STUDY PROTOCOL

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

Study code:	RE-ROS2002-2021
IND number:	CCI
Study reference number used by CRO:	N.A.
Phase:	IIa
Name of the investigational product:	Rifaximin Delayed-Release 250 mg tablet, a new formulation that contains enteric-coated microgranules of Rifaximin (Rifaximin-EIR).
Coordinating Investigator	PPD High Point, NC 27262, USA
Sponsor:	Alfasigma S.p.A.
CRO:	BIORASI 18851 NE 29th Ave, Suite 800 Aventura FL 33180, USA
Status / Date:	Final Version 2.0 – 25/03/2022

SIGNATURE PAGE (1/3)

The signatories are obliged to comply in all respects with:

- this clinical study protocol,
- the standards of Good Clinical Practice as defined in the "Guideline for Good Clinical practice E6 (R2)" (EMA/CHMP/ICH/135/1995)" and related Guidelines,
- all applicable regulatory requirements including national drug law and data protection law.

PPD
Clinical Research Physician
Alfasigma S.p.A.

PPD

PPD
Early Stage Clinical Development Head
Alfasigma S.p.A.

PPD

Date (dd/mm/yyyy) Signature

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Clinical Trial Operations Head
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Alfasigma S.p.A.

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Quality Assurance GCP/GVP/GLP Manager
Alfasigma S.p.A.

PPD

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STUDY COORDINATOR'S SIGNATURE

Declaration of Coordinating Investigator

Title: 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

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice, and other applicable national and local laws and regulations.

Prof./Dr. _____
_____
_____
_____
_____

Tel: _____
e-mail: _____

_____ _____
Date (dd/mm/yyyy) Signature

SIGNATURE PAGE (3/3)

Declaration of the Investigator

Title: 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

All documentation for this study supplied to me, which has not been previously published, will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, Case Report Forms, and other scientific data.
The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC).
No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the subjects.
I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local study centre:

Name:

Title:

Institution:

Telephone:

Date (dd/mm/yyyy)	Signature

EMERGENCY INSTRUCTIONS

- **All Serious adverse events**
- **All adverse events that could affect the safety of the study participants or the conduct of the trial must be reported within 24 hours to one of the following:**

Alfasigma S.p.A., PPD
E-mail: PPD

Fax: PPD

During an emergency the investigator may unblind the subject via interaction response technology (IRT) if the knowledge of the assigned treatment is necessary to treat the subject adequately.

Definition of Serious Adverse Events and Serious Adverse Drug Reaction:

A serious adverse event (SAE) or serious adverse drug reaction (SADR) is any untoward medical occurrence that at any dose is:

- fatal
- life-threatening
- resulting in or prolonging in-patient hospitalisation
- severely or permanently disabling or incapacitating
- a congenital anomaly/birth defect
- other event(s) considered medically important

SYNOPSIS

Study Title:	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
Study Code:	RE-ROS2002-2021
Study Centre(s):	Approximately 40 centers in the USA
Drug on the study	Rifaximin is a negligibly absorbed, broad spectrum oral antibiotic currently used to treat specific intestinal infections. Rifaximin-EIR is a delayed release formulation of Rifaximin that contains enteric-coated microgranules of Rifaximin. The new formulation used in this trial, Rifaximin-EIR 250mg tablet (not yet on the market), has been developed to optimize Rifaximin intestinal concentration and therapeutic effect.
Rationale and Objectives:	Rosacea is a common chronic inflammatory relapsing-remitting skin condition almost exclusively affecting the central area of the face and the eyes. Based on its main clinical manifestations rosacea has been classically divided into the following four subtypes: i) erythematotelangectic, ii) papulopustular, iii) phymatous, and iv) ocular. Recent modifications of international clinical guidelines however have focused on individual features rather than combination of features. This contemporary “phenotype” approach is based on two rosacea features that are independently diagnostic (persistent central facial erythema and phyma), and other major features that are diagnostic when in combination (i.e. papules and pustules, facial telangiectasia excluding alar involvement, transient central facial erythema, otherwise known as flushing, and specific ocular manifestations). Preliminary evidence suggests that treatment with rifaximin, a poorly absorbed oral antibiotic drug may be beneficial in patients with rosacea, specifically in those with papulopustular phenotype. The objective of this study is twofold: 1) to explore the safety and efficacy of two doses of oral Rifaximin delayed-release (750mg/day and 1500mg/day) administered t.i.d. for 30 days versus placebo in adults with moderate-to-severe papulopustular rosacea. 2) in a sub-group of patients, to assess the pharmacokinetics (PK) of the 750mg/daily and 1500mg daily dose of Rifaximin-EIR 250mg administered using a t.i.d. schedule.
Phase	IIa
Experimental design:	This is Proof of Concept, phase IIa, multicenter, double-blind, placebo-controlled, randomized clinical trial.

	Two hundred one (201) eligible subjects are planned to participate in the main study while a subgroup of 18-36 (a minimum of 6 and a maximum of 12 subjects per arm) are planned to participate in the PK sub-study. Patients participating in the PK study will be randomly selected from the whole population. At randomization visit, subjects will be 1:1:1 randomized to receiving any of the two daily doses of active treatment or placebo. TID doses of the assigned treatment will be administered from Day 1 to Day 30. On Day 30, just the first daily dose will be administered. The randomization will be stratified based on Lactulose Breath Test (L-BT) status, i.e. negative (L-BT result with H_2 ≤10 ppm by 90 minutes compared to time 0) vs. positive (L-BT H_2 >10 ppm) and PK sub-study participation (yes or not). Subjects participating in the PK sub-study, at Day 30 will stay overnight to complete the PK assessments. Venous blood samples for PK analysis will be collected on: • Day 1: Pre1st dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post dosing. • Day 30: 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. Prospective participants can be recruited using two different strategies: • social media recruitment; • on-site recruitment Based on the recruitment strategy, subjects will follow a different path (Study Schedule A [social media recruitment] or B [on-site recruitment]). **A - Social media recruitment** Specific advertisements targeted to papulopustular rosacea subjects posted in selected social media will re-direct subjects interested in participating in the study to a dedicated study website. Remote data recording (V0): In a dedicated study website, a *prospective participant* will be asked to: • review a concise study description, • provide online pre-screening informed consent, • provide contact details, • provide demographics (e.g., age, gender, etc.), • complete an online medical history and concomitant medication questionnaire, • upload either three pictures of their face (one frontal and two

	lateral [right and left side] photos) or a short video of the face.
	
	Central Reader Validation:
	This task will be performed no later than 2 working days after V0. A Central reader (*expert dermatologist*) will:
	• check preliminary compliance with inclusion/exclusion criteria based on the
	  ○ review of personal and clinical data (including medical history, concomitant medications etc.) posted on the website,
	  ○ review of patient's images downloaded on the website,
	• confirm the preliminary eligibility of pre-screened candidates and refer them via a dedicated website to the clinical team.
	
	Phone contact with the subject:
	This task will be performed no later than 1 week after the candidate validation has been occurred.
	The investigational team will call the prospective participant and in case the subject accepts to move forward, a clinic visit will be scheduled (V1).
	
	On the Screening visit (V1) the Site Investigator will conduct formal informed consent process and proceed with the procedures foreseen by the visit. All subsequent visits (Randomization visit, V2; End of Treatment visit, V3; End of Study visit, V4) will be identical for both recruitment paths (social media and on site).
	
	B - On-site recruitment
	
	Subjects presenting at the Investigational Site with moderate or severe papulopustular rosacea will be informed about the possibility to participate in the trial and if they are interested the Investigator will provide further information on the study and, if the subject is interested to participate, he will schedule the Screening visit (V1). All other visits will be performed as the social media recruitment path (see above).

Number of Subjects:	As described in Section 16.1 (Determination of Sample Size), two hundred one (201) eligible subjects (67 per arm) with moderate-to-severe papulopustular rosacea, with a cap of 33 subjects per arm having negative L-BT at screening (i.e., a test showing an increase ≤10 ppm of H_2 by 90 minutes compared to time 0) are planned to be enrolled. Plasma samples will be collected in a sub-group of 6-12 subjects (minimum and maximum, respectively) per arm for pharmacokinetics assessments.
Study Population:	Inclusion criteria

	All the following criteria must be met both at the Screening and Randomization visits unless otherwise specified. 1. Men and women aged 18 years or older at screening (V1) 2. Female participants are eligible if they are: of non-childbearing potential, i.e.: i) post-menopausal (at least 2 years without spontaneous menses), or ii) surgically sterile (bilateral tubal occlusion, or hysterectomy), or iii) ablation of both ovaries), or of childbearing potential with a negative pregnancy test result at screening and randomization and agreeing to use a highly effective method of contraception (i.e. with failure rate of less than 1% per year) until 72 hours after taking the last study treatment dose. *Note 1*. Based on Clinical Trial Facilitation Group recommendations, highly effective methods of contraception are the following: • intrauterine device (IUD); • intrauterine hormone-releasing systems (IUS) or; • combined hormonal contraceptives (i.e. estrogen and progestogen) in oral, intravaginal or transdermal form, with inhibition of ovulation as primary mode of action or; • progestogen-only hormonal contraceptives in oral, injectable or implantable form, with inhibition of ovulation as primary mode of action or; • absolute and continuous sexual abstinence from Day 1 included (first day of treatment) until 72 hours after taking the last study treatment dose. *Note 2*. In each case of delayed menstrual period (over one month between menstruations), female participants of child-bearing potential will be strongly recommended a confirmation of absence of pregnancy. This recommendation applies also to women of child-bearing potential with infrequent or irregular menstrual cycles. 3. Presence of rosacea, papulopustular phenotype, defined as papules and/or nodules with or without pustules of rosacea, without comedones, plus: centrofacial persistent erythema or phyma or transient episodic facial erythema (flushing) or facial telangiectasia (excluding peri-alar regions) or major ocular rosacea features (i.e. lid margin telangiectasia, interpalpebral conjunctival injection, spade-shaped infiltrates in the cornea, scleritis and sclerokeratitis). 4. Presence of ≥11 and ≤ 70 facial papules and/or pustules. 5. Moderate (Grade 3) or severe (Grade 4) rosacea based on Investigator's Global Assessment based on Investigator's judgement. 6. Patients accepting to provide and legally capable of providing

	free and informed consent to all procedures included in the protocol (including facial skin photography). Exclusion criteria None of the following criteria must be met both at the Screening visit and at the Randomization visit (Day 1) unless otherwise specified: 1. Granulomatous rosacea or rosacea fulminans. 2. Erythematoteleangectatic, phymatous or ocular rosacea only. Patients with these subtypes associated with papulopustular rosacea can be enrolled. 3. Rosacea with IGA grade ≤2 based on Investigator's judgment. 4. Anticipated need for proctoscopy or colonoscopy within two weeks after lactulose breath test. *Note 3*: Subjects undergoing unanticipated proctoscopy or colonoscopy within a week from lactulose breath test should have a thorough bowel cleansing with a non-fermentable solution. 5. Subjects requiring a low galactose diet. 6. Hypersensitivity or intolerance to lactulose or any excipient of the lactulose preparation to be used for L-BT. 7. History of inflammatory bowel disease (Crohn's disease or ulcerative colitis) or other conditions characterized by severe intestinal ulcers. 8. History of coeliac disease. 9. Patients with intestinal obstruction or partial intestinal obstruction. 10. Presence of diarrhoea associated with fever and/or blood in the stool. 11. Severe kidney impairment (i.e. estimated glomerular filtration rate <30 ml/min). 12. Severe hepatic impairment (i.e. Child-Pugh B or C). 13. Cancer or any cancer-related treatment within 5 years prior to screening (excluding non-melanoma skin-cancer). 14. History of alcohol or drug abuse within a year prior to screening, based on Investigator's judgement. 15. Facial skin conditions that can interfere with reliable assessment of rosacea throughout the study (e.g. facial hair, tattoos, other facial adornments, keloids, hypertrophic scarring, recent facial surgery, excessive sun exposure including use of tanning beds) 16. Any other significant health condition (e.g. cardiovascular, respiratory, renal, hepatic, neurologic, psychiatric, hematologic, oncologic, immune etc.) or non-health condition that in the investigator's judgement may: *i)* jeopardize the patient's safe participation in the trial or *ii)* make unlikely the patient's completion of the study or *iii)* make unlikely the patient's compliance with the study

	procedures (e.g. highly anticipated need of non-permitted treatments, terminal illness, etc.). 17. History of hypersensitivity to rifaximin, rifamycin-derivatives, any of the rifaximin-EIR or placebo excipients. 18. Treatment with biologic immunomodulatory and/or immunosuppressive drugs (e.g. anti-TNF drugs) within 6 months prior to randomization. 19. Treatment with non-biologic immunomodulatory and/or immunosuppressive drugs (e.g. cyclosporine, methotrexate etc.) within 30 days prior to randomization. 20. Treatment with warfarin (or other coumarins) within 14 days prior to randomization. 21. Treatment with niacin within 30 days prior to randomization. 22. Topical facial or systemic antibiotics within 30 days before randomization; 23. Treatment with neomycin or other low-absorbable oral antibiotics (such as marketed rifaximin) within 90 days before randomization. 24. Topical facial, inhaled or systemic corticosteroids within 30 days prior to randomization. 25. Topical facial retinoids within 30 days before randomization. 26. Systemic retinoids within 6 months before randomization. 27. Any other topical or systemic treatment for rosacea within 30 days before randomization (including also laser and pulsed light, etc.). 28. Over-the-counter intestinal or topical skin probiotics (functional food is allowed), within 30 days before randomization. 29. Any experimental treatment within 6 months prior to randomization. 30. Current swab-positive or suspected (under investigation) Covid-19 infection; or fever and one or more of the following respiratory disease signs or symptoms: cough, sputum production, shortness of breath within the last 14 days; or contact with people with Covid-19 infection within the last 14 days. 31. Women who are pregnant, breast-feeding or planning a pregnancy during the trial period. 32. Subjects who are investigational site staff members and their family members, site staff members otherwise supervised by the investigator, or patients who are Alfasigma's employees.

Test products (dose and mode of administration) and reference therapy/placebo (dose and mode of administration):	Rifaximin-EIR 250 mg tablets or placebo. Patients will be randomly allocated into the following treatment arms: • Group A: two tablets of Rifaximin-EIR 250 mg TID (1500 mg daily) for 29 days (morning, afternoon, night); on Day 30 they will receive two tablets of Rifaximin-EIR 250 mg in single administration in the morning. • Group B: one tablet of Rifaximin-EIR 250 mg (750 mg daily) + one tablet of placebo TID for 29 days (morning, afternoon, night); on Day 30 they will receive one tablet of Rifaximin-EIR 250 mg and one tablet of placebo in single administration in the morning. • Group C: two tablets of placebo TID for 29 days (morning, afternoon, night); on Day 30 they will receive two tablets of placebo in single administration in the morning.
Prohibited and permitted medications:	Prohibited Medications Initiation of beta-blocker treatment is not allowed from the screening visit (V1) to the end of follow-up (Day 60; V4) included. Patients already on chronic treatment with beta-blockers at the screening visit can be enrolled in the study. Initiation of any oestroprogestinic or progestogen contraceptive or oestroprogestinic or progestogen replacement therapy is not allowed from V1 to V4 included. Patients already treated with oestroprogestinic or progestogen contraceptives or oestroprogestinic or progestogen replacement therapy before V1 can be enrolled in the study. The following medication will not be permitted from V1 to V4 included: • Biologic or non-biologic immunomodulatory or immunosuppressive drugs; warfarin (or other coumarins); niacin; topical facial or systemic antibiotic treatments; neomycin or any other low-absorbable oral antibiotics (such as marketed rifaximin); topical facial, inhaled or systemic corticosteroids; topical and systemic retinoids; any other topical or systemic treatment for rosacea (including also laser and pulsed light, etc.). • Any cancer-related treatment. • “over the counter” formulations including topical skin or intestinal probiotics. • Any other experimental treatment Permitted Medications: All other medications for concomitant chronic conditions are allowed and should be maintained constant throughout the whole study.

<table>
<tr><td></td><td></td></tr>
<tr><td>Duration of the study:</td><td>Overall, including all parts from sites activation, the study will last approximately 14 months (study period for a single subject: 1 month for screening, 1 month for treatment, 1 month for follow-up).</td></tr>
<tr><td>Criteria for evaluation:</td><td><u>Co-primary efficacy endpoint:</u>

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

1. Absolute change from baseline in number of rosacea inflammatory lesions (papules and pustules) at the end of treatment (Day 30) AND
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 at the end of treatment (Day 30).

Investigator’s Global Assessment (IGA) score
<table><tr><th>Grade</th><th>Description</th><th>Amount and size of inflammatory lesions present</th></tr><tr><td>0</td><td>Clear</td><td>None</td></tr><tr><td>1</td><td>Almost Clear</td><td>Very few, small papules/pustules</td></tr><tr><td>2</td><td>Mild</td><td>Few small papules/pustules</td></tr><tr><td>3</td><td>Moderate</td><td>Several small or large papules/pustules</td></tr><tr><td>4</td><td>Severe</td><td>Numerous small and/or large papules/pustules</td></tr></table>
<u>Secondary efficacy endpoints:</u>

1. Mean change from Baseline (V2) in number of inflammatory lesions (papules and pustules) at V3.
2. Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at V3 and V4.
3. Percent of participants with IGA score of 0 (clear) at V3 and V4.
4. Change from Baseline (V2) in the following rosacea additional features at V3 and V4:
• 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 at V3 and V4 (absent=0, mild=1, moderate=2, severe=3).
6. Change from baseline in abdominal pain score at V3 and V4.
7. Change from baseline in abdominal distension score at V3</td></tr>
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	and V4. 8. Change from baseline in bowel habit satisfaction score at V3 and V4. 9. Change from baseline in global severity of abdominal symptoms score at V3 and V4. 10. Percent of participants showing treatment success according to a Modified IGA scale excluding papules/pustules but including erythema (i.e. score of 0 or 1) at V3 and V4. 11. Percent of participant showing treatment success according to a Modified IGA scale including erythema and papules/pustules at V3 and V4. Pharmacokinetic assessments (PK sub-study only): 12. 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) of Rifaximin-EIR 250 mg.
Safety	Safety assessments: • Complete Physical Examination including height, weight, BMI; • Vital signs (including heart rate, blood pressure, and body temperature); • Routine laboratory parameters (haematology, biochemistry, urinalysis); • Adverse events (AEs); • Withdraw of subjects from study due to adverse events.
Statistical Methods:	A detailed Statistical Analysis Plan (SAP) will be issued after the study starts. SAS software (V.9.3 or subsequent) will be used for the statistical analyses. Continuous variables will be summarized by descriptive statistics (number of cases, mean and standard deviation, median, minimum, 1st and 3rd quartile, maximum). Categorical variables will be summarized using absolute frequencies and percentages. All the statistical tests will be conducted at the two-sided $\alpha = 0.05$ significance level. Due to the explorative purposes of this trial, no adjustment of significant level for multiplicity will be applied. Sample size estimation 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 [redacted] :

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Based on these assumptions, 51 patients were to be enrolled in each treatment arm as shown in the tables below.

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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 (Control Clin Trials, 1990): 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.

Analysis Populations

The following analysis populations will be defined:

Screened Population, defined as all patients enrolled into a screening phase after informed consent.

Safety Analysis Set (SAF), defined as all randomised patients having taken at least one dose of the investigational treatment.

	Full Analysis Set (FAS), defined as all patients in the SAF who have at least one post-baseline measurement for any primary endpoint. Modified Full Analysis Set (mFAS), defined as all patients in the FAS excluding the initial 30 patients randomized. Per Protocol Set (PPS), defined as all patients in the FAS who fulfil the study protocol requirements with no major deviations that may affect study results. PK Set, defined as all randomized patients who received at least one dose of Rifaximin and had a suitable PK profile. Analysis 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. Analysis of the safety and tolerability endpoints will be performed on the SAF. <u>Efficacy Statistical Analysis</u> Each active group will be compared with the placebo group. Statistical tests will be performed at the two-sided α =0.05 level. Considering the Proof of Concept purposes of this trial, no adjustment for multiplicity will be applied. The first co-primary efficacy endpoint (i.e.: mean change from Baseline in number of inflammatory lesions at Day 30) will be analysed by means of an Analysis of Covariance (ANCOVA) model, with change from Baseline to Day 30 as dependent variable, treatment group and stratification factor as explicative factors, and baseline number of inflammatory lesions as covariate. The adjusted mean differences will be presented with 95% CIs and p-values. The second co-primary efficacy endpoint (i.e.: percent of patients showing treatment success (IGA score of 0 or 1) with at least a 2-grade improvement from baseline at Day 30) will be analysed by means of a stratified Cochran Mantel-Haenszel (CMH) chi square test. The Breslow-Day test 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) will also be computed. The primary statistical analysis on the FAS will be performed according to the actual treatment received. In addition, two sensitivity analyses will be implemented: • The first by analyzing the FAS according to the “randomized treatment”, as originally planned • The second on the mFAS according to the “randomized treatment”.

	PK analysis PK parameters will be assessed according to a non-compartmental approach. At least the following PK parameters will be calculated: • C_{max}, T_{max}, $AUC_{0\text{-}tau}$, $t_{1/2}$; • Accumulation factor after the last dose; Rifaximin-EIR individual and mean plasma concentrations at each sampling time point will be presented by listings and descriptive summary statistics including arithmetic means, geometric means, medians, quartiles, ranges, standard deviations and coefficients of variation (arithmetic and geometric). Individual and mean concentrations versus time will be plotted on linear and log-linear scales. Descriptive statistics and graphs will be also performed for the PK parameters. Safety analysis With reference to the safety parameters, standard statistical analyses will be conducted on vital signs, physical examination, laboratory parameters and AEs at the relevant visits.

Note: Full details of study design and schedule of assessment are reported in Section 11 of the study protocol (“Study Procedures”)

TABLE OF CONTENTS

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1. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
AL	Alfasigma S.p.A.
b.i.d.	*Bis In Die* (twice a day)
BMI	Body Mass Index
CDAD	Clostridium Difficile Associated Diarrhoea
CH_4	Methane
CI	Confidence Interval
CRF	Case Report Form
DLQI	Dermatology Life Quality Index
GCP	Good Clinical Practice
CRO	Contract Research Organization
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicine Agency
ePRO	Electronic Patient Reported Outcome
FAS	Full Analysis Set
FDA	Food and Drug Administration
G-BT	Glucose Breath Test
IB	Investigator's Brochure
IBD	Irritable Bowel Disease
IBS	Irritable Bowel Syndrome
IBS-SSS	Irritable Bowel Syndrome - Symptom Severity Scale
ICF	Informed Consent Form
ICH	International Council on Harmonization
H_2	Hydrogen
IEC	Independent Ethics Committee
IGA	Investigator’s Global Assessment
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
L-BT	Lactulose Breath Test
MedDRA	Medical Dictionary for Regulatory Activities
PMC	Pseudomembranous Colitis
ppm	Parts Per Million
PPS	Per Protocol Set
PT	Preferred Term
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAF	Safety Analysis Set
SIBO	Small Intestinal Bacterial Overgrowth
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
t.i.d.	Three Times Daily

2. ETHICS

2.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

Before initiating the trial, Alfasigma S.p.A. (AL) and the investigator(s)/institution(s) must obtain written and dated approval/favourable opinion from the IRB(s)/IEC(s) for the trial protocol, written informed consent form, subject recruitment procedures (e.g. adverts), and any other written information to be provided to subjects. As part of the investigator(s)'/institution(s)' written application to the IRB/IEC, Alfasigma will provide the IRB(s)/IEC(s) with a current copy of the Investigator's Brochure (IB). If the IB is updated during the trial, AL will supply a copy of the updated IB to the IRB(s)/IEC(s).
Alfasigma S.p.A and the investigator(s)/institution(s) must obtain approval/favourable opinion from the IRB(s)/IEC(s) for change(s) to any aspect of the trial, such as modification(s) of the protocol, written Informed Consent Form (ICF), written information to be provided to subjects, and/or other procedures.
Alfasigma S.p.A must promptly report any new information that may affect the safety of the subjects or the conduct of the trial to the IRB(s)/IEC(s).
Alfasigma S.p.A will submit safety update reports to the IRB(s)/IEC(s) and to the Competent Authorities periodically, in accordance with the applicable laws. Upon completion of the trial, Alfasigma S.p.A will provide the IRB(s)/IEC(s) and the Competent Authorities with a brief report of its outcome (synopsis).
(See also chapter "EMERGENCY INSTRUCTIONS")

2.2 Ethical Conduct of the Study

The Guidelines of the World Medical Association's Declaration of Helsinki in its revised edition (64th WMA General Assembly, Fortaleza, Brasil, October 2013), the Guidelines of Good Clinical Practice (EMA/CPMP/ICH/135/1995 R2, 1 December 2016) and the Directives 2001/20/EC and 2005/28/EC as well as demands of the national drug and data protection laws will be strictly followed.
(See also chapters: "Insurance for Subjects, "Data Protection" and "Documentation of Subjects' Participation"

2.3 Subject Information and Consent

The investigator is responsible for not admitting subjects to the trial before informed consent has been given. Consent means that the person involved has the legal capacity to give consent and is able to exercise free power of choice. Consent should be given as written informed consent after receiving detailed information. The subjects who refuse to give informed consent must not be included in this trial.
Subjects will be given a written "Subject information and consent form".

Before signing the ICF the subject will be informed in detail by a physician about the following items:

a. the aim and rationale of the trial
b. the nature of the treatment and the allocation of subjects to the different treatment groups
c. other therapeutic alternatives
d. expected therapeutic effects of the medication
e. known adverse drug reactions and other risks or inconvenience during the clinical trial
f. the trial procedures to be followed, including all invasive procedures
g. compensation and/or treatment available to the subject in the event of trial-related injury
h. anticipated prorated payment and expenses to the subjects for participating in the trial (if applicable)
i. subject's right to withdraw at any time without justification and without penalty or loss of benefits to which the subject is otherwise entitled
j. the subject's responsibilities

k. availability of more detailed information before and during the trial
l. information about data protection
m. person(s) to contact in the event of trial-related injury
n. foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated
o. the expected duration of the subject's participation in the trial
p. the approximate number of subjects involved in the trial

The subject must be given ample time to inquire about the details of the trial. The ICF is signed by the informing physician and by the subject. Persons who withdraw their informed consent must not continue the trial.

The filled-in and signed ICF will be kept and archived in original by the investigator in the "Investigator's Study File". (See chapter 17.1). The subject will be provided with a copy.

3. GENERAL INFORMATION

Sponsor
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CRO:
Biorasi

Investigators
A list of all Investigators involved in the Study will be provided as a separate document.

Laboratories
PK bioanalysis: Pyxant
Clinical Laboratory: Biorasi (or delegated laboratory)

4. BACKGROUND AND RATIONALE

Rosacea

Rosacea is a common chronic skin disease that almost exclusively affects the central area of the face and the eyes. This condition affects 0.09 to 20% of the population (depending on the geographical areas and genetic predisposition) with typical age of onset of 30 to 50 years although can be found also in younger adults. Rosacea is slightly more prevalent among women, populations of Northern Europe and/or Celtic heritage, and less frequent among Asians and African Americans (Steinhoff et al., 2011). In a recent systematic review using available global data on rosacea epidemiology (including 32 studies, 41 populations, more than 26 million individuals), the global prevalence of rosacea has been estimated to be 5.5%. (Gether et al., 2018).

Based on its main clinical manifestations rosacea has been classically divided into the following four phenotypes: 1) erythematotelangjectatic, 2) papulopustular, 3) phymatous, and 4) ocular (Wilkin et al 2014, Steinhoff et al., 2013).

Recently, a phenotype system has been developed to address shortcomings of the previous diagnosis and subtyping classification system. With the phenotype system, diagnosis of rosacea can be established by the following: either fixed centrofacial erythema or phymatous changes independently; or in their absence, the presence of any 2 major features (flushing, papules and pustules, telangiectasias and specific ocular manifestations). Minor features (burning, stinging, edema, dryness, scaling) are not diagnostic (Gallo et al. 2018, Tan J et. al. 2017)). The advantage of the latter is that it allows diagnosis and treatment more closely based on signs and symptoms as well as pathophysiology (Buddenkotte and Steinhoff, 2018).

While the pathogenesis of rosacea is still largely unknown, there is convincing evidence that several genetic and environmental factors may contribute to the development of rosacea, such as adaptive and innate immune dysregulation, neurovascular changes, and chronic inflammation (Holmes and Steinhoff., 2017). Among the environmental factors, microorganisms and/or their products may have an important part in the genesis of the disease: *Helicobacter pylori*, *Staphylococcus epidermidis*, *Chlamydia pneumoniae*, *Bacillus olenorius* and, more convincingly, *Demodex folliculorum*, a skin parasite, have been hypothesized to have a significant role in the pathogenesis of rosacea (Lazaridou et al., 2011), particularly in patients with papules and pustules, the phenotype with the most intense inflammatory component (Holmes and Steinhoff., 2017). Thus, involvement of bacteria in the pathogenesis is possibly one of the reasons why antibiotics have been traditionally proven to be effective in patients with rosacea, particularly for the papulopustular phenotype. In addition, the anti-inflammatory capacity of certain antibiotics such as tetracyclines, doxycycline and minocycline, have been shown to reduce inflammatory mediators associated with rosacea. Systemic and topical metronidazole, systemic tetracyclines and macrolides and others have been used for moderate-to-severe forms of disease, although slow-release 40mg doxycycline (a member of the tetracyclines family) is the only systemic antibiotic approved for this indication. However, since long-term treatment is needed in more severe patients, up to several months, the risk of adverse events and antibiotic resistance is high. at least at higher dosages. Potential other side effects of the antibiotics used in rosacea are summarized in a Cochrane review and include esophagitis, colitis, facial melasma (hard-to-treat), for example (van Zuuren 2015).

Rosacea can be triggered by several factors including heat, hot/spicy food, chemicals, UV radiation, exercise, and bacteria and/or microbial products (Holmes and Steinhoff., 2017, Steinhoff 2013, Yamasaki 2007). In addition, it has been suggested that quantitative changes of the small intestinal

microflora, such as small intestinal bacterial overgrowth (SIBO) may also trigger rosacea (Parodi et al. 2008, Weinstock and Steinhoff 2013, Drago et al. 2016).

SIBO is a heterogeneous syndrome and a quantitative change of the gut microbiota characterized by an increased number of the bacteria normally populating the small bowel. SIBO can be assessed using lactulose hydrogen/methane (H_2/CH_4) breath test (L-BT) or glucose hydrogen/methane (H_2/CH_4) breath test (G-BT).

L-BT is a well-established non-invasive, office-based test for assessing malabsorption/fermentation of carbohydrates in the small intestine as a consequence of local overgrowth of bacteria. An increased number of intestinal bacteria results in increased carbohydrate fermentation in the gut, leading to the increased production of H_2 (and/or CH_4), eventually released with the expiratory breath. Breath testing consists of timed and standardized measurement of exhaled H_2 and CH_4 after the ingestion of a solution of water and lactulose. Although, classically, lactulose breath testing has been performed using a 4-hour collection time (Gasbarrini et al., 2009), a recent consensus (Rezaie et al., 2017) and the American College of Gastroenterology (ACG) clinical guideline (Pimentel et al., 2020) suggest that a 2-hour procedure is sufficient to detect a significant abnormal colonization of the small intestine. Lactulose breath test is usually performed by collecting breath samples every 15 minutes for 120 minutes (Rezaie et al., 2017). Current guidance (Rezaie et al., 2017, Pimentel et al. 2020) recommend a rise of >20 ppm of H_2 and/or >10 ppm of CH_4 by 90 minutes as diagnostic for SIBO. However, a threshold of >10 ppm of H_2 has also been used in clinical studies and associated with rosacea (Parodi et al., 2008), suggesting that also lower degrees of quantitative microbiota changes, with a rise of H_2-producing bacteria may trigger rosacea, although not entirely meeting the current operational definition of small intestinal bacterial overgrowth. However, this need to be determined.

A recent meta-analysis on the sensitivity and specificity of L-BT and G-BT has been recently published (Losurdo et al. 2020). This metanalysis included 4 studies performed with L-BT meeting the pre-specified inclusion criteria. The results showed that the pooled sensitivity of L-BT was 42.0% and pooled specificity was 70.6%. Slightly better results were obtained with G-BT (sensitivity 54% and specificity 82%).

Rifaximin

Rifaximin is a semi-syntetic Rifamycin-derivate with a large antimicrobial spectrum covering gram-positive and gram-negative bacteria as well as aerobes and anaerobes (e.g. *Clostridium* and *Bacteroides*). Rifaximin is a member of the rifamycin class and, like the other components of the group, it inhibits the RNA-synthesis binding to the beta-subunit of the DNA-dependent RNA enzyme of bacteria.

Rifaximin is an original molecule discovered and patented by Alfa Wassermann S.p.A. (a Company currently merged into Alfasigma S.p.A.) in 1980; it was first registered in Italy in 1985 as 200 mg tablets and 2 g/100 mL granules for oral suspension (60 mL bottle) and marketed in 1987.

Rifaximin is currently approved in 68 countries and marketed, under several trade names, for the treatment of bacterial intestinal infections (200-400 mg every 6-8 hours) and for the treatment of hepatic encephalopathy (HE) (400 mg). In 2015, Rifaximin 550 mg three times daily was approved by the Food and Drug Administration agency (FDA) for treating IBS with diarrhea (IBS-D) in adults. The approval of rifaximin in the IBS-D setting is based on the existing understanding that one possible underlying cause of IBS is perturbation of the microbiome.

Systemic exposure of Rifaximin following oral administration is minimal in all populations studied and its action is mainly focused on the intestinal tract. Being virtually non-absorbed, Rifaximin bioavailability within the gastrointestinal tract is rather high, with intraluminal and faecal drug concentrations largely exceeding the minimal inhibitory concentration values observed in vitro against a wide range of pathogenic organism.

Rifaximin in rosacea

Preliminary evidence shows that Rifaximin may have a beneficial effect in patients with papulopustular rosacea.

In an open-label study by Parodi and collaborators (Parodi et al., 2008), 52 patients with rosacea and either positive L-BT or glucose breath test were randomized to receive Rifaximin 400 mg every 8 hours (1200 mg daily) (n = 32) or placebo (n = 20) for 10 days. Patients treated with placebo were subsequently switched to Rifaximin therapy. Patients underwent a second L-BT or glucose breath test a month after stopping therapy to assess the normalization of the intestinal microbiota. At the same time, participants underwent a dermatologic visit to assess the outcome. In the Rifaximin-treated arm, eradication of the microbiota alteration was achieved in 28 of 32 patients (87.5%) and was associated with a complete resolution of rosacea inflammatory lesions in 20 of the 28 eradicated patients (71.4%). Significant improvement of rosacea was observed in 6 out of the 28 patients (21.4%). Eighteen of 20 patients treated with placebo (90%) did not show any improvement and 2 (10%) worsened (P<0.001). The 20 placebo-treated patients were subsequently switched to rifaximin and 17 of them had their intestinal condition eradicated. In this group, a resolution of the inflammatory cutaneous lesions occurred in 15 of the 17 of the latter patients and a relevant improvement in the 2 remaining cases. Overall, after a 10-day course of Rifaximin, eradication of the microbiota alteration was observed in 45 of 52 (86.5%) patients. Of those, 35 (78%) achieved complete clearance of the rosacea inflammatory lesions and 8 (17.7%) were substantially improved. After Rifaximin treatment, 13 of 16 patients with negative breath did not show any meaningful improvement of rosacea.

A study, performed by the same research group (Drago et al., 2016), showed that approximately 65% of the patients achieving clinical remission were still in remission three years later.

In another clinical study by Weinstock et al. (Weinstock and Steinhoff, 2013), 28 of 32 patients with rosacea and small intestinal bacterial overgrowth, defined using L-BT, were treated with Rifaximin. Of them, 46% showed cleared or markedly improved rosacea, 25% reported moderately improved rosacea, and 11% showed mildly improved rosacea. All 4 patients with ocular rosacea and SIBO reported marked improvement. Rosacea was unchanged in 18% of patients.

Another study by Gravina et al. (Gravina et al., 2015) found a non-significant benefit of Rifaximin (odds ratio= 6.9, 95% confidence interval= 0.3 to 136.2), but the sample size was probably too small (N=9) to infer any conclusion.

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This body of evidence provides a rationale and an initial proof of concept for the use of Rifaximin in the treatment of rosacea.

Rifaximin-EIR

A novel, not yet marketed, delayed release formulation of Rifaximin, **Rifaximin Delayed-Release** (Rifaximin-EIR) 250 mg tablet, will be used in this study. **Rifaximin Delayed-Release** (Rifaximin-EIR) is a patented technology by which a microgranulation of Rifaximin is coated with a gastro-resistant polymer insoluble at pH lower than 5, in order to delay the release of Rifaximin. Rifaximin-EIR has been formulated to by-pass the stomach and optimize the effect of the active principle into the intestine; the intent of the formulation is in fact to provide a more uniform coverage of the active drug in the intestinal lumen and to maximize contact with the intestinal mucosa.

A Rifaximin-EIR formulation with a higher dose (400 mg tablet) is currently investigated in a European phase II trial in subjects with moderate-to-severe papulopustular rosacea. In this trial the investigated daily doses are 800 mg (400 mg BID) and 1600 mg (800 mg BID).

However, a lower dose of Rifaximin-EIR, lower than those investigated in the ongoing European phase II trial (i.e. Rifaximin-EIR 250 mg TID), is planned to be moved forward in this indication (i.e. patients with papulopustular rosacea). However, the effect of this lower dose, particularly when administered TID, has not yet been assessed. Moreover, the bioavailability of Rifaximin-EIR in patients suffering from papulopustular rosacea has never been investigated.

Pharmacokinetic profile of Rifaximin-EIR

Pharmacokinetic data for Rifaximin Delayed-Release (Rifaximin-EIR) have been collected in healthy volunteers and patients with Crohn's Disease.

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Overall, the pharmacokinetic profile of Rifaximin-EIR is similar to that of the marketed form of Rifaximin, which has been extensively assessed in studies of healthy volunteers and patients with gastrointestinal diseases (Crohn's disease, ulcerative colitis, pouchitis, IBS), after single and multiple oral doses.

5 STUDY OBJECTIVES AND PURPOSE

The effect of the 250 mg tablets formulation of Rifaximin-EIR, particularly when used at low dosages and administered three times daily (i.e. 750 mg daily, 250 mg t.i.d.), has not yet been assessed for the treatment of subjects with papulopustular rosacea. Moreover, the bioavailability of Rifaximin-EIR at doses of 750 mg or 1500 mg daily (i.e. 750 mg daily, 250 mg t.i.d. or 1500 mg daily, 500 mg t.i.d., respectively) in subjects with papulopustular rosacea is not known.

This study is therefore aimed at:

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

Data collected with this study will provide key information to be used in the development of Rifaximin-EIR 250mg tablet in rosacea.

5.1 Co-primary efficacy endpoint

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

1) Absolute change from baseline in number of rosacea inflammatory lesions (papules and pustules) at the end of treatment (Day 30) AND
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 at the end of treatment (Day 30).

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

5.2 Secondary endpoints

Secondary efficacy endpoints:

1) Mean change from Baseline (V2) in number of inflammatory lesions (papules and pustules) at V4.
2) Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at V3, V4.
3) Percent of participants with IGA score of 0 (clear) at V3, V4.
4) Change from Baseline (V2) in the following rosacea additional features at V3, V4.

- 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 at V3, V4 (absent=0, mild=1, moderate=2, severe=3).
6) Change from baseline in abdominal pain score at V3, V4.
7) Change from baseline in abdominal distension score at V3, V4.
8) Change from baseline in bowel habit satisfaction score at V3, V4.
9) Change from baseline in global severity of abdominal symptoms score at V3, V4.
10) Percent of participants showing treatment success according to a Modified IGA scale excluding rosacea inflammatory lesions but including erythema (i.e. score of 0 or 1) at V3, V4.
11) Percent of participant showing treatment success according to a Modified IGA scale including erythema and inflammatory lesions at V3 and V4.

Pharmacokinetic assessments (PK sub-study only):

12) 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).

6. OVERALL STUDY DESIGN AND PLAN

This is a randomized, double-blind, placebo controlled, phase IIa clinical trial.

As described in Section 16.1 (Determination of Sample Size), two hundred one (201) eligible subjects are planned to participate in the main study while a subgroup of minimum 18 and maximum 36 subjects are planned to participate in the PK sub-study. A maximum of 12 (minimum 6) subjects per arm is established for the PK sub-study.

At randomization visit (V2, Day 1), subjects will be 1:1:1 randomized to receiving any of the two daily doses of active treatment or placebo. TID doses of the assigned treatment will be administered from Day 1 to Day 29. On Day 30 (V3) just the first daily dose will be administered, and PK assessed.

Patients will be stratified at randomization based on their L-BT status (negative vs. positive) and PK sub-study participation (yes or not). A negative L-BT is defined as a test showing an increase ≤10 ppm of H_2 by 90 minutes compared to time 0.

The primary endpoint will be evaluated at end of treatment (V3, Day 30).

Subjects participating in the PK sub-study at V3 (Day 30) will stay overnight to complete the PK assessments.

Venous blood samples for PK analysis will be collected on:

- Day 1: Pre1st dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post dosing.
- 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 until Day 31 as appropriate.

Details on the procedures performed with the different recruitment options are described in Section 11 (Study Procedures).

Participants will be enrolled at approximately 40 US Centers in USA.

7. DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

This study is a double-blind, placebo-controlled, randomized clinical trial.

In the present phase IIa trial, the use of placebo is considered appropriate to test the effect size of two dosages of Rifaximin-EIR in patients with moderate-to-severe papulopustular rosacea. While not always the use of placebo is ethically acceptable, in this specific trial the use a placebo arm is deemed ethically acceptable according to the art. 33. Of the Declaration of Helsinki and the EMEA/17424/01. In fact, withholding/postponing the use of an established intervention for rosacea of approximately one month does not “entail any additional risks of any irreversible harm to the participant”.

Participants in the placebo arm, the 33% of the study sample, might be exposed to a temporary discomfort in terms of a lack of improvement or even a slight worsening of the symptoms.

Although, preliminary evidence shows that Rifaximin may be beneficial particularly in patients with rosacea and quantitative microbiota changes, considering the methodological limits of L-BT testing and that the role of microbiota quantitative and qualitative composition in the pathogenesis of rosacea has not yet been fully clarified, according to the FDA recommendation, patients with negative L-BT will also be enrolled. In fact, it cannot be excluded that the benefit of Rifaximin in patients with rosacea could be mediated by the effect of the drug on factors different from or just associated with SIBO.

In any case, patients can decide to withdraw from the clinical study at any time with no penalties. Similarly, the Investigator can decide to withdraw the patient in his/her best interest, at any time, to pursue alternate treatments or no treatment at all.

8. BENEFIT-RISK EVALUATION

Pharmacological and efficacy studies with Rifaximin (see Investigator's Brochure) have shown that the benefits of Rifaximin include:

- The negligible absorption of Rifaximin (less than 1%) means it has an excellent safety profile and can be administered at high doses for long period time.
- No potential for either inhibition or induction of human hepatic cytochrome P450 at expected clinical plasma concentrations.
- No drug-drug interactions with oral contraceptives at expected clinical plasma concentrations.
- Rifaximin can be administered with or without food.
- A large antimicrobial spectrum covering most gram-positive and -negative bacteria, including aerobes and anaerobes and therefore good activity against the microflora normally present in both the small intestine and in the colon.
- Rifaximin is widely used in gastroenterology (i.e. Traveller's diarrhoea, Hepatic Encephalopathy and Irritable Bowel Syndrome with diarrhoea IBS-D).
- Bioavailability studies with Rifaximin-EIR 1200 mg (3 x 400 mg tablet) administered TID for 7 days showed that absorption is negligible in subjects with Crohn's Disease (CD). Absorption is negligible also in subjects with IBS receiving Rifaximin 550 mg TID for 14 days.
- Rifaximin works by decreasing the triggers of immune activation as well as enteric inflammatory response, rifaximin may reduce the uncontrolled immune response to enteric bacteria.
- Based on the available evidence summarized in a recent meta-analysis on 32 clinical trials through March 2015, Rifaximin is effective and safe in eradicating SIBO and resolving symptoms (overall success with an intention-to-treat: 70.8%) (Gatta, 2017). According to Gatta and colleagues, SIBO eradication rates increase linearly with increasing Rifaximin dose, in the 600-to-1600 mg daily dose range. With regards to safety data, in the above-mentioned meta-analysis the 4.6% of SIBO patients treated with Rifaximin reported AEs, but only the 0.47% of them had to discontinue the therapy.
- Data from randomized clinical studies, evaluating the efficacy of Rifaximin 200 mg TID (1200 md/day) in rosacea patient's positive for SIBO, demonstrated that eradication of SIBO induced an almost complete regression of cutaneous lesions among patients achieving glucose breath test negativity after treatment (Parodi, 2008) (Weinstock, 2013).

The safety of Rifaximin evaluated in clinical trial and during post-marketing surveillance indicates that Rifaximin is extremely safe. In details:

- The safety of Rifaximin-EIR has been evaluated in 6 studies, 3 Phase II studies involving 379 patients with mild to moderate Crohn's disease, 1 PK study on 18 healthy volunteers and 2 PK studies on 30 patients with active Crohn's disease. The safety analysis has shown a favourable safety profile of Rifaximin-EIR in the treatment of mild to moderate active Crohn's disease.
- The safety of Rifaximin (200 mg or 550 mg formulations) has been extensively established in approximately 7000 subjects enrolled in clinical studies (IBS-D: 4020 subjects; Hepatic Encephalopathy: 697; Traveller's Diarrhoea: 1527; healthy volunteers: 221) and of Rifaximin 600 – 1800 mg in approximately 125 patients (receiving rifaximin 1100 mg/day for 2 years),
- Post-marketing experience confirmed that Rifaximin is extremely safe.

Overall, in clinical trials (including those investigating Rifaximin-EIR), nearly all adverse reactions recorded were uncommon (<1%). The most frequently reported adverse events were related to

gastrointestinal, nervous, skin and general disorders, in addition to those revealed by laboratory findings.
The incidence of adverse events in people treated with rifaximin was comparable to or lower than that in the placebo-treated patients, as one would be expected from a non-absorbable antibiotic. In some of the cases, gastrointestinal adverse events recorded during clinical studies were also symptoms of the intestinal disease to be treated with the investigational drug.
In addition to those identified during clinical trials, further potential risks may include:

- Cases of *Clostridium Difficile* Associated Diarrhoea (CDAD) have been reported with Rifaximin use, ranging in severity from mild diarrhoea to fatal colitis. The potential association of Rifaximin treatment with CDAD and Pseudomembranous Colitis (PMC) cannot be ruled out.
- Allergic reactions have also been reported during post-approval use. Systemic absorption of Rifaximin is approximately 0.4% of the orally administered dose; however, even these very low quantities are sufficient to trigger an allergic reaction, particularly in hypersensitive subjects. Therefore, serious unpredictable reactions such as anaphylactic shock or severe skin reactions may occur.
- Cases of both increases and decreases in International Normalised Ratio (INR) with a temporal association with Rifaximin administration were reported in patients maintained on warfarin and Rifaximin with a temporal association with rifaximin administration. Therefore, closer INR monitoring and/or dose adjustment of warfarin is required around the time of Rifaximin administration.
- In healthy subjects, clinical drug interaction studies demonstrated that rifaximin did not significantly affect the pharmacokinetics of CYP3A4 substrates, however, in hepatic impaired patients it cannot be excluded that Rifaximin may decrease the exposure of concomitant CYP3A4 substrates administered (e.g. antiepileptics, antiarrhythmics), due to the higher systemic exposure with respect to healthy subjects.
- In healthy subjects, co-administration of a single dose of cyclosporine (600 mg), a potent P-glycoprotein inhibitor, with a single dose of Rifaximin (550 mg) resulted in 83-fold and 124-fold increases in Rifaximin mean C_{max} and AUC_{∞}. The clinical significance of this increase in systemic exposure is unknown.
- There is no experience regarding administration of Rifaximin to subjects affected by an undiagnosed systemic bacterial infection potentially treatable with rifampicin.

Experts have agreed that the systemic absorption of Rifaximin is too low to select resistant strains, and in fact, two recent surveys carried out in Italy, where Rifaximin has been widely used since 1987, found no evidence of development of resistance by *M. tuberculosis* or *N. meningitides*. Therefore, the clinically relevant selection of cross-resistant *M. tuberculosis* or *N. meningitides* is considered not probable.

In summary, the properties of Rifaximin, including its broad range of activity against bacteria, the supposed immunomodulatory activity, its low systemic availability, the excellent safety profile, the clinical efficacy shown in Proof of Concept studies in the treatment of rosacea, support a positive risk/benefit ratio.

9. SELECTION OF STUDY POPULATION

9.1 Number of Participants

As described in Section 16.1 (Determination of Sample Size),two hundred one (201) eligible subjects are planned to be enrolled in the main study while a subgroup of 18-36 (a minimum of 6 and a maximum of 12 subjects per arm) are planned to participate in the PK sub-study.

At the randomization visit (V2), subjects will be 1:1:1 randomized to receiving any of the two daily doses of active treatment or placebo. TID doses of the assigned treatment will be administered from Day 1 to Day 30. On day 30 (V3) just the first daily dose will be administered, and PK assessed (for the PK sub-study only).

Patients will be stratified at randomization based on their L-BT status (negative vs. positive) and PK sub-study participation (yes or not).

Duration of the study:

- overall (including all parts of the study) approximately 14 months from sites activation;
- study period for a single subject: approximately 3 months (time interval for screening, treatment and follow-up).

9.2 Inclusion Criteria

All the following criteria must be met both at the Screening and Randomization visits unless otherwise specified.

1. Men and women aged 18 years or older at screening (V1)
2. Female participants are eligible if they are:
   of non-childbearing potential, i.e.: i) post-menopausal (at least 2 years without spontaneous menses), or ii) surgically sterile (bilateral tubal occlusion, or hysterectomy), or iii) ablation of both ovaries),

   or

   of childbearing potential with a negative pregnancy test result at screening and randomization and agreeing to use a highly effective method of contraception (i.e. with failure rate of less than 1% per year) until 72 hours after taking the last study treatment dose.

Note 1. Based on Clinical Trial Facilitation Group recommendations, highly effective methods of contraception are the following:

- intrauterine device (IUD);
- intrauterine hormone-releasing systems (IUS) or;
- combined hormonal contraceptives (i.e. estrogen and progestogen) in oral, intravaginal or transdermal form, with inhibition of ovulation as primary mode of action or;
- progestogen-only hormonal contraceptives in oral, injectable or implantable form, with inhibition of ovulation as primary mode of action or;
- absolute and continuous sexual abstinence from Day 1 included (first day of treatment) until 72 hours after taking the last study treatment dose.

Note 2. In each case of delayed menstrual period (over one month between menstruations), female participants of child-bearing potential will be strongly recommended a confirmation of absence of pregnancy. This recommendation applies also to women of child-bearing potential with infrequent or irregular menstrual cycles.

3. Presence of rosacea, papulopustular phenotype, defined as papules and/or nodules, with or without pustules of rosacea, without comedones, plus: centrofacial persistent erythema or phyma or transient episodic facial erythema (flushing) or facial telangiectasia (excluding peri-alar regions) or major ocular rosacea features (i.e. lid margin telangiectasia, interpalpebral conjunctival injection, spade-shaped infiltrates in the cornea, scleritis and sclerokeratitis).
4. Presence of ≥11 and ≤70 facial papules and/or pustules.
5. Moderate (Grade 3) or severe (Grade 4) rosacea based on Investigator's Global Assessment (IGA) based on Investigator's judgement.
6. Patients accepting to provide and legally capable of providing free and informed consent to all procedures included in the protocol (including facial skin photography).

9.3 Exclusion Criteria

None of the following criteria must be met both at the Screening visit and at the Randomization visit (Day 1) unless otherwise specified:

1. Granulomatous rosacea or rosacea fulminans.
2. Erythematoteleangectatic, phymatous or ocular rosacea only. Patients with these subtypes associated with papulopustular rosacea can be enrolled.
3. Rosacea with IGA grade ≤2 based on Investigator's judgment.
4. Anticipated need for proctoscopy or colonoscopy within two weeks after lactulose breath test.

Note 3: Subjects undergoing unanticipated proctoscopy or colonoscopy within two weeks from lactulose breath test should have a thorough bowel cleansing with a non-fermentable solution.

5. Subjects requiring a low galactose diet.
6. Hypersensitivity or intolerance to lactulose or any excipient of the lactulose preparation to be used for L-BT.
7. History of inflammatory bowel disease (Crohn's disease or ulcerative colitis) or other conditions characterized by severe intestinal ulcers.
8. History of coeliac disease.
9. Patients with intestinal obstruction or partial intestinal obstruction.
10. Presence of diarrhoea associated with fever and/or blood in the stool.
11. Severe kidney impairment (i.e. estimated glomerular filtration rate <30 ml/min).
12. Severe hepatic impairment (i.e. Child-Pugh B or C).
13. Cancer or any cancer-related treatment within 5 years prior to screening (excluding non-melanoma skin-cancer).
14. History of alcohol or drug abuse within a year prior to screening, based on Investigator's judgement.
15. Facial skin conditions that can interfere with reliable assessment of rosacea throughout the study (e.g. facial hair, tattoos, other facial adornments, keloids, hypertrophic scarring, recent facial surgery, excessive sun exposure including use of tanning beds)
16. Any other significant health condition (e.g. cardiovascular, respiratory, renal, hepatic, neurologic, psychiatric, hematologic, oncologic, immune etc.) or non-health condition that in the investigator's judgement may:

    iv) jeopardize the patient's safe participation in the trial or
    v) make unlikely the patient's completion of the study or
    vi) make unlikely the patient's compliance with the study procedures (e.g. highly anticipated need of non-permitted treatments, terminal illness, etc.).

17. History of hypersensitivity to rifaximin, rifamycin-derivatives, any of the rifaximin-EIR or placebo excipients.
18. Treatment with biologic immunomodulatory and/or immunosuppressive drugs (e.g. anti-TNF drugs) within 6 months prior to randomization.
19. Treatment with non-biologic immunomodulatory and/or immunosuppressive drugs (e.g. cyclosporine, methotrexate etc.) within 30 days prior to randomization.
20. Treatment with warfarin (or other coumarins) within 14 days prior to randomization.
21. Treatment with niacin within 30 days prior to randomization.
22. Topical facial or systemic antibiotics within 30 days before randomization;
23. Treatment with neomycin or other low-absorbable oral antibiotics (such as marketed rifaximin) within 90 days before randomization.
24. Topical facial, inhaled or systemic corticosteroids within 30 days prior to randomization.
25. Topical facial retinoids within 30 days before randomization.
26. Systemic retinoids within 6 months before randomization.
27. Any other topical or systemic treatment for rosacea within 30 days before randomization (including also laser and pulsed light, etc.).
28. Over-the-counter intestinal or topical skin probiotics (functional food is allowed), within 30 days before randomization.
29. Any experimental treatment within 6 months prior to randomization.
30. Current swab-positive or suspected (under investigation) Covid-19 infection;
    or fever and one or more of the following respiratory disease signs or symptoms: cough, sputum production, shortness of breath within the last 14 days;
    or contact with people with Covid-19 infection within the last 14 days.
31. Women who are pregnant, breast-feeding or planning a pregnancy during the trial period.
32. Subjects who are investigational site staff members and their family members, site staff members otherwise supervised by the investigator, or patients who are Alfasigma's employees.

9.4 Premature Discontinuation from the Study per Subject

The subject has the right to withdraw from the study at any time without providing any reason.

Participants may be withdrawn at any time when any of the following conditions occur:

- adverse events; when during the observation period adverse events occur that, in the Investigator's or the patient's opinion, are of such a nature or severity to recommend treatment withdrawal;
- failure to comply with major requirements of the protocol (e.g. inclusion error or evidence of non-compliance with exclusion/inclusion criteria arisen during the study, subject misses study visits or/and is not compliant with key study procedures);
- explicit withdrawn of patient's consent to participate or failure to report to study visits;
- patient's loss to follow-up;
- Investigator's opinion; the Investigator may choose to withdraw a subject from the study if, in the investigator's opinion, continuing the participation in the study for any reason would compromise the safety or well-being of the participant;
- other reasons, different from the ones previously specified.

Reason and date of patient's withdrawal must be recorded in the Patient's Clinical Chart and the e-

CRF.

The study may be terminated prematurely if:

- the Sponsor feel that the number and/or severity of AEs justify discontinuation of the study;
- the Sponsor considers the applied doses of the study drug to be no longer relevant;
- data not known before becoming available and raise concern about the safety of the study drug so that continuation would pose potential risks to the subjects.

Premature termination of the study should be mutually agreed upon by the Coordinating Investigator and the Sponsor and must be documented. However, study results must be reported according to the requirements outlined in this protocol as far as applicable.

The data of subjects who are withdrawn will be considered for evaluation both for efficacy, PK and safety.

Subjects who discontinue treatment due to SAE will be followed up until the event resolves or stabilises.

Subjects withdrawn from the study will not be replaced.

10. TREATMENTS

10.1 Treatments to be Administered

Eligible subjects will be allocated in a blinded fashion to one of the three treatment groups, according to a computer-generated randomisation list.

- Group A: two tablets of Rifaximin-EIR 250 mg formulation t.i.d. (1500 mg daily) for 29 days; on Day 30 subjects will receive two tablets of Rifaximin-EIR 250 mg in single administration (500 mg daily).
- Group B: one tablet of Rifaximin-EIR 250 mg formulation t.i.d. (750 mg daily) + one tablet of placebo t.i.d. 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 daily).
- Group C: two tablets of placebo t.i.d. for 29 days; on Day 30 subjects will receive two tablets of placebo in single administration.

The Investigator at each study site will be responsible for the handling and storage of the study material (in accordance with the Sponsor's indications).

Important note: Investigational products are only allowed to be administered to subjects selected for this clinical trial according to the study protocol.

10.2 Investigational Products

10.2.1 Test preparation

Rifaximin-EIR will be supplied as tablets containing 250 mg of active ingredient.

One Rifaximin–EIR 250 mg tablet (Rifaximin Delayed-Release tablet) contains:
Active ingredient: rifaximin 250 mg.
CCI [redacted].

One placebo tablet contains:
Active ingredient: none.
CCI [redacted]

10.2.2 Labelling and Packaging

Test drug and matched placebo bulk manufacturing will be performed in Glatt Air Techniques Inc 20 Spear Road, Ramsey, NJ 07446 US.

All clinical material will be packaged in 95-tablets bottles. Investigational drug will be provided in boxes ("Patient's kits") including the number of bottles required for a 30-days treatment period.

Each patient kit will contain two bottles characterized by labels of two different colours (white and yellow). Bottles with labels of different colours (white and yellow) can contain either tablets of Rifaximin-EIR 250 mg or tablets of placebo depending on the treatment arm. Each patient kit will contain two bottles (one white-labelled bottle and one yellow-labelled bottle) to be utilised for the 29

days of t.i.d. treatment plus one single dose to be administered on Day 30.

Glatt Air Techniques Inc., 20 Spear Road, Ramsey, NJ 07446 US will be responsible for the IMP release

10.2.3 Storage, Dispensing, Use and Disposal of the compound during and at the end of the study

An adequate amount of Patient's kits will be initially supplied to each study centre. According to the actual rate of recruitment of the centre, additional supplies will be performed.
All investigational products must be kept in a locked place with restricted access and maintained under controlled temperature conditions at ≤ 25°C. Do not refrigerate.

At visits V2, each eligible subject will receive a patient's kit containing two "treatment bottles", covering the treatment necessary for 30 days of study. Treatments will be assigned according to the randomization procedure.

At visits V3, each patient will return to the Investigator all the unused and partially used investigational product and bottles contained in the treatment box assigned for the treatment.

The Investigator must not supply the investigational product to people other than the enrolled subjects or the authorized study personnel in charge of distribute it.

Under no circumstances, without written authorisation from the Sponsor, the Investigator is allowed to supply the study drug to other study centres (or Investigators working for other centres involved in the study). Similarly, the Investigator is not allowed to or will not instruct people to use or handle the investigational drug in a way different from that described in this protocol.

The investigational products should be stored safely and properly, and they must not be used after the expiry date.

The investigational products will be self-administered by the patient, following instruction provided by the Investigator or personnel authorised by the Investigator.

The final accountability records will reconcile shipment records with those of used and returned investigational product. Any discrepancy will be accounted for. All unused investigational products will be returned or destroyed locally, based on the local regulation, but return and destruction will not occur until authorised by the Sponsor.

10.3 Method of Assigning Subjects to Treatment Groups / Randomisation

A unique subject's identification number will be assigned to all subjects consenting to be enrolled in the study obtained concatenating the center number with the screening number.
A subject who fails to meet the protocol eligibility criteria will be identified as screening failure; his/her identification number will not be reallocated.
Randomisation will occur at visit V2 after all baseline procedures have been performed and eligibility for the study has been confirmed.
Participants will be randomised, on a 1:1:1 basis, to Rifaximin-EIR 250 mg three times a day (750 mg daily) for 29 consecutive days and Rifaximin-EIR 250 mg single dose on Day 30, Rifaximin-EIR 500 mg three times a day (1500 mg daily) for 29 consecutive days and Rifaximin-EIR 500 mg single dose on Day 30, or placebo for 30 consecutive days.
Blocks will be used to assure treatment balance.

Patients will be stratified at randomization based on their L-BT status (negative vs. positive) and PK sub-study participation (yes or not).

Randomization will occur throughout an IWR system integrated into the e-CRF.
At V2, the randomization system will assign an individual Patient's kit number based on a pre-defined randomisation list.
The enrolment will be competitive.
Decoding of randomization in case of emergency is described in chapter 11.4.

The subjects will be offered participation to the PK sub-study. If they agree, they will be randomized according to the relevant stratum maintaining treatment balance among groups; otherwise, they will enter the main study.

10.4 Selection of Doses in the Study

Based on the available evidence summarized in a recent meta-analysis, Rifaximin seems to be effective and safe for the treatment of intestinal dysbiosis (Gatta et al., 2017), a condition hypothesized triggering rosacea, showing a dose-response relationship in the dose-range between 800 and 1600 mg per day (Gatta, 2017).

Rifaximin-EIR 250 mg tablet will be tested in this study. Its peculiar formulation may allow a t.i.d. administration. Therefore, in the current study, we aim to test the minimum (750 mg daily) and maximum (1500 mg daily) doses that have been shown to act at pathophysiological level (e.g. SIBO eradication).

In agreement with the published studies, the duration of treatment in this study will be 29 days for both the Rifaximin-EIR 1500 mg/daily and 750 mg/daily dosages plus Rifaximin-EIR 500mg or Rifaximin-EIR mg 250 mg single dose on Day 30, respectively.

10.5 Selection and Timing of Dose for each Patient

The experimental treatment should be taken three times a day approximately 8 hours apart, in the morning, in the afternoon and in the evening, with a small amount of water, with or without food. Treatment bottles will be characterized by labels of two different colours, white and yellow.

The participant will be instructed to start the study drug intake from the morning of V2 (Day 1), immediately after randomization and preferably early in the morning (around 8:00 AM), taking one tablet from the bottle with white label and one tablet from the bottle with yellow label, as well as one tablet from the bottle of white label and one tablet from the bottle of yellow label in the afternoon (around 4:00 PM) as well as one tablet from the bottle of white label and one tablet from the bottle of yellow label late in the evening (around 12:00 PM).

Bottles with labels of different colours can contain either Rifaximin-EIR 250 mg or placebo depending on the assigned treatment arm.

For the subjects included in the PK sub-study the first drug intake must be performed at the investigational site in occasion of the randomization visit (V2, Day 1), immediately after the first blood drawing (for PK analysis) and preferably early in the morning (around 8:00 AM). Also, at V3 (Day 30) subjects participating in the PK sub-study will stay overnight at the centre to complete the PK procedures.

10.6 Blinding

Blinding is intended to limit the occurrence of conscious and unconscious bias in the conduct and interpretation of a clinical trial arising from the influence which the knowledge of treatment may have on the recruitment and allocation of subjects, their subsequent care, the attitudes of subjects to the treatments, the assessment of end-points, the handling of withdrawals, the exclusion of data from analysis, and so on.
In accordance with the double-blind design, both the Investigator and the patient will be unaware of the treatment being dispensed in each case. The placebo will be indistinguishable from the active substance, i.e. will have the same weight, appearance, colour, smell and flavour.
Only in case of an emergency, when knowledge of the study medication is essential for the patient's safety, the Investigator may unblind a patient's treatment assignment (see section 11.4 "Decoding of randomisation in case of emergency").

10.7 Treatment Compliance

The investigational product will be provided to patients by the Investigator, who will thus monitor compliance at each visit.

To monitor treatment compliance, patients will be asked to bring back the two bottles provided at the randomization visit (V2), including all unused tablets.

The investigational drug supplies, the unused returned investigational drug will be duly recorded in the drug accountability forms, as source documents, by the authorized study centre personnel and then reported in the e-CRFs. Any discrepancy must be clearly documented.

A level of compliance >80% during the treatment period will be considered satisfactory.

10.8 Prior and Concomitant Therapy

Prohibited Treatment

Initiation of beta-blocker treatment is not allowed from the screening visit (V1) to the end of follow-up (Day 60; V4) included. Patients already on chronic treatment with beta-blockers at the screening visit can be enrolled in the study.
Initiation of any oestroprogestinic or progestogen contraceptive or oestroprogestinic or progestogen replacement therapy is not allowed from V1 to V4 included. Patients already treated with oestroprogestinic or progestogen contraceptives or oestroprogestinic or progestogen replacement therapy before V1 can be enrolled in the study.

The following medication will not be permitted from V1 to V4 included:

- Biologic or non-biologic immunomodulatory or immunosuppressive drugs; warfarin (or other coumarins); niacin; topical facial or systemic antibiotic treatments; neomycin or any other low-absorbable oral antibiotics (such as marketed rifaximin); topical facial, inhaled or systemic corticosteroids; topical and systemic retinoids; any other topical or systemic treatment for rosacea (including also laser and pulsed light, etc.).
- Any cancer-related treatment.
- "Over the counter" formulations including intestinal and topical skin probiotics.

- Any other experimental treatment

Concomitant Permitted Treatments

All other medications for concomitant chronic conditions are allowed and should be maintained constant throughout the whole study.

11. STUDY PROCEDURES

11.1 Visit Schedule

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

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	X[4]
Five-points Investigator's Global Assessment (IGA) of Rosacea Severity	X	X	X	X	X	X[4]
Evaluation of erythema, telangiectasia, ocular manifestations and phymatous changes	X	X	X	X	X	X[4]
Visual Analogue Scale (VAS) for pain, burning/stinging and itching	X	X	X	X	X	X[4]
Abdominal symptoms questionnaire (IBS-SSS)[1]		→	→	→	→	
Dermatology Life Quality Index Questionnaire[1]		→	→	→	→	
Clinical chemistry, haematology, urinalysis	X		X	X	X	X[4]
Contraception recommendations and/or adherence^		X				
Serum pregnancy test^	X					
Urine pregnancy test^		X	X	X	X	X[4]
Lactulose Breath test	X		X		X	X[4]
Inclusion/exclusion criteria	X	X				
Randomization		X				
Blood sampling for PK analysis^^		X[2]	X[3]			
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 an electronic diary (ePRO).

[2]Sampling to be performed right before and after the first morning dose of Rifaximin-EIR at V2 (Day 1) at the following 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 following time points: immediately pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6 and 8, 12, 22, 24, 30 hours post-dosing (single dose 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

11.2 Study Evaluations/Procedures

Procedures for Social Media recruitment path

Specific advertisements targeted to rosacea subjects posted in selected social media will re-direct subjects interested in participating to a dedicated study website.

11.2.1. Remote data recording (visit V0)

In a dedicated study website, a *prospective participant* will be asked to:

- review a concise study description;
- provide online pre-screening consent;
- provide contact details;
- provide demographics (e.g. age, gender, ethnicity, educational level, profession, etc.);
- provide data on health-related behaviour (e.g. smoking, alcohol consumption);
- complete an online medical history and concomitant medication questionnaire;
- upload either three pictures of their face (one frontal and two lateral [right and left side] photos) or a short video of the face.

11.2.2 Central Reader Validation

This task will be performed no later than 2 working days after V0. A Central reader (*expert dermatologist*) will:

- check preliminary compliance with inclusion/exclusion criteria based on the
  - review of personal and clinical data (including medical history, concomitant medications etc.) posted on the website,
  - review of patient's images downloaded on the website,
- address all the suitable pre-screened candidates to the closest investigational site for scheduling the remote screening. The Study Navigator may assist with referring the candidates to the closest investigational site after the central reader confirms the candidates' preliminary eligibility.

11.2.3 Phone Contact with the Subject

This task will be performed no later than 1 week after the candidate validation has been occurred.
The investigational team will call the prospective participant and in case the subject accepts to move forward, a clinic visit will be scheduled. If the site considers also to perform the lactulose breath test (L-BT) at the clinic in this occasion, detailed patient's instruction on L-BT preparation will be provided.
In case the subject does not accept to move forward, the investigational site will record the reason for refusing participation.

Procedures for On-site recruitment path

Subjects presenting at the Investigational Site with moderate or severe papulopustular rosacea will be informed about the possibility to participate in the trial and if they are interested the Investigator will provide further information on the study and, if the subject is interested to participate, will schedule the Screening visit at the Investigational site (V1, see below).

Common procedures for both Social Media and On-site recruitment paths

11.2.4 Screening Visit (V1, from Day -30 to Day -1)

The following procedures will be performed during the screening period:

1. Informed consent process and signature of the informed consent form (ICF). For subjects participating in the PK sub-study, the "PK-specific" consent signature should also be collected;
2. Demographics collection (age, sex, race) and health-related behaviour assessment (smoking habit, alcohol consumption, alcohol or drug abuse);
3. Relevant rosacea-associated medical history collection (including but not limited to family history for rosacea, age of onset etc.);
4. Relevant non-rosacea-associated medical history collection (attention will be paid to those medical conditions included in the exclusion criteria;
5. Record of previous and concomitant medications (attention will be paid to drug-associated exclusion criteria and prohibited treatments);
6. Vital signs recording (heart rate, blood pressure, body temperature);
7. Complete physical examination (including also measurement of height);
8. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules);
9. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity;
10. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes);
11. Visual analogue scale (VAS) for pain, burning/stinging and itching;
12. Blood collection and performance of the following laboratory tests (to be performed as close as possible to the randomisation visit [V2]):
    - haematological tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count)
    - clinical chemistry tests (glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate)
    - if not provided by the local laboratory, estimation of glomerular filtration rate
    - C-reactive protein
9. Urinalysis (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, and microscopic examination [if available]);
10. Serum pregnancy tests (women of child-bearing potential only);
11. Lactulose Breath Test (L-BT): L-BT can be performed either at the study centre under Investigator's supervision or directly by the subject at subject's home. In both cases, the test has to be performed within 14 days before the randomization visit (V2);
12. Check of inclusion and exclusion criteria;
13. Schedule Randomization visit (V2, if eligible);
14. Start Adverse Events collection.

11.2.5 Randomization Visit (V2, Day 1)

The following procedures will be performed at the randomization visit:

Procedures for all eligible patients:

1. Concomitant medications recording (change from the previous visit, attention will be paid to prohibited treatments);
2. Vital signs recording (heart rate, blood pressure, body temperature);
3. Complete physical examination (including also weight and BMI);
4. Urine pregnancy tests (women of child-bearing potential only);
5. Contraception recommendations and/or adherence (women of child-bearing potential only);
6. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules);
7. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity;
8. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes);
9. Visual analogue scale (VAS) for pain, burning/stinging and itching;
10. Confirmation of inclusion and exclusion criteria;
11. Randomization (if still eligible);
12. Instruct the subject on how to complete the electronic Patient Reported Outcome (ePRO), which encompasses the abdominal symptoms questionnaire (IBS-SSS) and Dermatology Life Quality Index questionnaire (DLQI).
13. Start of recording IBS-SSS related answers to be collected every 10 days through ePRO up to visit V4;
14. Start of recording DLQI related answers to be collected every 10 days through ePRO up to visit V4;
15. Treatment dispensing (if subject has been randomised);
16. Adverse Events collection.

Additional procedures for eligible patients participating also in the PK sub-study

17. Collection of blood samples for PK analysis;
18. On-site administration of the first investigational drug dose (it must be performed early in the morning after the baseline blood drawing).

11.2.6 End of Treatment Visit (V3, Day 30±1)

The following procedures will be performed at End of Treatment visit:

Procedures for all randomized patients:

1. Concomitant medications recording (change from the previous visit);
2. Vital signs recording (heart rate, blood pressure, body temperature);
3. Complete physical examination (including also weight and BMI);
4. Urine pregnancy tests (women of child-bearing potential only);
5. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules);
6. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity;
7. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes);

8. Visual analogue scale (VAS) for pain, burning/stinging and itching;
9. Blood collection and performance of the following laboratory tests:
    - haematological tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count)
    - clinical chemistry tests (glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate)
    - if not provided by the local laboratory, estimation of glomerular filtration rate
    - C-reactive protein
    - Urinalysis (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, and microscopic examination [if available]);
10. Check subject's recording of IBS-SSS and DLQI questionnaires via ePRO (to be ended on visit V4);
11. Lactulose Breath Test (L-BT): L-BT can be performed either at the study centre under Investigator's supervision or directly by the subject at subject's home. In the latter case, the test has to be performed within 3 days after the V4 visit;
12. Adverse Events collection;
13. Study drug accountability.

Additional procedures for randomized patients participating also in the PK sub-study

14. Collection of blood samples for PK analysis;
15. Administration of the last investigational drug dose (it must be performed early in the morning after the baseline blood drawing);
16. Overnight stay (discharge on Day 31 after the last blood drawing).

11.2.7 End of Study Visit (V4, Day 60±3)

The following procedures will be performed at the V4 visit:

1. Concomitant medications recording (change from the previous visit);
2. Vital signs recording (heart rate, blood pressure, body temperature);
3. Complete physical examination;
4. Urine pregnancy tests (women of child-bearing potential only);
5. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules);
6. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity;
7. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes);
8. Visual analogue scale (VAS) for pain, burning/stinging and itching;
9. Blood collection and performance of the following laboratory tests:
    - haematological tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count)
    - clinical chemistry tests (glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate)

- if not provided by the local laboratory, estimation of glomerular filtration rate
- C-reactive protein
- Urinalysis (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, and microscopic examination [if available]);

10. Check subject's recording of IBS-SSS and DLQI questionnaires via ePRO;
11. Last recording of IBS-SSS and DLQI questionnaires through ePRO;
12. Adverse Events collection;
13. End of study.

11.2.8 Early Termination Visit (ETV, from Day 1 to Day 60)

The following procedures will be performed at the ETV visit:

1. Concomitant medications recording (change from the previous visit);
2. Vital signs recording (heart rate, blood pressure, body temperature);
3. Complete physical examination (including also weight and BMI);
4. Urine pregnancy tests (women of child-bearing potential only);
5. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules);
6. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity;
7. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes);
8. Visual analogue scale (VAS);
9. Blood collection and performance of the following laboratory tests:
    - haematological tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count)
    - clinical chemistry tests (glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate)
    - if not provided by the local laboratory, estimation of glomerular filtration rate
    - C-reactive protein
    - Urinalysis (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, and microscopic examination [if available]);
10. Recording of IBS-SSS and DLQI questionnaires via ePRO;
11. Lactulose Breath Test (L-BT): L-BT can be performed either at the study centre under Investigator's supervision or directly by the subject at subject's home. In the latter case, the test has to be performed within 3 days after the ETV visit;
12. Adverse Events collection.

11.2.9 Unscheduled Visit (UV, from Day 1 to Day 60)

The following procedures will be performed at the UV visit:

1. Concomitant medications recording;
2. Vital signs recording (heart rate, blood pressure, body temperature);
3. Complete physical examination;

4. Urine pregnancy tests (women of child-bearing potential only), if judged necessary by the Investigator;
5. Rosacea-associated facial skin inflammatory lesion count (i.e. papules and pustules), if judged necessary by the Investigator;
6. Five-points Investigator's Global Assessment (IGA) of Rosacea Severity), if judged necessary by the Investigator;
7. Other rosacea-associated lesions assessment (i.e. facial erythema, facial telangiectasia, ocular manifestations, phymatous changes), if judged necessary by the Investigator;
8. Visual analogue scale (VAS), if judged necessary by the Investigator;
9. Blood collection and performance of the following laboratory tests, if judged necessary by the Investigator:
   - haematological tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count)
   - clinical chemistry tests (glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate)
   - if not provided by the local laboratory, estimation of glomerular filtration rate
   - C-reactive protein
   - Urinalysis (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, and microscopic examination [if available]);
10. Lactulose Breath Test (L-BT), if judged necessary by the Investigator;
11. Adverse Events collection.

11.3 Laboratory Tests

During the study period, it can be estimated that a total volume of approximately 132 ml of blood for each patient will be drawn.

Haematology, chemical chemistry
The standard procedures to perform laboratory analyses will be followed, and the usual amount of blood necessary to carry out the tests will be taken. The following tests will be analysed by the local clinical laboratory, unless otherwise indicated.

- Approximately 20 ml of blood will be drawn three times (at V1, V3 and V4 visits, for total of 60 ml) during the study period to perform standard laboratory tests (haematology and standard chemistry):
  - Laboratory tests (haematocrit, haemoglobin, red blood cell count, white blood cell count with differential count, platelet count, glycaemia, total cholesterol, triglycerides, serum creatinine, urea (or BUN), sodium, potassium, chloride, AST, ALT, Gamma-GT, alkaline phosphatase, total and fractioned bilirubin, erythrocyte sedimentation rate and C reactive protein) will be performed at the screening visit (V1), at the end of treatment visit (V3) and at the end of the study (V4) to assess systemic tolerability.
    Glomerular Filtration Rate will be estimated at the screening visit only for enrolment purposes.

- At the screening visits, other 5 ml of blood (approximately) will be collected to perform a serum pregnancy test (serum human chorionic gonadotropin assay).

PK assessment

Only for those subjects participating in the PK sub-study, additional 132 ml of blood will be drawn during the study for the following pharmacokinetic evaluations:

- At randomization (V2, Day 1), sampling will be performed before and after the first morning dose of Rifaximin-EIR at the following time points: immediately pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post-dosing.
- At the end of treatment (V3, Day 30), sampling will be performed right before and after the morning dose of Rifaximin-EIR at the following time points: immediately pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6 and 8, 12, 22, 24, and 30 hours post-single dose at day 11 (the sampling started at V3 will end after overnight stay, as appropriate).

PK samples handling procedures

The whole process of blood sampling, processing and storage of plasma samples collected from the subjects participating in the PK sub-study is detailed below.

Blood tube	A 6 mL Vacutainer tube (or equivalent) containing Na-Heparin as anticoagulant
Blood handling and processing	Draw about 6 mL blood into the tube (the first 0.5 mL of blood withdrawn via cannula will be discarded), invert tube gently to mix and place immediately in ice/water bath. Centrifuge at 4°C at 1500*g* for 10 min within 1h from collection.
Plasma aliquot volumes	Aliquot plasma evenly into two (2) polypropylene cryovial tubes. One aliquot should be retained at the site.
Plasma storage conditions	Store at below -65°C until transfer and ship on dry ice.

Samples will be analyzed within 185 days from the date of collection.

Urinalysis

Urinalysis (visual examination (specific gravity, pH, protein, glucose, ketones, haemoglobin, nitrite [if available], bilirubin, urobilinogen, microscopic examination [if available]) will be performed at the screening visit (V1), at the end of treatment visit (V3) and at the end of follow-up visit (V4) to assess systemic tolerability.

Urine pregnancy test will be performed on the sample of urines collected at V2, V3 and V4.

Lactulose breath test

Lactulose Breath Test (L-BT) is a non-invasive test for assessing malabsorption/fermentation of carbohydrates in the small intestine as consequence of SIBO. Overgrowth of colonic bacteria results in increased carbohydrate fermentation in the gut, leading to a production of hydrogen (H_2) and methane (CH_4), eventually released with the expiratory breath. Breath testing consists of times and standardised measurement of exhaled H_2 and CH_4 after the ingestion of a solution of water and lactulose. Although classical L-BT has been performed using a 4-hour collection time (Gasbarrini et

al. 2009), a recent consensus suggests that a 2-hour procedure is sufficient to detect a significant small intestine colonization (Rezaie et al. 2017).

During the screening period (V1), L-BT will be performed to all subject recruited for participating to the clinical study within the last 2-weeks before randomization (V2). Lactulose breath test will be also repeated at the end of treatment (V3) or at the Early Termination Visit (if applicable) within 3 days from the Visit.

L-BT can be performed either at the study center or directly by the subject at home. All study subjects will be advised to follow dietary restrictions and life-style habits required for the test performance (i.e. avoid complex carbohydrate rich diets one day before the test, 12-hours as fasting period, smoking and physical exercise were not allowed 2-hours before and during the test, etc.).

Study investigators will be instructed on how to accurately inform subjects to perform L-BT.

In summary, a first breath sample will be collected to evaluate the basal breath of H_2 level (in ppm) after a 12-hours of fasting. Thereafter, subjects will be asked to drink a 10-grams of lactulose solution. Subsequently, levels of breath H_2 and CH_4 will be estimated every 15-20 minutes for a total duration of 120 minutes.

After timely delivery, H_2 and CH_4 levels will be determined by a centralised laboratory (Quintron).

11.3.1 Handling of Biological Samples

Blood and urine sample for hematology, chemical chemistry, and urinalysis will be handled according to a centralized laboratory procedure. Breath samples for centralized L-BT will be handled according to directions provided in a separate “Centralized Laboratory Test Manual”. Detailed instructions for managing blood samples for PK analysis will be also provided in a separate “PK Laboratory Manual”.

11.4 Decoding of randomisation in case of emergency

The overall randomization code will be broken by the Sponsor at the study end, once all final clinical data have been entered onto the database and all data queries have been resolved.

During the study, **ONLY** the Sponsor’s Pharmacovigilance (PV) may unblind, and **ONLY** for safety reasons upon PV Clinical Safety Unit Manager and EU-QPPV decision or for regulatory purposes (when SUSAR has to be submitted to RAs and ECs).

Only in the case of an emergency safety issue, the Investigator may unblind a subject’s treatment assignment, when knowledge of the study medication is essential for the clinical management or welfare of the subject,

After the emergency blind-breaking, the Investigator must notify the unblind, as soon as possible, to the PV Clinical Safety Unit Manager without share the treatment information to other people involved in the study (see contact details in the “Emergency Instructions” chapter at the beginning of the protocol). In addition, the Investigator will record the date and reason for revealing the blinded treatment assignment for that subject in the appropriate data collection tool.

As a rule, the Investigator can break the blind using the online tool embedded in the eCRF system.

The blindness should be maintained for persons responsible for the ongoing conduct of the study (such as management, monitors, sub-investigators); if not the subject must be withdrawn from the study and procedures accompanying withdrawal are to be performed. In cases where there are ethical reasons for the patient to remain in the study, the Investigator must obtain specific approval from the Sponsor for the subject to continue in the study.

11.5 Subject Diary

Diary data will be collected 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). The subjects will fill the diary information regarding gastrointestinal symptoms (IBS-SSS questionnaire) and dermatologic quality of life (DLQI questionnaire) every ten days from V2 to V4 (included).

This e-PRO will be integrated in the electronic data capture system utilized for the collection of all the other study data. The clinical site staff will review periodically the e-PRO data collected and in case of missing data will be responsible for reminding the subject about appropriate completion. The diary data collected during the different study periods will be also revised by the clinicians in occasion of each scheduled study visit.

12. EFFICACY AND SAFETY ASSESSMENTS

12.1 Assessment of efficacy

Rifaximin-EIR has currently being investigated in a phase 2 trial in subjects with rosacea and positive L-BT at daily doses of 800mg (400 mg b.i.d.) and 1600mg (800 mg b.i.d.). A lower dose of Rifaximin-EIR, lower than those investigated in this ongoing trial (i.e. 250 mg t.i.d., overall 750mg daily), is planned to be moved forward in this indication. However, the effect on rosacea lesions of this lower dose, particularly when administered t.i.d., has not yet been assessed. Moreover, known the bioavailability of Rifaximin-EIR in patients with papulopustular rosacea has not been investigated.
This study is therefore aimed at:

1) exploring the safety and efficacy of two doses of oral Rifaximin-EIR (750 mg/day and 1500 mg/day) versus placebo in adult subjects with moderate-to-severe papulopustular rosacea, and
2) in a sub-group of subjects, evaluating the PK of two doses of Rifaximin-EIR 250 mg tablet (250 mg t.i.d. [750 mg/daily] and 500 mg t.i.d. [1500 mg/daily])

administered t.i.d. for 29 consecutive days and one last morning dose on Day 30.

Data collected with this study will provide key information to be used in the development of Rifaximin-EIR in rosacea.

12.1.1 Primary Efficacy Endpoint

Co-primary efficacy endpoint:

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

1) Absolute change from baseline in number of rosacea inflammatory lesions (papules and pustules) at the end of treatment (V3, Day 30) AND
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 (V2) at the end of treatment (V3, Day 30).

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

12.1.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints:

1) Mean change from Baseline (V2) in number of inflammatory lesions (papules and pustules) at V4;
2) Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at V3 and V4;

3) Percent of participants with IGA score of 0 (clear) at V3 and V4;
4) Change from Baseline (V2) in the following rosacea additional features at V3 and V4:
   - 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 at V3 and V4 (absent=0, mild=1, moderate=2, severe=3);
6) Change from baseline in abdominal pain score at V3, V4;
7) Change from baseline in abdominal distension score at V3, V4;
8) Change from baseline in bowel habit satisfaction score at V3, V4;
9) Change from baseline in global severity of abdominal symptoms score at V3, V4.
10) Percent of participants showing treatment success according to a modified IGA scale excluding papules/pustules but including erythema (i.e. score of 0 or 1) at V3 and V4.
11) Percent of participant showing treatment success according to a Modified IGA scale including erythema and papules/pustules at V3 and V4.

Pharmacokinetic assessments (PK sub-study only):

12) 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) of Rifaximin-EIR 250 mg.

Clinical assessment of rosacea lesions

At each study visits (from V1 to V4 [or ETV]) the Investigators will perform a clinical assessment of the rosacea facial skin lesions. The following evaluation will be performed:

- Number of papules and pustules;
- Presence and severity of erythema (absent=0, mild=1, moderate=2, severe=3);
- Presence and severity of telangiectasia (absent=0, mild=1, moderate=2, severe=3);
- Presence and severity of ocular manifestations (absent=0, mild=1, moderate=2, severe=3);
- Presence and severity of phymatous changes (absent=0, mild=1, moderate=2, severe=3).

The assessment will be performed in 3 areas of the face and reported in the Rosacea Patient Lesion Assessment form (see Appendix 4).

Patients will be asked by the Investigators to perform a “Pain, Burning or stinging and itching” self-evaluation at each study visits (from V1 to V4 [or ETV]) using a 0-10 cm Visual Analogue Scale VAS) (see Appendix 5).

Investigators will also be required to provide a global assessment using the Investigator Investigator’s Global Assessment (IGA) of Rosacea Severity (see Appendix 1) as well as Modified IGA scales (see Appendices 2 and 3) to be reported in the Rosacea Patient Lesion Assessment form (Appendix 4).

In order to standardize assessment, Investigators will be instructed on how to perform the evaluation and visual aids with photographs of different lesion severity will be provided.

Five-point Investigator's Global Assessment (IGA) Scale

The Investigator's Global Assessment (IGA) scoring system utilized in the study is a re-formatting of a Standardized Grading System used for grading of rosacea severity. The Standardized Grading System provides a widely used basic framework for disease quantification (see Appendix 1).

Visual Analogue Scale (VAS)

A Visual Analogue Scale is a psychometric measuring tool meant to document the characteristics of a symptom severity in individual patients and is used to obtain a statistically measurable classification of symptom severity. In the present study, a 10 cm VAS (a straight 10 cm horizontal line where the left-hand side end represents the presence of "no pain, stinging/burning or itching" and the right-hand side end represents the "worst pain, stinging/ burning or itching ever experienced") will be used to document the presence/severity of pain, burning/stinging or itching in participants with rosacea. Patients will be instructed to mark on the line the point that they feel represents their perception of their current state. The measure (in cm and millimetres) of the distance between the left-hand side end and the patient's mark will be the measure of the symptom (see Appendix 5).

Irritable Bowel Syndrome - Symptom Severity Scale (IBS-SSS)

IBS-SSS is the most frequently used severity measure for evaluating IBS severity. Items relate to pain, bowel dysfunction and overall well-being. This is a composite score of abdominal pain, number of days with abdominal pain, bloating/distension, satisfaction with bowel habits, and IBS-related quality of life (QoL). The IBS-SSS questionnaire consists of 5 questions referred ''to the last ten days'' (see Appendix 6).

Dermatology Quality of Life Index (10-item DLQI)

The Dermatology Life Quality Index (DLQI) represents a valuable method for measuring the impact of skin disease on patients' lives. The DLQI consists of 10 questions concerning symptoms and feelings, daily activities, leisure, work, and school, personal relationships and treatment. Each question is answered by a tick box: ''not at all'', ''a little'', ''a lot'' or ''very much''. Each question is scored from 0 to 3 and the scores summed, giving a range from 0 (no impairment of life quality) to 30 (maximum impairment). All the questions are referred ''to the last week''. The DLQI was designed to be used in adults over the age of 18 years. Information belonging from DLQI can enhance the quality of care provided (see Appendix 7).

12.2 Assessment of safety

Safety will be evaluated by collecting the following parameters:

- Complete Physical Examination including height, weight, BMI;
- Vital signs (including heart rate, blood pressure, and body temperature);
- Routine laboratory parameters (haematology, coagulation, biochemistry, urinalysis, serum and urine pregnancy tests);
- Adverse events (AEs);

- Withdraw of subjects from study due to adverse events.

Accurate monitoring of adverse events will be performed by each Investigator by questioning the patient, at each visit during treatment, and on the final assessment visit.

12.3 Appropriateness of Measurements

Systolic and diastolic blood pressure will be measured at rest in a seated position. Blood pressure should be measured by the auscultation method using a mercury sphygmomanometer; however, the use of an automatic sphygmomanometer is also permitted. The same assessment method will be maintained throughout the study.

Heart rate will be measured at rest in a seated position using the pulse at the wrist assessed for one minute. The same assessment method will be maintained throughout the study.

Body temperature will be measured at rest, at oral, axillary, or auditory canal level, according to local procedures. The same assessment method will be maintained throughout the study.

Body weight (in kilograms) will be measured using local procedures. The same assessment method will be maintained throughout the study.

13. PHARMACODYNAMIC ENDPOINTS

Not assessed.

14. PHARMACOKINETIC ENDPOINTS

Pharmacokinetic of the new formulation of Rifaximin-EIR (250 mg tablets) has been included in the secondary endpoints. In particular, the PK of the two groups receiving repeated doses of Rifaximin-EIR (250 mg t.i.d. and 500 mg t.i.d., respectively) after the initial administration (Day 1) and at the end of treatment (Day 30) will be assessed.

14.1 Pharmacokinetics blood sampling

Venous blood samples (about 6 mL each) will be collected from the subjects by a trained member of the clinical team. Consent will be collected from the subjects for use of these samples for the purposes of the proposed study. Samples will be processed to isolate plasma and PK analysis will be carried out on plasma samples.
Plasma samples will be sent for laboratory testing in linked anonymised form (subject number only).
Venous blood samples (heparin) will be withdrawn via an indwelling cannula or by venepuncture.
For all subject randomized in the three PK sub-groups, approximately 22 venous blood samples for PK analysis 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 exact 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.

Procedures for processing and shipping PK samples are summarised will be described in detail the PK sample processing manual.
Bioanalysis for the quantification of rifaximin in plasma samples will be carried out by using a validated HPLC-MS/MS method. Bioanalysis will be performed by a bioanalytical laboratory (Pyxant).

14.2 Pharmacokinetics parameters

The pharmacokinetics of rifaximin after the repeated administration of Rifaximin-EIR at two different dose levels (250 mg t.i.d. and 500 mg t.i.d.) will be assessed after the first and last dosing (Day 1 and Day 30) as secondary study endpoint.

The PK analysis will be done for all randomized subjects who received at least one dose of the study drug and had a suitable PK profile. Both continuous and categorical variables will be used.

Pharmacokinetic analysis of the plasma concentration-time data for rifaximin will be performed using appropriate non-compartmental techniques to obtain estimates of the PK parameters presented below, where appropriate and possible.

Parameter	Day	Definition
T_{max}	1 & 30	Time of maximum observed concentration
C_{max}	1 & 30	Maximum observed concentration
$AUC_{0\text{-}tau}$	1 & 30	Area under the curve for the defined interval between doses (tau)
$AUC_{0\text{-}\infty}$	30	Area under the curve from time 0 extrapolated to infinity
$AUC_{0\text{-}last}$	30	Area under the curve from time 0 to the time of last measurable concentration
$t_{1/2}$	30	Terminal elimination half-life
Lambda-z	30	First order rate constant associated with the terminal (log-linear) portion of the curve
AR C_{max}	30	Accumulation Ratio based on Day 30 C_{max} /Day1 C_{max}
AR $AUC_{0\text{-}tau}$	30	Accumulation Ratio based on Day 30 $AUC_{0\text{-}tau}$/Day1 $AUC_{0\text{-}tau}$

Further details of the PK data analysis will be included in the Statistical and Analysis Plan (SAP).

15. SAFETY ASPECTS

15.1 Adverse Events

15.1.1 Definition of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

An AE does include any:

- Exacerbation of pre-existing illness.
- Increase in frequency or intensity of a pre-existing episodic event or condition.
- Condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Symptoms associated with disease not previously reported by the patient.

An AE does not include a/an:

- Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE.
- Planned medical or surgical procedures.
- Pre-existing diseases or conditions present or detected at the start of the study that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalization for cosmetic elective surgery, social and/or convenience admissions).
- The disease or disorder being studied, or sign or symptom associated with the disease or disorder unless more severe than expected for the patient condition.
- Overdose of other study drug or concurrent medication without signs or symptoms.
- Symptoms associated with the disease that are consistent with the patient usual clinical course unless the symptom(s) meet(s) the criteria for “serious” except for what detailed in chapter. 15.1.3.

15.1.2 Definition of Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product which does not necessarily have a causal relationship with this treatment. A serious adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product and results in:

- ➢ death;
- ➢ is life-threatening;
- ➢ requires in-patient hospitalisation or the prolongation of existing hospitalisation;
- ➢ results in persistent or significant disability/incapacity;

- ➢ is an important medical event that may not be immediately life-threatening or result in hospitalisation or death, but may jeopardise the subject, or may require intervention to prevent one of the occurrences listed above;
- ➢ is a congenital anomaly/birth defect.

The term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it had been more severe.
Examples of important medical events which could be reported as serious are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

An unexpected adverse event is an AE whose **nature, severity or outcome** is not consistent with the information given into the Reference Safety Information (RSI). Reference documents in this study is the Investigator's Brochure.

Expected serious adverse reactions should be **considered always unexpected** if:

- ➢ have an unexpected outcome (e.g. a fatal outcome);
- ➢ have an increase in the rate of occurrence, which is judged to be clinically important.

Expectedness evaluation is performed by the Sponsor based on Reference Safety Information (RSI).

15.1.3 Protocol-Specified Serious Adverse Events

In the population under study, there are no SAEs that are anticipated to occur independent of drug exposure and therefore should not be reported as SAEs.

Hospitalisation for treatment planned prior to randomisation and hospitalisation for elective treatment of a pre-existing condition will not be considered as SAEs.

Pregnancy
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.

Female patients will be instructed to notify the Investigator in case they become pregnant.

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.

The report shall be made using a specific "*In Pregnancy/In Utero Exposure*" form, for the notification of pregnancy, which must be sent by email or fax to the same contact who receives the SAE notifications.

In case of an onset of SAE or an unfavorable outcome occurs (abortion, abnormalities in the fetus, early termination of pregnancy) a SAE form also must be filled-in by the Investigator and immediately sent to the Sponsor.

Also, a follow-up of the pregnancy, until the childbirth, will be performed to document its outcome and the state of health of the newborn.

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.

15.1.4 Change of Laboratory Parameters

Whether a change of parameters in the safety laboratory investigations (i.e. haematocrit, liver enzymes etc.) is observed and the parameter shifts from normal to a *clinically significant* abnormal value, or a basal abnormal value worsens during treatment with the investigational drug respect pre-treatment value, can also represent an ***adverse event***.
If the change is *clinically significant*, when evaluating such changes, variation from the normal range, the extent of deviation from normal and time taken to return to normal after discontinuing treatment, should be taken into consideration.
If there are pathological values at the end of the treatment phase which were not present before, further clinical or laboratory investigations must be performed until the values return to normal or, a plausible explanation is found for the pathological laboratory value (e.g. concomitant disease).
The Investigator will decide if a change in laboratory parameter is clinically relevant and therefore represents or not an adverse event (serious/not serious) according to the above criteria and the clinical state of the subject.

15.1.5 Classification and Coding of the Causal Relationship and Severity

The Investigator must assign, for each event, the **causal relationship** with study treatment according to the following categories:

[**0 = Not related / No Reasonable possibility**]: to be used for an AE/SAE which is not suspected to be related to the study treatment.
[**1 = Related/Reasonable possibility**] to be used for an AE/SAE which is suspected to be related to the study treatment. The definition implies a reasonable possibility of a causal relationship between the event and the study treatment. This means that there are facts (evidence) or arguments to suggest a causal relationship.

Moreover, Investigators must assign the possible grade of **Severity** to the observed AEs using Common Terminology Criteria for Adverse Event (CTC-AE) V5.0 (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf), as follows:

- **Mild**: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Moderate**: minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL (Activities of Daily Living).
- **Severe:** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (Activities of Daily Living).
- **Life-threatening** consequences: urgent intervention indicated.
- **Death.**

Categories “Severe”, “Life-threatening” and “Death” will be considered as seriousness criteria per se.

Outcome: “recovered”, “recovered with sequelae”, “not yet recovered”, “death”, “unknown/lost to follow up”.

15.1.6 Documentation of Adverse Events

All AEs occurring during the study, after patient signature of the informed consent (with the exception described in chapters 15.1.1. and 15.1.3., events recorded in appropriate e-CRF pages), must be assessed and documented by the investigator on the AE pages of the subjects' e-CRF. If they meet the criteria for "serious" require also the completion of a SAE Report Form that must be immediately (within maximum 24 hours) reported to the Sponsor (section 15.1.7).
The Investigator will always provide his/her assessment of causality at the time of the initial report.
Each AE will be assessed and documented by the investigator according to the following categories:

- Study protocol number.
- Study Centre number.
- Patient identification number.
- Description of the sign and symptom/event.
- Severity as previously defined in Section 15.1.5.
- Duration: Record the onset and resolution time and dates of the event.
- Action taken.
- Causality to study treatment/individual component: as previously defined in section 15.1.5.
- Outcome
- Seriousness.

All AEs reported during the study must be followed-up by the Investigator until the event resolves, stabilises or the patient is lost to follow-up (see sec. 15.1.9.).
AEs still present at the end of the study period and for the subsequent 30 days must be followed until the final outcome is determined.
An AE that is initially reported as non-serious and later meets the criteria for a SAE, it must be reported as a SAE as soon as the information regarding the presence of one or more seriousness criteria become known to the Investigator.

The Investigator is responsible to ensure that the follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical, the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
The Sponsor may request the Investigator to perform or arrange for the conduct of supplemental measurements and/or evaluations.

15.1.7 Prompt Reporting of SAE to the Sponsor

Any SAE occurring during the study, whether related to the investigational product or not, must be reported immediately (i.e., within 24 hours) to Alfasigma PV Clinical Safety.

The SAE form must be faxed or mailed using the following contact details:

E-mail: PPD
or
Fax: PPD

One “SAE form” should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same “SAE form” preferably as a diagnosis.
The SAE form must be completed as thoroughly as possible with all available details of the event, signed by the Investigator (or appropriately qualified designee), and reported immediately to the Sponsor, within 24 hours from first awareness of the event.
If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before compiling the SAE form and notifying the event to the Sponsor, although the following minimal information is required:

- Study Protocol number
- Study Centre number
- Patient identification number
- Sex, and age
- Investigational product(s)
- Description of the adverse event
- Causal relationship by the investigator.
- Name, affiliation, telephone and fax number of the reporting Investigator

The additional information as soon as available will be recorded in a separate SAE form completed in every part that will be sent as a follow-up report.

The Investigator will **always** provide his/her assessment of causality at the time of the initial report. If follow-up obtained subsequently, lead the investigator to change the assessment of causality, the SAE form may be appropriately amended, signed and dated, and resubmitted to the Sponsor as follow up report.
If a patient dies during participation in the study or during a recognized follow-up period, the Investigator should send to the Sponsor, together with SAE Form, any other available *post-mortem* information, including autopsy and histopathology.
In accordance with local Independent Ethics Committee (IEC) requirements, the Investigator might have to notify to IEC also any SAEs.

15.1.8 Reportable events

Reportable events are those serious adverse events that are causally related to the study drug, and that are both serious and unexpected (SUSAR). Such events are subject to expedited reporting to regulatory authorities and they must be reported within the timelines foreseen by the relevant rules by the Sponsor or a suitably qualified designee.
The Sponsor is responsible for reporting SUSAR to the relevant regulatory authorities in the time frames and methodology applicable according to local Law and Regulations.

15.1.9 Post study AEs and SAEs

Serious and non-serious AEs will be recorded, after patient signature of informed consent, from the Screening visit (V1) to the End of study visit (V5) or Early termination visit, regardless of whether the participant is on treatment.
SAEs will be recorded and reported as appropriate and followed-up until resolution or the patient is lost to follow up. Non-serious AEs will be recorded as appropriate and followed-up until End of Study

visit (V5, ESV) or Early Termination Visit (ETV) with event's outcome reporting.
SAEs will be recorded and reported as appropriate and followed-up until Resolution, Chronicity or the patient is lost to follow up.
SAEs still present at the end of the study period and for the subsequent 30 days, must be followed until a final outcome is determined or the patient is lost to follow up.
A post-study AE/SAE is defined as any event that occurs outside the AE/SAE detection period as defined previously.
Investigators are not obliged to actively seek AEs or SAEs after study termination. However, if the Investigator learns of any SAE, including death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the Investigational Product, the Investigator will promptly notify the Sponsor, according to the process explained at section 15.1.7.

15.2 Warnings and Precautions

Rifaximin is a safe and well tolerated drug as demonstrated by Clinical Studies and years of post-marketed experiences in registered indications. Due to its pharmacodynamics and pharmacokinetics characteristics, life-threatening, serious AEs are not expected.

Patients should be informed that despite the negligible absorption of the drug (less than 1%), like all rifamycin derivatives, rifaximin may cause a reddish discolouration of the urine.
Clostridium Difficile associated diarrhoea (CDAD) has been reported with use of nearly all antibacterial agents, including rifaximin. The potential association of rifaximin treatment with CDAD and pseudomembranous colitis (PMC) cannot be ruled out.

16. STATISTICAL PLAN AND DETERMINATION OF SAMPLE SIZE

The following describes the statistical analysis as it is at the time of planning the trial. A detailed Statistical Analysis Plan (SAP) will be issued after the study starts.

SAS software (V.9.3 or subsequent) will be used for all statistical analyses.

The primary objective of the study is to evaluate the effect of Rifaximin Delayed-Release 500 mg t.i.d. or Rifaximin-EIR 250 mg t.i.d. vs placebo in terms of

- absolute change from baseline in number of rosacea lesions (papules and pustules) at the end of treatment (V3, Day 30), and
- rate 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 (V3, Day 30).

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

A unique randomization list will be created. The randomization list will be stratified by L-BT status (negative vs positive) and by PK sub-study participation (yes or not).

16.1 Determination of 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

Based on these assumptions, 51 patients were to be enrolled in each treatment arm as shown in the tables below.

CCI

CCI [redacted]

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 (Control Clin Trials, 1990): 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 H_2 by 90 minutes compared to time 0) is established.

Sample size for the sub-study on PK is not based on formal statistical assumptions. A cap of 12 subjects per arm is established. This is considered a good value for this kind of study. In any case, in order to have an adequate sample size, it is advisable to have at least 6 subjects per arm.

16.2 Definition of Study 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.

16.2.1 Screened Population

Screened Population is defined as all subjects enrolled into a screening phase 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.

16.2.2 Safety Population

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.

16.2.3 Full Analysis Set (FAS)

The primary efficacy analysis population will be the Full Analysis Set (FAS), defined as all randomised patients having taken at least one dose of the investigational treatment.

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).

16.2.4 Modified Full Analysis Set (mFAS)

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.

16.2.5 Per Protocol Set (PPS)

Per Protocol Set (PPS) 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 PPS will be performed according to the actual treatment received. Patients randomized to a wrong stratification factor level will not be excluded from the PPS and will be analysed according to the actual stratum (not the randomized one).

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.

16.2.6 PK Set

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

16.2.7 Disposition

The number of subjects screened, randomized, treated and completing the trial (and reason for not completing) will be presented in frequency tables by treatment group.

The number of subjects in each analysis population (SAF, FAS, mFAS and PPS) will be summarized by treatment group.

All major protocol violations will be presented as a frequency table by treatment group.

16.2.8 Demographic and Other Baseline Variables

Baseline values are the last recorded values collected prior to treatment initiation.

Subject demographics, medical history and disease characteristics, previous and concomitant medications, and others baseline characteristics measured before randomization will be summarized descriptively by treatment group on the FAS. Selected summaries will be provided by treatment group and stratification factor (details to be provided prospectively in the SAP).

If the PPS comprises less than 80% of the FAS, selected baseline data will be also summarized on the PPS.

Concomitant diseases at baseline and relevant previous diseases will be coded using the latest available version of Medical Dictionary for Regulatory Activities (MedDRA).

Relevant previous and concomitant treatments will be coded using the latest available version of the World Health Organization dictionary of medical codes (WHO).

16.3 Statistical and Analytical Plans

The statistical analyses will be performed at the end of the study, when the data base has been cleaned and locked.
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”.

16.3.1 Primary Efficacy Endpoints and Statistical Model of Analysis

The co-primary efficacy endpoints, clinically assessed by the Investigator, are the absolute change from baseline in 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 (i.e.: mean change from Baseline [V2] in number of inflammatory lesions at Day 30 [V3]) will be analysed by means of an Analysis of Covariance (ANCOVA) model, with change from Baseline to Day 30 as dependent variable, treatment group and stratification factor as explicative factors, and baseline number of inflammatory lesions as covariate. The adjusted mean differences will be presented with 95% CIs and p-values.

The second co-primary efficacy endpoint (i.e.: percent of patients showing treatment success) will be analysed by means of a stratified Cochran Mantel-Haenszel (CMH) chi square test. The Breslow-Day test 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) will also be computed.

All statistical tests will be conducted at the two-sided $\alpha = 0.05$ significance level. Due to the explorative purposes of this trial, no adjustment of significance level for multiplicity will be applied.

16.3.2 Secondary Endpoints and Statistical Model of Analysis

16.3.2.1 Secondary Efficacy Endpoints and Statistical Model of Analysis

Exploratory secondary endpoints are the following:

1) Mean change from Baseline (V2) in number of inflammatory lesions (papules and pustules) at V4.
2) Percent of participants showing treatment success (i.e. IGA score of 0 or 1) at V3, V4.
3) Percent of participants with IGA score of 0 (clear) at V3, V4.
4) Change from Baseline (V2) in the following rosacea additional features at V3, V4:
   - 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 at V3, V4 (absent=0, mild=1, moderate=2, severe=3).
6) Change from baseline in abdominal pain score at V3, V4.
7) Change from baseline in abdominal distension score at V3, V4.
8) Change from baseline in bowel habit satisfaction score at V3, V4.
9) Change from baseline in global severity of abdominal symptoms score at V3, V4.
10) Percent of participants showing treatment success according to Modified IGA scale excluding papules/pustules but including erythema (i.e. score of 0 or 1) at V3, V4.
11) Percent of participant showing treatment success according to a Modified IGA scale including erythema and papules/pustules at V3 and V4.

The change from baseline to the relevant visit in secondary endpoints 1 and from 4 to 9 will be analyzed by means of an Analysis of Covariance (ANCOVA) Model, with change from Baseline to the relevant visit for the current endpoint as dependent variable, treatment group and stratification factor as explicative factors and baseline value of the current endpoint as covariate. The adjusted mean differences will be presented with 95% CIs and p-values.

The secondary endpoints expressed as percentages (endpoints 2, 3, 10 and 11) will be analyzed by means of a two-sided Cochran Mantel-Haenszel Chi-square test stratified by L-BT status. 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 active group vs placebo) will also be computed.

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

16.3.2.2 Pharmacokinetics Methods and Statistical Data Analysis

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.
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.
Pharmacokinetic parameters will be calculated for all subjects who belong to the pharmacokinetic population (see paragraph 16.2.5), using standard non-compartmental methods as described in paragraph 14.2.

No value of derived parameters such as Lambda-z, $t_{1/2}$, $AUC_{0-\infty}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile. All times used in the calculation of pharmacokinetic 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. Determination of PK parameters will be performed using appropriate software.

Pharmacokinetics parameters will be summarised by descriptive statistics by dose day, study groups, gender and overall.

16.3.3 Safety Laboratory Statistical Analysis

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

Where appropriate, the reported laboratory values and the corresponding reference ranges will be converted into SI units.

For laboratory data and vital signs, the numeric values and corresponding changes from baseline will be summarized using descriptive statistics (number of subjects, mean, standard deviation, minimum, median, maximum) by parameter and visit.

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. This classification will be summarized by means of shift tables from baseline to post-baseline assessments.

16.3.4 Statistical Analysis of 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.

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.

TEAEs relationship to study medications will be investigated with frequency tables. Missing classifications concerning study drug relationship will also be considered as treatment-related.

The following tables will be presented:

1. Overview of AEs and TEAEs showing the number of AEs and TEAEs and the number of patients with any AEs and TEAEs, treatment-related TEAEs, serious TEAEs, treatment-related serious TEAEs and TEAEs leading to study withdrawal;
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 causing early discontinuation of the study treatment by Primary SO and PT.

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

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

16.3.5 Statistical Analysis of Lactulose Breath Test Results

Results relevant to the lactulose breath test (Positive/Negative SIBO) will be summarized by treatment group, stratification factor and visit by means of frequency tables (counts and percentages).

16.3.6 Statistical Analysis of Vital Signs and Body Weight

Vital signs will be summarised by treatment group and visit using descriptive statistics.

Changes from Baseline to the relevant visits will be summarised by treatment group using descriptive statistics.

Also, body weight will be summarised by treatment group and visit using descriptive statistics.

16.3.7 Statistical Analysis of 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).

16.3.8 Handling of Missing and Incomplete 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 Lactulose medication 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. With reference to the statistical analyses performed on the FAS, the last observation carried forward (LOCF) strategy will be employed for dealing with any missing continuous data for the relevant primary and secondary endpoints. For categorical data (i.e. Success/Failure) missing data will be considered as Failures, unless differently specified in the SAP.

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 otherwise specified in the SAP.

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 replaced.

16.4 Additionally Planned Statistical Analyses

16.4.1 Interim Analyses and Data Monitoring Committee

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

16.4.2 Multicentre study

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.

16.4.3 Examination of Subgroups

There are no pre-defined sub-groups in this study. However, there is one sub-study on PK data.

17. DOCUMENTATION, RECORD ACCESS AND ARCHIVING

17.1 Documentation of Essential Documents/Supplements at Study Centre during the Trial

An “Investigator Study File” will be established at the study centre at the beginning of the trial. The investigator/institution must maintain the trial documents as specified in the Guideline for Essential Documents for the Conduct of a Clinical Trial (ICH E6 (R2) - EMA/CHMP/ICH/135/1995) and the applicable regulatory requirement(s).

17.2 Screening/Enrollment Log

The date of screening of all subjects fulfilling the inclusion requirements before any study related action is taken will be documented on the “Screening/Enrolment Log”.
The Investigator will document that the subject satisfies the inclusion and exclusion criteria, and therefore if the subject is enrolled or is screening failure.
The screening list must not permit the identification of subjects. The original list will be handed to the sponsor, and a copy will be archived in the "Investigator's Study File"
With this list the investigator documents the relationship between the general patient population in the specific indication and the study population.

17.3 Documentation of Subjects’ Participation

The investigator must record all subject identification data (full name, initials, date of birth, screening number, randomisation number, hospital admission-number and date of admission [if relevant], study termination date) for all subjects who have given informed consent - whether the subject has received any investigational product(s) or not - in the "Confidential Subject Identification List". The subject identification list must allow the definite identification of subjects who take part in this study. The subject identification list is kept by the investigator in his “Investigator Study File” and archived according to the requirements of the applicable national/international regulations, in particular in accordance with art. 32 of the EU Regulation 2016/679.

17.4 Data Protection

Personal data are securely stored to prevent unauthorized access, disclosure, dissemination, alteration or loss of information and unauthorized personal data processing. Access to personal information is restricted so that only personnel who are required to access personal data as part of their job role can do so. All personnel who access personal information are bound by a duty of confidentiality.
Technical arrangements surrounding the electronic storage and use of data are as follows:

- Computers storing electronic personal data are protected by antivirus software and the network on which computers are linked are protected by industry grade firewalls;
- Off-site personnel can only access networked computers through a virtual private network;
- All data are stored on password protected computers.

Organisational arrangements are as follows:

- All buildings are secured by key-card access;
- Manual files of personal data are stored within locked cabinets that can only be accessed by authorized personnel;
- Data security and/or confidentiality provisions are utilized in agreements with third parties;
- Documented Back-up and disaster recovery procedures are in place;

- Internal audit and compliance functions provide regulatory oversight.

The personal data of patients will be pseudonymized in that they will only include health status, patients code and demographics (age, gender and ethnicity) and cannot be linked back to the individual by the recipient. The sponsor and the clinical center shall be the independent data controller in respect of the personal data of the study subjects collected in connection with the study and shall act in accordance with the relevant data protection laws in relation to the collection and processing of those personal data. The study subjects' pseudonymized personal data shall be collected and processed for the purposes of the study and may also be added to research databases and used in the future by the sponsor and its affiliates for certain additional clinical research, for product regulation and safety reporting purposes and for ensuring compliance with legal requirements. The study subjects' pseudonymized personal data may be processed for such purposes by other parties including: the sponsor's affiliates and licensing partners, its business partners, regulatory agencies and other health authorities, and ECs. Additionally, the CRO personnel are contractually bound by a duty of confidentiality obligation through a data processing agreement and the standard contractual clauses if it comes from extra EU.

17.5. Personal Data Breach

The Clinical Centre and the CRO have a comprehensive process to identify, assess, resolve and report any potential data security breaches. All staff are trained by Sponsor or delegate to identify potential data security breaches. Potential personal data breaches are handled by appropriately trained personnel according to Sponsor/CRO SOPs. Each incident and violation of personal data must be communicated to the Sponsor within 48 hours at the latest and, in any case, without undue delay after becoming aware of a personal data breach.

Notification must contain at least the following information:

- a description of the nature of the personal data breach including the following elements:
  - the categories and the approximate number of data subjects involved;
  - the categories and the approximate number of personal data involved;
  - the data breach impact level;
  - the safety measures implemented;
  - the name and contact details of the Data Protection Officer or other contact point from which further information may be obtained;
  - the name of the Data Protection Officer or appropriate privacy office of any sub-responsible parties involved in the processing that can be contact for further information.
- a description of the measures adopted and/or the measures that intends to adopt to remedy the personal data breach, including, where it is appropriate, measures to mitigate the possible negative consequences;
- a description of the probable consequences of the personal data breach;
- any further information necessary to notify the competent control Authority of the breach.

If it is not possible to provide all this information simultaneously, the information may be communicated at a later moment, without undue delay.
Every party shall be obliged to notify any case of personal data breach, regardless of its degree of severity. Any assessment of the seriousness of a possible personal data breach is assessed

exclusively by the sponsor.

17.6 Source Documents

Source documents consist of inpatient hospital charts, clinic notes, outpatient records, original test results, laboratory data, worksheets, drug accountability records, consent forms, patient's diaries, etc. Source documents must be available for review and inspection during on-site monitoring of the study by the Sponsor, its designees, IRB/IEC, and/or appropriate regulatory authorities.

17.7 Subjects' Records

If subjects' files are in electronic format, the system used must be compliant with the minimum requirements of the "Computerised system used in clinical trials" regulation.

If the above-mentioned minimum requirements are not fulfilled, the Investigator must print-out, sign and date the patient's data that will become the source documents The Investigator must assure in writing that the data on the print-outs are identical to the electronic data and are complete. These print-out must to be archived in the Investigator's Study File. No e-CRF print-out will serve as source documentation.

All data collected from screened subjects will be verified against the source documents.

This will include all entries in subjects' e-CRF and all laboratory data. Data documented in the e-CRF at the baseline visit, during treatment and in the follow-up period will be verifiable through the original source documents (i.e. patient's notes, laboratory records).

It is the Investigator's responsibility to ensure all relevant data are entered in the patient's medical file, for example medical history/concomitant diseases, date of study enrolment, visit dates, results of examinations and AEs.

The Investigator(s)/Institution(s) will permit study-related monitoring, audits, IRB/IEC reviews, and regulatory inspections, and provide direct access to source data/documents.

17.8 Case Report Forms

An electronic CRF (e-CRF) is used to record clinical trial data and is an integral part of the trial and subsequent reports. They must reflect patient's status at each phase during the course of the trial.

All information requested on the eCRF should be entered. If one is not available or is not applicable, this must be indicated.

The Investigator must ensure the accuracy, the completeness and the consistency of the data entered in the e-CRF. A User Manual with detailed instructions about e-CRF filling in will be provided to each Investigator and a specific training will be performed.

Subjects must not to be identified on the e-CRF by name, but by subject's identification number. The e-CRF is specifically designed to record the data required by this protocol. They must be kept up-to-date so that they always reflect the latest observations on the subjects enrolled in the study; the e-CRF should be filled in immediately after the conclusion of each subject's visit, preferably within 24 hours but no later than five working days after the visit.

The principal investigator will electronically sign and date each e-CRF attesting to his/her responsibility for the quality of all data included therein, and that the data represented a complete and accurate record of each subject's participation in the study.

The eCRFs system will foresee an audit trail allowing the tracking of all the changes and corrections performed to the eCRFs, with the indication of date and author of entry and of correction.

17.9 Data Management

The contract research organization (CRO) Data Management will identify and implement the most effective data acquisition and management strategy for the clinical trial protocol and deliver datasets which support the protocol objectives. Subject's data will be entered into a defined eCRFs and then combined with data provided by other sources (e.g. Central Labs, ePRO etc). Clinical data management will be performed in accordance with CRO standards and data cleaning procedures with the objective of removing errors and inconsistencies in the data, which would otherwise impact on the analysis and reporting objectives, or the credibility of the Clinical Study Report. Adverse events and concomitant medications terms will be coded using validated dictionaries such as MedDRA and WHO-Drug.

This study will conform to SDTM standards and ADaM and will be fully CDISC compliant adhering to the latest CDISC standards including all associated documentation to aid review such as the study data reviewers guide, the analysis data reviewers guide, metadata and Define.xml for both SDTM and ADaM.

17.10 Database Processing

Clinical data will be captured using a study specific eCRF using a validated and Code of Federal Regulations (CFR) Part 11 compliant Electronic Data Capture (EDC) system. Sites will receive training and have access to the study specific eCRF completion guidelines.

All eCRFs should be completed by designated trained site staff and reviewed, electronically signed and dated by the Investigator.

Pre-defined data validation checks will be run within the eCRF as the data are entered and submitted by authorised site staff. The resulting data queries will be reviewed by the clinical site and resolved. An electronic audit trail of all changes made to the eCRF will be kept within the EDC system. This audit trail identified the user making the change and date and time of change.

At the end of the study, each site will receive their subject's data in an electronic readable format (i.e., PDF format) burned on an adequate media (e.g., CD or DVD). The site will receive a PDF file for each enrolled subject; the file will contain the patient's data and its audit trail. Data files will be archived with the site study records

17.11 Archiving Requirements for Sponsor and Investigator

Essential documents (as defined in the Guideline for Good Clinical practice E6 (R2) EMA/CHMP/ICH/135/1995) must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents must be retained for a longer period however if required by the applicable regulatory requirement(s) or if required by Alfasigma S.p.A.

The subject identification list must be retained according to the requirements of the applicable national/international regulations after the completion, or discontinuation, of the study.

Alfasigma S.p.A will notify the Investigator(s)/Institution(s) in writing when the trial related records are no longer needed.

If an investigator moves, withdraws from a trial or retires, the responsibility for maintaining the records may be transferred to another investigator who accepts this responsibility. Notice of this transfer must be given to and agreed upon by Alfasigma S.p.A.

18 PROJECT MANAGEMENT

18.1 Investigator Information and Training

The Investigators and essential support staff will be trained by the Sponsor or its designee with regards to the International Council on Harmonization (ICH) GCPs and all aspects of protocol application and study management. It is the responsibility of the Investigator to train ancillary study staff and to document such training.

18.2 Quality Assurance and Quality Control

The sponsor will implement and maintain quality assurance and quality control systems with written Standard Operating Procedures (SOPs) in accordance with the Guidelines for Good Clinical Practice E6 (R2) (EMA/CHMP/ICH/135/1995).

18.2.1 Audit and Supervision of the Study

At its own discretion the Sponsor or its designee may conduct a quality assurance audit of this study. If such an audit occurs, the Investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and his/her staff to the auditor to discuss findings on any relevant issue. In the event that on-site auditing visits cannot occur, alternative measures (e.g.: remote audits) may be considered, as allowed by local regulations and according to the relevant SOPs.

An independent representative for quality assurance will ensure quality by auditing the conformity of protocol, monitoring data handling and archiving (trial master file) with the Guidelines for Good Clinical Practice E6 (R2) (EMA/CHPMP/ICH/135/19-95), national drug law(s) and SOP(s).

In addition, regulatory agencies may conduct a regulatory inspection of this study. If such an inspection occurs, the Investigator agrees to allow the inspector direct access to all relevant documents and to allocate his time and his staff to the inspector to discuss findings and any relevant issue. Inspections may be performed by regulatory authorities.

18.2.2 Monitoring

The Sponsor may engage a Contract Research Organization (CRO) to monitor the study, according to the Sponsor’s or the CRO’s SOPs. Monitors from this agency have the same rights and responsibilities as monitors of the sponsor.

This study will be monitored by the Sponsor or its designee, in accordance with ICH GCPs Topic E6 guideline "Note for Guidance on Good Clinical practice". By signing this protocol, the Investigator agrees to periodic, on-site monitoring of all appropriate study documentation.

The monitors will establish contact between the investigator and the sponsor.

The monitors will evaluate the competence of each study centre and inform the sponsor of any problems relating to the facilities and technical staff. During the study the monitors will check that informed consent was obtained from all subjects, that the data are recorded correctly and completely, the Investigator providing direct access to source data/documents for data verification, and that the investigator complies with the protocol (and any amendments), GCPs, and all applicable regulatory requirements.

The clinical monitor will check whether the envelopes have been opened during the monitoring visits.

In case of restrictions to on-site monitoring visits due to SARS-CoV2 pandemic, remote monitoring activities will be implemented in order to remotely check the correspondence of the data inputted in the e-CRF throughout the sharing of Pseudonymized copy of the source data, protecting the subjects' privacy.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring, assessment of the impact of the envisaged processing operations on the protection of personal data), methods, responsibilities and requirements, including handling of non-compliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

18.3 Trial steering committee

No trial steering committee has been planned for this study.

18.4 Data monitoring committee

No data monitoring committee has been planned for this study.

18.5 Amendments to the Protocol

Modifications of the signed protocol are only possible by protocol amendments with the agreement of all responsible persons.

The investigator should not implement any deviation from, or changes to the protocol without agreement by Alfasigma S.p.A. and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial.

Protocol amendments must be submitted to the appropriate regulatory authorities.
Alfasigma S.p.A. and the Investigator/Institution must have approval/favourable opinion from the IRB/IEC for any amendment to the protocol.
Any protocol amendment must be distributed to those who received the original protocol and be appended to it.

18.6 Closure or Discontinuation of Study and Site

Upon completion of the study, the following activities, when applicable, must be conducted by the monitor in conjunction with the Investigator, as appropriate:

- Return of all study data to the Sponsor or its designee
- Data clarifications and/or resolutions
- Accounting, reconciliation, and final disposition of used and unused study drug
- Review of site study records for completeness.

18.7 Premature Discontinuation of the Study in a Trial Site

The study site can be discontinued at the request of the Sponsor, the Investigator, or regulatory authorities.
Conditions that may warrant discontinuation of the study site include, but are not limited to the following:

- The centre cannot include an adequate number of subjects within the planned time.

- Serious and/or persistent non-compliance with the protocol.
- Careless or premeditated false documentation in the CRFs.
- Inadequate co-operation with the Sponsor.
- Non-compliance with GCP, SOPs or regulatory requirements.
- The investigator asks to discontinue the trial.
- The submission of knowingly false information from the research facility to regulatory authorities

If the trial is prematurely terminated or suspended for any reason, the subjects will be informed promptly, appropriate therapy and follow-up will be assured and where required the relevant regulatory authorities will be informed. The IRB/IEC will be promptly informed and provided with a detailed written explanation.

18.8 Premature Discontinuation of the Whole Study

If the trial is prematurely ended or suspended, Alfasigma S.p.A. will promptly inform the Investigators/Institutions, and the regulatory authorities with appropriate justifications. A written explanation will be promptly sent to the IRB/IEC by Alfasigma S.p.A. or the Investigator/Institution, as specified in the regulatory requirements.

18.9 Insurance for Subjects

Alfasigma S.p.A will arrange insurance for all study subjects. The following guidelines must be taken into account:

- Any serious adverse event which may be correlated or not to investigational product or diagnostic procedures during the trial must be reported to the sponsor or the CRO immediately.
- The insurance company is entitled to ask all doctors involved in the treatment of a subject, other insurance companies and the national insurance for information which might help to clarify the cause of injury. Before enrolment in the study a subject must be informed of insurance against trial-related injuries and who to contact for compensation.

The terms of the insurance will be included in the Investigator's Study File.

18.10 Disclosure of all Information and Results

By signing the study protocol, the investigator agrees to keep confidential all information and results concerning the study and the investigational product, until the data are published.

18.11 Publication policy

The Sponsor declare its intent to publish the results of the study, after completion of the regulatory Report.
The personal data especially sensitive data regarding the clinical trial, will be only dissemination in strictly anonymous form.
The Sponsor will work with the protocol development team and will identify a lead author for the manuscript development. The authors for the manuscript will be determined by the amount of effort and participation each Investigator contributes towards the study design, the study conduct, as well as the analysis of study results.
All of the parties agree to provide the other with the text sufficiently in advance to allow examination,

prior to submission to a scientific journal.
It is understood by the Investigator that the information developed in the clinical study may be disclosed, as required, to other clinical Investigators, and regulatory authorities.

18.12 Ownership

All data and records provided by the Sponsor or generated during the study (other than a subject's medical records) and all inventions discovered in the course of conducting the study are the property of the Sponsor. If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed than contract's ownership provisions shall apply rather than this statement.

18.13 Contracts, Finances

In addition to the protocol trial-related duties, functions and financial aspects must be specified in a separate contract between Alfasigma S.p.A., the investigator and any other parties involved in the clinical trial.

19. REPORTING

After completion of the study an "integrated" full report will be prepared (according to the ICH Harmonised Tripartite Guideline Topic E3 “Structure and Content of Clinical Study Reports.

20. REFERENCE LIST

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21. Appendices


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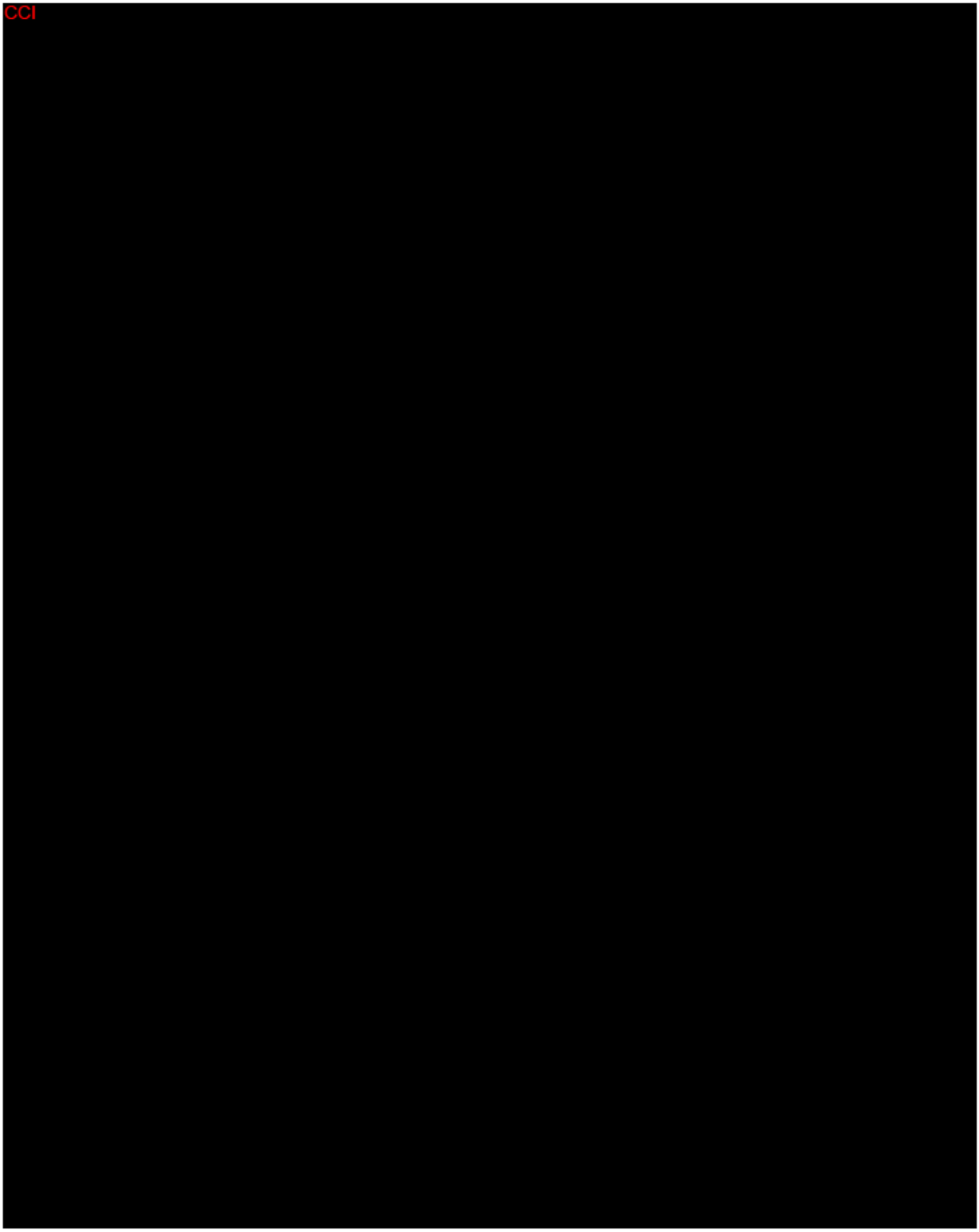

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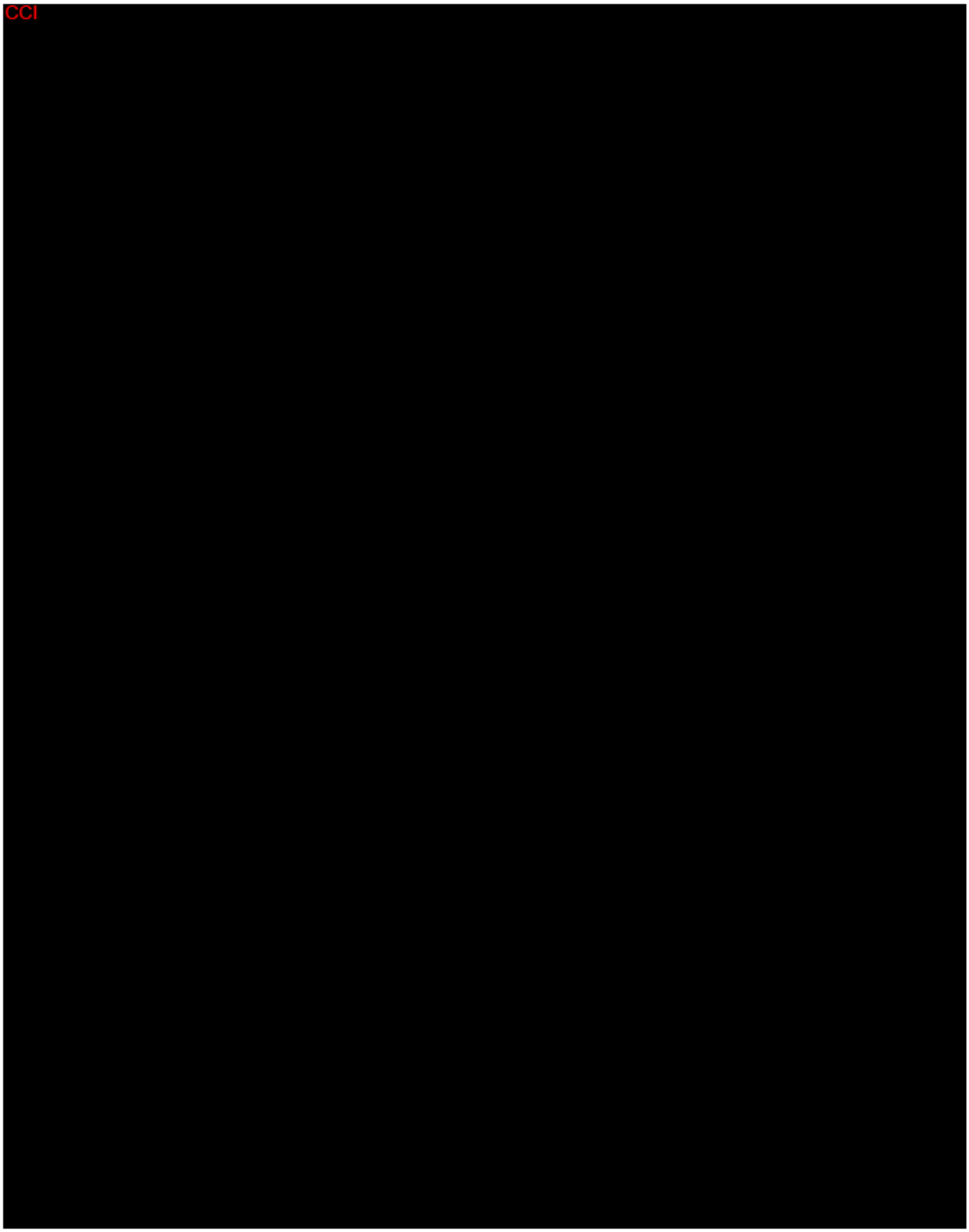
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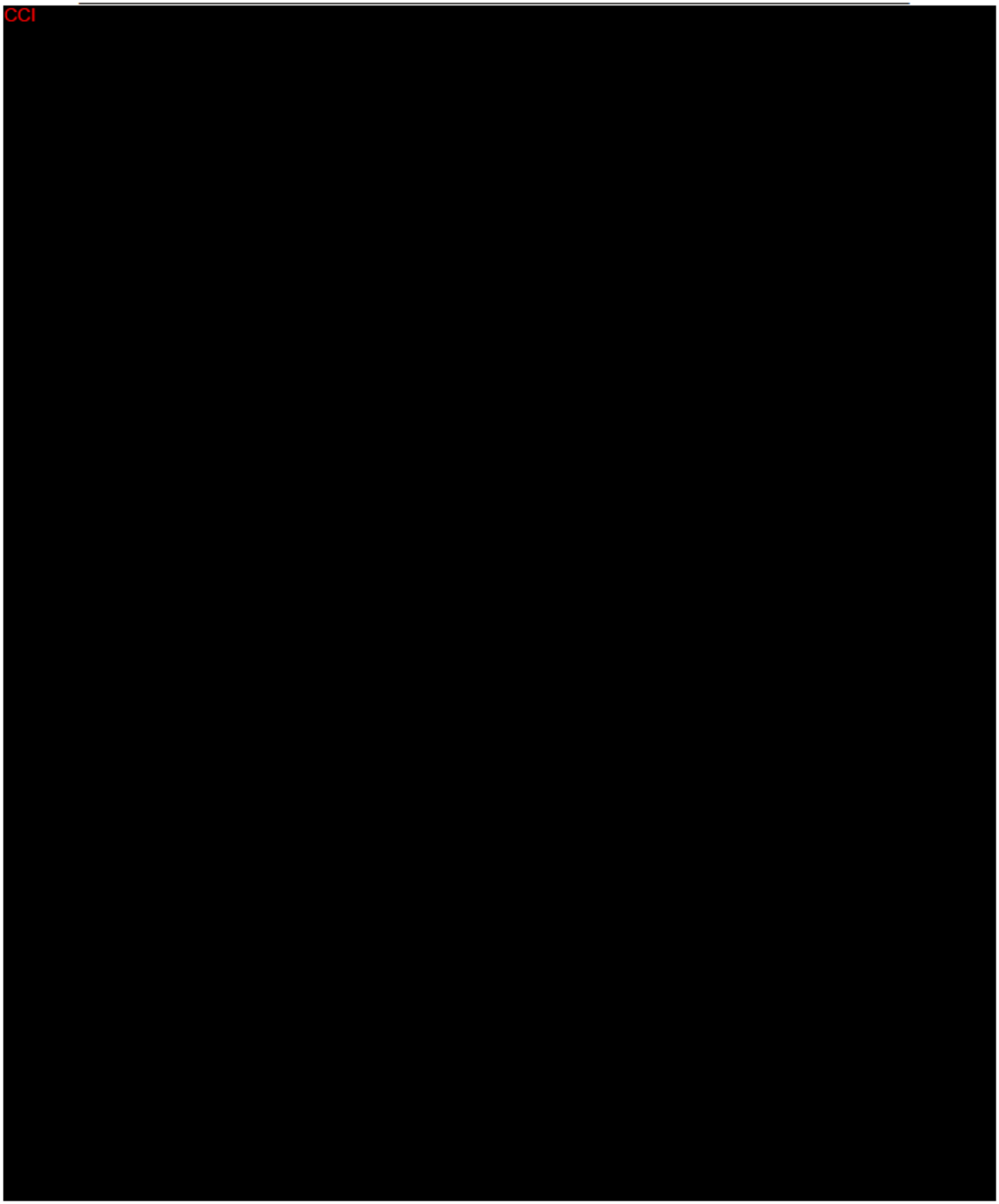

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