include missing data, unless otherwise specified in the table description. Percentages are rounded to 1 decimal place, unless otherwise specified in the table shell.

For continuous variables, the number of patients, mean (arithmetic), SD, median, interquartile range (IQR), minimum, and maximum values will be presented. The precision of summary statistics, unless otherwise specified, will be as follows: mean, median, and IQR to 1 more decimal place than the raw

data and SD to 2 decimal places more than the raw data. In general, the decimal places should not exceed 3 decimal places unless appropriate. CIs will be provided and will be rounded to 1 more decimal place than the raw data, unless otherwise specified in the table and listing shell.

For ordinal variables, the summary procedures for both categorical and continuous variables will be followed where appropriate.

5.2 Data Conventions

The precision of original measurements will be maintained in summaries, when possible.

For tables where rounding is required, rounding will be done at the final stage of the calculation to the nearest round-off unit. For example, when rounding to the nearest integer, values ≥XX.5 will be rounded up to XX + 1 (eg, 97.5 will round up to 98), while values <XX.5 will be rounded down to XX (eg, 97.4 will be rounded down to 97).

For frequency counts of categorical variables, categories whose counts are zero will be displayed for completeness. For example, if none of the patients discontinue due to “lost to follow-up,” this reason will be included in the corresponding table with a count of 0. Percentages based on frequency counts of case report form (CRF) collections (eg, demographic categories) will be presented to one decimal place.

Quantitative laboratory tests containing less than (<) and greater than (>) symbols are test results that are below and above quantifiable limits, respectively. In order to retain these values for analysis purpose, values will be imputed as the numeric portion of the result.

5.3 Timing of Analyses

The statistical analyses will be performed at the end of the study, after the database has been cleaned and locked.

5.4 Analysis Populations

Study populations definitions are provided below. The numbers of subjects in each population and reasons for exclusion will the summarized.

All decisions on populations will be taken during a Blind Data Review Meeting and will be detailed in the relevant documents.

The analyses of the main efficacy endpoints will be performed on the FAS and the PP set. Results on the FAS will be considered primary. Results on the PP set will be used as supportive. The mFAS population will be used for sensitivity analyses. For details as to which populations will be used to analyze each endpoint please refer to the TLF Shells document.

5.4.1 Screened Population

Screened Population is defined as all subjects enrolled into a screening period after informed consent. A screen failure is defined as a subject who has not been randomized to treatment. Data from screen failures will only be listed as indicated.

5.4.2 Randomized Population

The randomized population is defined as the set of all randomized subjects.

5.4.3 Safety Analysis Set

All safety and tolerability analyses will be carried-out in the Safety Analysis Set (SAF), consisting of all randomized subjects having received at least once the investigational treatment.

Analysis on the SAF will be performed according to the actual treatment received.

5.4.4 Full Analysis Set

The primary efficacy analysis population will be the Full Analysis Set (FAS), defined as all randomized patients having taken at least one dose of the investigational treatment and have at least one post baseline assessment for any of the two co-primary endpoints.

To mitigate the impact of the treatment kit misallocations, the primary statistical analysis on the FAS will be performed according to the actual treatment received (not according to the "randomized" treatment, as per ITT principle).

5.4.5 Modified Full Analysis Set

Modified Full Analysis Set (mFAS) is defined as all patients in the FAS excluding the initial 30 patients randomized.

The analysis on the mFAS will be performed according to the "randomized" treatment.

5.4.6 Per Protocol Analysis Set

Per Protocol Analysis (PP) set is defined as all subjects in the FAS who fulfil the study protocol requirements with no major deviations that may affect study results.

Analysis on the PP set will be performed according to the actual treatment received. Patients randomized to a wrong stratification factor level will not be excluded from the PP set and will be analyzed according to the actual stratum (not the randomized one).

5.4.7 Pharmacokinetic Set

The Pharmacokinetic (PK) set will include all randomized subjects within the PK Sub-study strata who received at least one dose of Rifaximin. The PK set will be defined with approval from Alfasigma after receipt of plasma concentration data, database lock and unblinding.

PK set will include all subjects who have received at least 1 dose of IMP and, specifically that they satisfy the following criteria for at least 1 profile:

- No missing samples at potential critical time points.
- No relevant events (e.g. protocol deviations or adverse events) that may impact the study objectives with respect to the PK endpoints.
- Subjects who received placebo will not be included in the PK population.

5.5 Baseline Definition

Baseline is defined as the last non-missing assessment prior to the first administration of study medication (including unscheduled assessments) in the study.

5.6 Derived and Transformed Data

Variables requiring calculation will be derived using the following formulas:

- Days – A duration expressed in days between a date (date1) and another later date (date2) will be calculated using the following formula:
  duration in days = date2 – date1 + 1
- Months – A duration expressed in months is calculated as the number of days divided by 365.25/12 (approximately 30.4)
- Years – A duration expressed in years is calculated as the number of days divided by 365.25
- Change from baseline will be calculated as:

Change = post baseline value – baseline value

5.7 Subgroups

The following subgroup analyses will be performed:

5.7.1 Co-primary endpoints

The co-primary endpoints will be analysed by the following subgroups:

- Stratification factor: L-BT Positive, L-BT Negative
- Breath H2 concentrations at Screening (increase of ≥20 ppm of H2 within 90 minutes compared to baseline, increase of <20 ppm of H2 within 90 minutes compared to baseline)
- Breath CH4 concentrations at Screening (increase of > 10 ppm of CH4 within 90 min, increase of ≤10 ppm of CH4 within 90 minutes compared to baseline)

Treatment effect within subgroups will be presented by descriptive statistics (including 95%CI), inferential analysis and forest plots.

5.7.2 Secondary efficacy endpoints

All secondary endpoints will be analysed by stratification factor.

The following secondary endpoints will be analysed by Breath CH4 concentrations (increase of> 10 ppm within 90 min compared to baseline, increase of ≤ 10 ppm within 90 minutes compared to baseline) at Screening:

- Mean change from Baseline in number of inflammatory lesions at Day 60
- Percent of participants showing treatment success (i.e. IGA score of 0 or 1)
- Percent of participants with IGA score of 0 (clear)

Treatment effect within subgroups will be presented by descriptive statistics (including 95%CI) and inferential analysis.

5.8 Visit Windows

No visit windows will be applied for this study.

5.9 Missing Data

Summary statistics will generally be reported based upon observed data. Should a determination of treatment period (on treatment, pre-treatment) be required for adverse events or concomitant medications but the corresponding date is missing, or is a partial date, the event/medication will be considered on-treatment unless the portions of the date that are available indicate this is not possible.

Rules for imputation of start dates for AEs, concomitant medications and safety assessments are outlined in Table 6. Rules for imputation of end dates for AEs and concomitant medications are outlined in Table 7. Any AEs, concomitant medications and safety assessments with partial/missing dates will be displayed as such in the data listings.

Table 6: Imputation of start dates (AE, concomitant medications) and safety assessments (laboratory data, vital signs)

Missing element	Rule
year, or entire date	No imputation.

Missing element	Rule
day, month	If available year = year of study treatment start date then • If stop date contains a full date and stop date is earlier than study treatment start date then set start date = 01JanYYYY • Else set start date = study treatment start date. If available year > year of study treatment start date then 01JanYYYY. If available year < year of study treatment start date then 01JulYYYY.
day	If available month and year = month and year of study treatment start date then • If stop date contains a full date and stop date is earlier than study treatment start date then set start date= 01MMMYYYY. • Else set start date = study treatment start date. If available month and year > month and year of study treatment start date then 01MMMYYYY. If available month and year < month year of study treatment start date then 15MMMYYYY.

Table 7: Imputation of end dates (AE, concomitant medications)

Missing element	Rule
year, or entire date	No imputation. Notes: • If the AE or medication is ongoing, the stop date will remain missing. • The AE or medication will be included in all relevant time interval categories from its start day.
day, month	If partial end date contains year only, set end date = earliest of 31DecYYYY or end date of the study
day	If partial end date contains month and year, set end date = earliest of last day of the month or end date of the study

With reference to efficacy analyses performed on the FAS, the last observation carried forward (LOCF) strategy will be employed for dealing with any missing continuous post-baseline data for the relevant primary and secondary endpoints. For binary data (i.e. Success/Failure) missing data will be considered as Failures, unless differently specified in the appropriate analysis section for that data.

With reference to other categorical variables, the number of patients with missing data will be presented under the "unknown" category. Missing values will be included in the denominator count when computing percentages, unless differently specified in the appropriate analysis section for that data.

LOCF imputation will be used for the inferential analysis of categorical variables.

When other continuous data will be summarized, only the non-missing values will be evaluated for computing summary statistics.

Missing data in safety variables will not be imputed/replaced unless otherwise specified in the analysis section.

5.10 Interim Analyses and Data Monitoring

No interim analysis or assignment of a data monitoring committee is planned.

5.11 Multi-Center Studies

Considering that the number of subjects per center will be low, the center will not be considered as an explicative factor in the statistical analyses.

5.12 Multiple Testing

Due to the proof-of-concept purpose of this trial, no alpha-level adjustment for multiplicity will be applied.

5.13 Dry Run

Blinded dry-run(s) will occur prior to final database lock and unblinding in order to allow a review of clinical trial data and tables, listings, and figures. Consequences on the statistical analysis will be discussed and documented.

6 Summary of Study Data

6.1 Subject Disposition

Patient disposition will be tabulated overall, by treatment group and stratification factor and will include the number of patients who are screened via the social media recruitment path, screened via on-site recruitment path, randomized, completed the study, and discontinued the study during the double-blind period (including reasons).

The number and percentage of patients in each analysis set and reason for exclusion will be summarized overall, by treatment group and stratification factor.

Patient enrollment by study site will be tabulated by treatment group and overall.

A by-patient listing of inclusion into analysis sets and study completion information including reason for study drug discontinuation or early withdrawal from study, if applicable, will be presented.

6.2 Screen Failures

The reason(s) for screen failures will be listed.

6.3 Protocol Deviations

Protocol deviations for lack of compliance, mis-stratification, inadequate or missing informed consent, intake of study treatment other than the one assigned, incorrect doses and prohibited treatments, missed visits, missed assessments, out-of-window visits or assessments, and violations of inclusion/exclusion criteria (where possible) will be determined based on available data. All other protocol deviations will be collected by the clinical research associates.

Protocol deviations that could potentially affect the efficacy or safety conclusions of the study will be identified, and the assessment of determination of evaluable patients for analysis populations (FAS, mFAS, SAF, PP and PK) will be performed and approved by the Study Statistician, Medical Monitor, and Clinical Study Manager prior to database lock and unblinding of individual patient treatment information.

The process applied for the identification of protocol deviations will be described in a specific document.

All protocol deviations will be listed and summarized by treatment group. To identify deviation categories and sub-categories for summarization, the “Sponsor PD Number” variable in the excel workbook containing the list of protocol deviations will be used. The main category will be identified by the first number and the sub-category will be identified by the second number. As an example: if a code is “1.3”, “1” is the main category code and “3” is the sub-category code.

6.4 Demographic and Baseline Variables

Subject demographics and other baseline characteristics measured before randomization will be summarized descriptively by treatment group on the FAS and, if the PP set comprises less than 80% of the FAS, also on the PP set. Summaries will be provided by treatment group as well as overall. Summaries by L-BT status at screening will also be presented. A by-patient listing will also be provided.

Demographic variables will include the following:

1. Age at inform consent
2. Class age (≥18 and <30; ≥30 and <50; ≥50 and <65; ≥65) at informed consent

3. Sex
4. Race
5. Ethnicity

Other baseline characteristics will include the following:

1. Weight (kg)
2. Height (cm)
3. Body mass index (BMI) (kg/m^2)
4. Childbearing potential (for females only)
5. Alcohol consumption
6. Smoking habits
7. Drug abuse

Additionally, baseline disease characteristics and medical history questionnaire responses will be summarized descriptively and provided in a by-patient listing.

6.5 Medical History

Verbatim terms on eCRFs will be mapped to preferred terms (PTs) and system organ classes (SOCs) using Medical Dictionary for Regulatory Activities terminology (MedDRA).

Medical history will be summarized by SOC, PT, and treatment group (and overall) for the SAF. Summaries will be ordered by descending order (based on total) of incidence of SOC and PT within each SOC. A by-patient listing will also be provided. Conditions will be classified as 'previous' or 'ongoing' at baseline.

Summaries will be done for:

- Previous medical and surgical history not related to rosacea
- Ongoing medical and surgical history not related to rosacea

6.6 Concomitant Medications

Medications will be considered as prior if they stopped before the first study drug intake.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD) and will be classified according to the default Anatomical Therapeutic Chemical (ATC) classification system code (up to 4 levels), WHO-DD Drug Name, and Preferred Term (PT).

Prior and concomitant medications will be summarized separately for the SAF by the ATC Class Level 1 category, Level 2 category and PT. Subjects may have more than one medication per ATC category and PT. At each level of subject summarization, a subject will be counted once if he/she reports one or more medications at that specific level. The prior medication frequency distribution table will be displayed by the safety treatment arms and all subjects even though the prior medication information was collected prior to dispensing study drug.

Additionally, prohibited concomitant medications will also be summarized.

Prohibited concomitant medications from V1 to V4 include:

- Initiation of beta-blocker treatment (ATC = C07)
- Initiation of any oestroprogestinic or progestogen contraceptive or oestroprogestinic or progestogen replacement therapy (ATC = G03 [excluding G03XB], G02B)

- Biologic or non-biologic immunomodulatory or immunosuppressive drugs (ATC = L); warfarin (or other coumarins) (ATC = B01AA); niacin (ATC=A11HA); topical facial or systemic antibiotic treatments (ATC = J01, D01, D06); neomycin or any other low-absorbable oral antibiotics (such as marketed rifaximin) (ATC = A01AB, B05CA, J01GB, R02AB, A07A); topical facial, inhaled or systemic corticosteroids (ATC = D07, H02, A01AC, A07EA, D10AA, M01BA, N02CB, R01AB, R01AD, R03AK, R03AL, R03BA); topical and systemic retinoids (ATC = D05BB, D10AD, D10BA, L01XF); any other topical or systemic treatment for rosacea (including also laser and pulsed light, etc.) (ATC = D).
- Any cancer-related treatment (ATC = L) .
- "Over the counter" formulations including intestinal and topical skin probiotics (ATC=A07F).
- Any other experimental treatment.

6.7 Study Drug Accountability

Subjects will be dispensed blinded study drug according to the randomization schedule the Day 1 visit. Subjects will receive the study drug tablets in two bottles: a White bottle and a Yellow bottle. Possible tablet strengths are 0 mg and 250 mg. Subjects will be instructed to take 1 tablet of investigational product from each bottle three times daily by mouth, in order to maintain study blinding.

The following data will be collected and derived at the end of the Day 30 visit interval:

Parameter	Origin/Derivation
Tablets Dispensed Amount	CRF
Tablets Returned Amount	CRF
Expected Number of Tablets Taken	Derived: ((Date of Last Dose – Date of First Dose) x 6) + 2)
Estimated Number of Tablets Taken	Derived: Number of Tablets Dispensed Amount – Number of Tablets Returned Amount
Treatment Compliance (%)	Derived: 100 x Estimated Number of Tablets Taken / Expected Number of Tablets Taken (approximated to one number after the decimal point)

The Overall Treatment Compliance (%) will be calculated as:

> 100 x (Estimated Number of Tablets Taken) / (Expected Number of Tablets Taken), and approximated to one number after the decimal point.

The computed treatment compliance will be listed and summarized descriptively. Compliance input into the eCRF will not be listed or summarized.

The following compliance categorical ranges will be defined:

> <=80%, 80-100%, >100%, and Not Reported

All study drug accountability comments will be provided in a subject listing. Descriptive statistics of percent compliance, as continuous and categorical variable (<=80, 80-100, >100) will be presented by treatment group and stratification factor on the FAS and PP.

6.8 Study Drug Exposure

The study drug will be packaged in a way that each TID dose will be split between two separate colored bottles (White and Yellow), with the following blinded configuration for each treatment arm:

- 250 mg Rifaximin-EIR TID dose: one bottle of tablets will be placebo and one bottle of tablets will be 250 mg strength
- 500 mg Rifaximin-EIR TID dose: Both bottles of tablets will be 250 mg strength
- Placebo dose: Both bottles of tablets will be placebo

Due to the nature of this study and data collection, it will be assumed each tablet taken by the subjects will have equal treatment weight for study drug compliance and exposure. After database lock and when the study drug treatment is unblinded, each bottle number assigned to a subject will be checked to verify that correct treatment arm was given based on the randomization schedule. Any incorrect treatment given where the estimated number of tablets taken defined in Section 6.7 is greater than zero, will be defined as a major protocol deviation. Study drug exposure will be listed and summarized based on the treatment arm subjects received.

The number of study days of study drug exposure (overall treatment duration) will be calculated as the date of last dose minus the date of first dose + 1 and will be summarized on the SAF using descriptive statistics. The date of first dose will be collected in the Study Drug Administration Form. If the last dose date of study drug was not collected in the Study Drug Compliance eCRF page it will remain missing.

7 Efficacy Analyses

7.1 General Considerations

The primary statistical analysis on the FAS will be performed according to the actual treatment received.

In addition, two sensitivity analyses will be implemented for the primary analysis:

- The first by analyzing the FAS according to the “randomized treatment”, as originally planned
- The second on the mFAS according to the “randomized treatment”

Selected analyses of the efficacy endpoints will also be performed on the PP set as supportive analyses. For details as to which populations will be used for each analysis please refer to the TLF Shells document.

For the efficacy endpoints which use Analysis of Covariance (ANCOVA) models, least squares (LS) means will be used to compare each active treatment arm with placebo. The active treatment arms will not be themselves compared. The LS mean differences will be presented with 95% CIs and p-values.

The binary efficacy endpoints expressed as percentages will be analyzed by means of a two-sided Cochran Mantel-Haenszel Chi-square test stratified by L-BT status at Screening. The Breslow-Day test for stratified tables will be applied. A two-sided 95% CI for difference of success rate between each active treatment arm vs placebo will also be computed.

For efficacy endpoints addressing results at both end of treatment (V3) and end of study (V4), analysis for each visit will be conducted separately.

7.2 Primary Efficacy Analyses

The co-primary efficacy endpoints, clinically assessed by the Investigator, are the change from baseline in total number of rosacea inflammatory lesions (papules and pustules) at the end of treatment, and the percent of subjects showing treatment success, defined as an IGA score of 0 (clear) or 1 (almost clear) with at least a 2-grade improvement from baseline at the end of treatment.

The first co-primary efficacy endpoint (change from baseline in number of inflammatory lesions at end of treatment (Day 30)) will be analyzed by means of an Analysis of Covariance (ANCOVA) model (one model for each comparison to placebo), with change from Baseline to Day 30 as dependent variable, treatment group and L-BT status at screening as explicative factors, and baseline number of inflammatory lesions as covariate.

The adjusted mean differences with the corresponding 95% confidence interval will be estimated from the model. The 95% confidence interval for the difference between adjusted mean differences of each active treatment arm versus the placebo arm will be also presented.

The second co-primary efficacy endpoint (percent of patients showing treatment success) will be analyzed by means of a stratified (by L-BT status) Cochran Mantel-Haenszel (CMH) chi square test. The Breslow- Day test with Tarone's adjustment for stratified tables will be applied. A two-sided 95% CI for difference in success rate between the treatment groups (each active group vs placebo) overall and by L-BT status will also be computed.

For each co-primary endpoint, inferential statistical analyses will be completed for each subgroup described in Section 5.7.

7.3 Secondary Efficacy Analyses

Considering the explorative nature of the secondary endpoints, all the inferential results obtained on them will be descriptively interpreted.

7.3.1 Change from Baseline in number of inflammatory lesions (papules and pustules) at end of study (Day 60)

The change from baseline to V4 will be analyzed by means of an Analysis of Covariance (ANCOVA) Model (one model for each comparison to placebo), with change from Baseline to Day 60 in inflammatory lesions as dependent variable, treatment group and L-BT status at screening as explicative factors and baseline count of inflammatory lesions as covariate.

The adjusted mean differences with the corresponding 95% confidence interval will be estimated from the model. The 95% confidence interval for the difference between adjusted mean differences of each active treatment arm versus the placebo arm will be also presented.

7.3.2 Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at end of treatment (Day 30) and end of study (Day 60).

The percent of participants showing treatment success (IGA score of 0 or 1) at the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of a two-sided Cochran Mantel-Haenszel Chi-square test stratified by L-BT status at screening. The Breslow-Day test with Tarone's adjustment for stratified tables will be applied. A two-sided 95% CI for difference of success rate between the treatment groups (each treatment arm vs placebo) will also be computed.

7.3.3 Percent of participants with IGA score of 0 (clear) at end of treatment (Day 30) and end of study (Day 60)

The percent of participants with an IGA score of 0 at the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of a two-sided Cochran Mantel-Haenszel Chi-square test stratified by L-BT status at screening. The Breslow-Day test for stratified tables will be applied. A two-sided 95% CI for difference of success rate between the treatment groups (each treatment arm vs placebo) will also be computed.

7.3.4 Change from Baseline in additional rosacea features at end of treatment (Day 30) and end of study (Day 60)

The following rosacea features will be analyzed:

1. Pain, burning/stinging and itching (measured using a 0-10 cm Visual Analogue Scale (VAS))
2. telangiectasia (absent=0, mild=1, moderate=2, severe=3)
3. ocular manifestations (absent=0, mild=1, moderate=2, severe=3)
4. phymatous changes (absent=0, mild=1, moderate=2, severe=3)

For each of the above features, the change from baseline value to the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of an Analysis of Covariance (ANCOVA) (one model for each comparison to placebo) Model, with change from baseline value as dependent variable, treatment group and L-BT status at screening as explicative factors and baseline value as covariate.

The adjusted mean differences with the corresponding 95% confidence interval will be estimated from the model. The 95% confidence interval for the difference between adjusted mean differences of each active treatment arm versus the placebo arm will be also presented.

7.3.5 Change from Baseline in facial non-transient erythema at end of treatment (Day 30) and end of study (Day 60)

The change from baseline to the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of an Analysis of Covariance (ANCOVA) Model (one model for each comparison to placebo), with change from baseline in facial non-transient erythema as dependent variable, treatment group and L-BT status at screening as explicative factors and baseline facial non-transient erythema as covariate.

The adjusted mean differences with the corresponding 95% confidence interval will be estimated from the model. The 95% confidence interval for the difference between adjusted mean differences of each active treatment arm versus the placebo arm will be also presented.

7.3.6 Change from Baseline in Irritable Bowel Syndrome - Symptom Severity Scale Parameters

The following Irritable Bowel Syndrome - Symptom Severity Scale parameter will be analyzed:

1. abdominal pain score
2. abdominal distention score
3. bowel habit satisfaction score
4. global severity of abdominal symptoms score

For each of the above features, the change from baseline to the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of an Analysis of Covariance (ANCOVA) Model (one model for each comparison to placebo), with change from baseline value as dependent variable, treatment group and L-BT status at screening as explicative factors and baseline value as covariate.

The adjusted mean differences with the corresponding 95% confidence interval will be estimated from the model. The 95% confidence interval for the difference between adjusted mean differences of each active treatment arm versus the placebo arm will be also presented.

7.3.7 Percent of participants showing treatment success according to Modified IGA Scale

The following Modified IGA scales will be used:

- Modified IGA scale including erythema but excluding rosacea inflammatory lesions

- Modified IGA scale including erythema and rosacea inflammatory lesions

The percent of participants with a Modified IGA score of 0 or 1 (according to the previous definitions) at the end of treatment (Day 30) and end of study (Day 60) will be separately analyzed by means of a two-sided Cochran Mantel-Haenszel Chi-square test stratified by L-BT status at screening. The Breslow-Day test with Tarone's adjustment for stratified tables will be applied. A two-sided 95% CI for difference of success rate between the treatment groups (each treatment arm vs placebo) will also be computed.

7.4 Other Efficacy Analyses

Other efficacy parameters will be summarized descriptively only. These parameters will be collected via a diary and reviewed throughout the course of the study through an electronic Patient Reported Outcome (ePRO) from the randomization visit until the end of the follow up period (30 days after last dose).

7.4.1 Irritable Bowel Syndrome - Symptom Severity Scale

The Irritable Bowel Syndrome - Symptom Severity Scale (IBS-SSS), that has to be collected every ten days, will be summarized descriptively by Study Day for the FAS population. A by-patient listing will be provided.

7.4.2 Dermatology Quality of Life Index

The Dermatology Quality of Life Index (DQLI) consists of 10 questions concerning symptoms and feelings, daily activities, leisure, work, and school, personal relationships, and treatment.

Each question is scored on a four-point Likert scale:

- Very much = 3
- A lot = 2
- A little = 1
- Not at all = 0
- Not relevant = 0
- Question unanswered = 0

The DLQI, that has to be collected every ten days, is calculated by adding the score of each question, resulting in a maximum of 30 and a minimum of 0. The higher the score, the more quality of life is impaired.

The meaning of the DLQI total score is summarized below:

- · 0-1 - no effect at all on patient's life
- · 2-5 - small effect on patient's life
- · 6-10 - moderate effect on patient's life
- · 11-20 - very large effect on patient's life
- · 21-30 - extremely large effect on patient's life

The score will be summarized descriptively by Study Day for the FAS population. A by-patient listing will be provided. In addition, the following summary scores will be calculated, listed and summarized descriptively:

- Symptoms and Feelings: sum of questions 1 and 2 (maximum score: 6)
- Daily Activities: sum of questions 3 and 4 (maximum score: 6)

- Leisure: sum of questions 5 and 6 (maximum score: 6)
- Work and School: question 7 (maximum score: 3)
- Personal Relationships: sum of questions 8 and 9 (maximum score: 6)
- Treatment: question 10 (maximum score: 3)

If one question is unanswered, this is allocated a score of 0 and the DLQI score summed in the usual way. If two or more questions are unanswered, the questionnaire is not scored.

When using sub-scales, if the answer to one question in a sub-scale is missing, that sub-scale should not be scored.

Scoring of question 7

The first part of question 7 asks: 'Over the last week, has your skin prevented you from working or studying?'

If working or studying are not relevant to the subject, the response is 'Not relevant' (scored 0).

If the skin disease has prevented the subject from working or studying, the answer is 'Yes'. As 'prevention' is the biggest possible impact it is scored the maximum, 3.

If the skin disease has not prevented the subject from working or studying, the answer is 'No'. The subject is therefore asked the following question 'over the last week how much has your skin been a problem at work or studying?'. There are three possible responses to the question 'How much has your skin been a problem at work or studying': 'A lot' (scored 2), 'A little' (scored 1) or 'Not at all' (scored 0).

8 Safety Analyses

All safety measures will be descriptively summarized by treatment group in the SAF population.

8.1 Adverse Events

Adverse events will be presented for the Safety Analysis Set (SAF), by treatment group.

All adverse events will be assigned to a Preferred Term (PT) and will be classified by Primary SOC according to the latest MedDRA version.

Any adverse event which started at or after the first administration of study treatment will be considered as Treatment Emergent Adverse Event (TEAE). If the start date is missing for an AE, the AE will be considered to be treatment emergent. See Section 5.9 for details on partially missing AE start dates.

Adverse events will be reported on a per-patient basis. This means that even if a patient reported the same event repeatedly, the event will be counted only once. Also, the number of events will be presented.

TEAEs relationship to study medications and TEAE severity will be investigated with frequency tables.

In case of missing relationship/severity, the AE is considered related/severe, respectively.

The following tables will be presented:

1. Overview of TEAEs showing the number of TEAEs, the number of TESAEs and the number of patients with any TEAEs, treatment-related TEAEs, serious TEAEs, treatment-related serious TEAEs, severe TEAEs, TEAEs leading to treatment discontinuation and TESAEs with Outcome equal to Death;
2. Summary of TEAEs by Primary SOC and PT;
3. Summary of Related TEAEs by Primary SOC and PT;

4. Summary of TEAEs by Primary SOC, PT and maximum Severity;
5. Summary of Serious TEAEs by Primary SOC and PT;
6. Summary of Related and Serious TEAEs by Primary SOC and PT;
7. Summary of Serious TEAEs by Primary SOC, PT and maximum Severity;
8. Summary of TEAEs Leading to Early Discontinuation of the Study Treatment by Primary SOC and PT.
9. Summary of TEAEs Leading to Temporary Interruption by Primary SOC and PT.
10. Summary of Most Frequent AEs (at least 2% in any treatment group) by PT

No inferential statistical tests will be applied to compare the treatment arms in terms of incidence of adverse events.

By-patient listings will be created for the following:

1. All Treatment Emergent Adverse Events
2. Serious Treatment Emergent Adverse Events
3. Treatment Emergent Adverse Events Leading to Treatment Discontinuation or Temporary Interruption
4. All Treatment Emergent Adverse Events in Patients with Fatal TEAEs

Adverse events occurred before the first administration of study treatment will only be listed.

8.2 Pregnancies

Although pregnancy is a strict exclusion criteria and acceptance of methods to prevent pregnancy is an eligibility criterion, unexpected cases of pregnancy could occur during the study. If a pregnancy occurs during the course of the study, any treatment will be stopped and the Investigator shall notify, regardless whether a SAE is present or not, the Sponsor or whoever assumes the tasks delegated by the sponsor within 24 hours of knowing about the event. If the pregnancy outcome meets the SAE criteria or if the newborn presents a serious event, the procedures for reporting a SAE will be followed. Pregnancy test results and corresponding SAE information will be listed.

8.3 Clinical Laboratory Evaluations

Laboratory parameters will be summarized in the standard international system of units. Quantitative laboratory results will be summarized by treatment group using descriptive statistics at baseline and each post-baseline scheduled visit. Change from baseline will also be summarized using descriptive statistics by parameter and visit. Quantitative urinalysis results will not be summarized.

In addition, laboratory values will be classified as normal/high/low based on the reference range. Abnormal values will also be classified as clinically significant (CS) abnormalities and non-clinically significant (non-CS) abnormalities.

Clinical laboratory values outside normal ranges will be summarized by visit, overall and separately for the two categories: "outside normal ranges but not clinically significant" and "outside normal ranges and clinically significant".

Also shift tables from baseline to post-baseline assessments will be produced for chemistry and hematology.

If a laboratory value is above/below limit of quantification (less than/greater than a certain value), the limit of quantification will be used in the summaries.

No inferential statistical tests will be applied to compare the treatment arms.

Listings will be provided for all tests. In addition, for lab values outside normal ranges and clinically significant, a listing for all test values overtime will be presented for the subjects with such abnormalities.

8.4 Lactulose Breath Test

Results relevant to the lactulose breath test (Positive/Negative SIBO) will be summarized by treatment group using descriptive statistics (counts and percentages) at screening and end of treatment visits. The numerical test results (H2 and CH4) will also be presented.

Shift tables for L-BT results will be provided.

8.5 Vital Signs

The results and change from baseline to each post-baseline scheduled visits will be summarized for blood pressure (systolic and diastolic), heart rate, body temperature, height, weight and BMI.

8.6 Physical Examination

Physical examination (signs and symptoms) will be summarized by Body System, treatment group and visit by means of descriptive summaries (counts and percentages).

9 Pharmacokinetics Analyses

The pharmacokinetics of rifaximin after the repeated administration of Rifaximin-EIR will be assessed after the first and last dosing (Day 1 and Day 30) as a secondary study endpoint.

The PK analysis will be done on the PK set for randomized subjects who are enrolled in the PK Sub-study.

9.1 Time Collection

Venous blood samples will be collected from the subjects enrolled in the PK Sub-study.

For all subjects in the PK Set, approximately 22 blood samples will be collected over the course of the study according to the following scheme:

- Day 1 (V2): Pre dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post dosing (first dose in the morning)
- Day 30 (V3): Pre dose (single dose in the morning), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 22, 24, 30 hours post dosing (blood sampling will continue at Day 31 as appropriate). The actual time of PK sampling collection must be recorded in the CRF.

The acceptable deviations from the nominal blood sampling times are as follows:

- Day 1 only: The pre-dose samples will be taken ≤1 h before dosing;
- 0 to 1 h post-dose samples will be taken within ± 2 min of the nominal post-dose sampling time 1.5 to 8 h post-dose on Day 1 and 1.5 to 12 h post-dose on Day 30 samples will be taken within ± 10 min of the nominal post-dose sampling time;
- 22 to 30 h post-dose samples will be taken within ± 30 min of the nominal post-dose sampling time.

Rifaximin concentrations in plasma will be quantified using a validated HPLC-MS/MS method. (Bioanalytical lab: Pyxant).

Individual plasma concentrations and collection times will be listed by subject for all subjects including those excluded from the PK analysis due to non-completion.

9.2 Plasma PK Parameters: Definitions and Rounding Specifications

Pharmacokinetic analysis of the plasma concentration-time data for rifaximin will be performed using non-compartmental methods for extravascular administration in Phoenix® WinNonlin® version 8.2 or higher. The PK parameters presented below will be estimated where appropriate and possible.

Table 8: Plasma Pharmacokinetic Parameters

Parameter	Day	Definition	Unit	DP or SF	No. of DP/SF
T_{max}	1&30	Time of maximum observed concentration	h	DP	2
C_{max}	1&30	Maximum observed concentration	mass unit/mL	SF	3
$AUC_{(0\text{-}tau)}$	1&30	Area under the curve for the dosing interval (0-8 hours), calculated by the linear trapezoidal method defined interval between doses	mass unit.h/mL	SF	3
$AUC_{(0\text{-}last)}$	30	Area under the curve from time 0 to the time of last measurable concentration, calculated by the linear trapezoidal method	mass unit.h/mL	SF	3
$AUC_{0\text{-}\infty}$	30	Area under the curve from time 0 extrapolated to infinity, calculated as $AUC_{0\text{-}last}$ + Ct/Lambda-z, where Ct is the last measured concentration.	mass unit.h/mL	SF	3
$t_{1/2}$	30	Terminal elimination half-life, calculated as Ln(2)/ Lambda-z	h	DP	2
Lambda-z	30	First order rate constant associated with the terminal (log-linear) portion of the plasma concentration vs time curve	1/h	DP	4
AR C_{max}	30	Accumulation ratio based on Day 30 C_{max}/Day 1 C_{max}	NA	DP	2
AR $AUC_{(0\text{-}tau)}$	30	Accumulation ratio based on Day 30 $AUC_{(0\text{-}tau)}$/Day 1 $AUC_{(0\text{-}tau)}$	NA	DP	2

DP=decimal places; SF=significant figures; NA=not applicable

The nominal dose will be used in the calculation of relevant PK parameters.

The imputation of non-numerical (e.g. below the limit of quantification (BLQ)) or negative values (e.g. pre-dose sampling times) reported in the input data set will be performed as follows for calculation of PK parameters:

- Day 1 pre-dose sample times will be entered as zero
- Values that are BLQ obtained prior to C_{max} will be entered as zero
- Values that are BLQ after C_{max} will be treated as missing
- Values that are measurable after at least 2 consecutive BLQ values after C_{max} will be treated as missing for the calculation of PK parameters
- Values that are reported as “No Result” or “Not Reportable” (NR), “Not Calculated” (NC) or “No Sample” (NS) etc. will generally be considered missing

No imputation will be made for missing concentration values. Missing values will be treated as if they were not scheduled to be collected and PK parameters will be estimated based on the reported rifaximin concentrations for the subject.

If the pre-dose concentration value on Day 1 is greater than 5% of Cmax on that day, the subject will be dropped from pharmacokinetic analyses.

Plasma PK parameters will be estimated using standard Phoenix WinNonlin methods, details of which may be found in the documentation accompanying the WinNonlin software package. The rules specified in Table 9 will be applied:

Table 9: PK Parameter Estimation Details

Sampling times	Actual
Calculation method	Linear trapezoidal linear/log interpolation
Number of points used for Lambda-z	At least 3, not including C_{max}
Minimum requirements for AUC	At least 4 consecutive measurable concentrations

All times used in the calculation of PK parameters will be the actual elapsed time from treatment administration, with the exception of pre-dose data which will be given the nominal time of 0.00 hours.

In case of multiple peaks, T_{max} and C_{max} refer to the highest measured concentration in all circumstances. In the case of two or more samples with the same concentration (as supplied by the bioanalytical laboratory), T_{max} refers to the earliest occurrence.

For calculation of partial AUCs (i.e. $AUC_{(0\text{-}tau)}$), log-linear interpolation will be used if required to account for deviations from the nominal sampling schedule to obtain AUCs over the same interval for all subjects.

Where possible, the terminal elimination rate constant (Lambda-z) will be calculated for all subject profiles. The value of Lambda-z will be determined by the slope of the regression line of the natural log transformed concentrations vs time. The WinNonlin determined choice of data points for determination of Lambda-z will be reviewed by the pharmacokineticist who may adjust the selection in order to provide a more appropriate fit. The choice of data points for determination of Lambda-z for each profile will be confirmed following a documented peer review.

No value of derived parameters such as λ_Z, $t_{1/2}$, $AUC_{0\text{-}\infty}$ will be reported for cases that

- do not exhibit a terminal log-linear phase in the concentration versus time profile
- in the event that the adjusted rsq of regression is <0.85
- where the value of $t_{1/2}$ is physiologically implausible
- the time period used for regression analysis is less than 2-fold the calculated half-life

$AUC_{0\text{-}\infty}$ will be deemed unreliable in the event that the extrapolated portion of $AUC_{(0\text{-}inf)}$ >25% and excluded from the summary statistics.

9.3 PK Concentration and Parameter Summaries

Summaries of concentration data will include mean, standard deviation and coefficient of variation by dose day at each scheduled collection time, by dose. Mean concentration–time profiles will be presented graphically, with concentration shown on linear and logarithmic scales. Summary statistics (i.e., n, mean, SD, coefficient of variation (CV%), minimum, Q1, median, Q3, maximum, geometric mean, geometric SD and geometric CV%) of concentration data will be calculated for each active dose level (i.e. 250 mg and 500 mg), visit (i.e., Day 1 and Day 30) and time point for rifaximin in plasma. The number of BLQ values (n#) per time point will also be presented. Geometric statistics will not be calculated for Day 1, pre-dose concentrations.

Non-measurable values reported in the plasma concentration data (i.e., values that are BLQ), will be entered as zero for the determination of summary statistics with the exception of geometric means, geometric SD and geometric CV%, where BLQ values will be imputed as half the lower limit of quantification (LLOQ) value. Data recorded as NR, NS or NC will be handled as missing (i.e., no assumption will be made about the actual concentration).

Pharmacokinetics parameters will be summarized separately by dose day, treatment group, gender and overall. Summary statistics (i.e., n, mean, SD, CV%, minimum, Q1, median, Q3 and maximum) of plasma PK parameters will be calculated for rifaximin for each active dose level, visit, and gender. Geometric mean, geometric SD and geometric CV% will be presented for all plasma PK parameters except T_{max}.

10 Summary of Changes to the Protocol

1. In protocol section 16.2.5, it states that "The analyses of the efficacy endpoints will be performed on the FAS and the PPS. Results on the FAS will be considered primary. Results on the PPS population will be used as supportive." This has been changed in the SAP section 5.4 as follows: "The analyses of the main efficacy endpoints will be performed on the FAS and the PP set. Results on the FAS will be considered primary. Results on the PP set will be used as supportive. The mFAS population will be used for sensitivity analyses. For details as to which populations will be used to analyze each endpoint please refer to the TLF Shells document.

2. The definition of the PK Set described in protocol section 16.2.6 has been changed from:

   The PK analysis set will include all randomized subjects who received at least one dose of Rifaximin and had a suitable PK profile.

   To:

   The Pharmacokinetic (PK) set will include all randomized subjects within the PK Sub-study strata who received at least one dose of Rifaximin. The PK set will be defined with approval from Alfasigma after receipt of plasma concentration data, database lock and unblinding.

   PK set will include all subjects who have received at least 1 dose of IMP and, specifically that they satisfy the following criteria for at least 1 profile:

   - No missing samples at potential critical time points.
   - No relevant events (e.g. protocol deviations or adverse events) that may impact the study objectives with respect to the PK endpoints.
   - Subjects who received placebo will not be included in the PK population.

3. Subgroup analyses described in Section 5.7 were not planned in the Study Protocol and have been added to permit a comparison with the similar study conducted in Europe.

11 References

[1] US Federal Register. International Conference on Harmonisation; Guidance on Statistical Principles for Clinical Trials. Department of Health and Human Services: Food and Drug Administration [Docket No. 97D-0174]. Federal Register Volume 63, Number 179, pages 49583-49598. 16 September 1998. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E9/Step4/E9_Guideline.pdf

[2] American Statistical Association. Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, 07 August 1999. http://www.amstat.org/profession/ethicalstatistics.html

[3] Royal Statistical Society. The Royal Statistical Society: Code of Conduct, August 1993. http://www.rss.org.uk/about/conduct.html

12 Planned CSR Tables, Listings, and Figures

A separate document containing the list of tables, listings, and figures (TLFs) to be included in the post-text Appendix 14 of the CSR will be provided. TLFs may be modified with Sponsor's approval and as deemed necessary without update to the SAP.

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