CLINICAL STUDY PROTOCOL

Study CRO-16-130 - Sponsor code CB-01-11/28

A Phase II, multicentre, randomised, double-blind, placebo controlled, proof of concept study of efficacy and safety of Rifamycin SV-MMX® 600 mg tablets administered three or two times daily to patients with diarrhoea-predominant irritable bowel syndrome (IBS-D)

Multicentre, randomised, double-blind, proof of concept, dose finding study

EudraCT Number: 2016-004977-42

Test formulation:	Rifamycin SV-MMX® 600 mg modified release tablets, manufactured by Cosmo S.p.A., Italy
Reference formulation:	Rifamycin SV-MMX® matching placebo tablets, manufactured by Cosmo S.p.A., Italy
Sponsor:	Cosmo Technologies Ltd., Riverside II, Sir John Rogerson's Quay, Dublin 2, Ireland Phone: +353.1.8170370 Fax: +353.1.8230718
International study co-ordinator:	Jan Tack, MD, PhD Professor of Medicine, Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven Head, Department of Clinical and Experimental Medicine, University of Leuven Head of Clinic, University Hospital Gasthuisberg, Department of Gastroenterology Herestraat 49, BE-3000 Leuven, Belgium Phone: +32-16-344225 Fax: +32-16-344419 Email: jan.tack@med.kuleuven.be
Development phase:	Phase II
Version and date:	Final version 3.0, 20FEB18

This study will be conducted in accordance with Good Clinical Practice (GCP), ICH topic E6(R2)

This document comprises 87 pages

CLINICAL STUDY PROTOCOL AMENDMENT HISTORY

CSP version N.	Reason for change
Final version 1.0, 23DEC16	1) Final version 1.0 of the CSP foresaw Visit 4 on Day 14, while the t.i.d. treatment will last 14 days including the whole day 14. In detail: ➢ **Treatment phase** ▪ Ambulatory – visit 2: week 0, day 1, baseline ▪ Ambulatory – visit 3: week 1, day 7±1 ▪ Ambulatory – visit 4: week 2, day 14±1 ➢ **Follow-up** ▪ Telephonic – follow-up 1: week 3, day 21±2 ▪ Ambulatory – visit 5: week 4, day 28±2 ▪ Telephonic – follow-up 2: week 5, day 35±2 ▪ Telephonic – follow-up 3: week 6, day 42±2 ▪ Telephonic – follow-up 4: week 7, day 49±2 ▪ Ambulatory – visit 6: week 8, day 56±2 ▪ Telephonic – follow-up 5: week 9, day 63±2 ▪ Telephonic – follow-up 6: week 10, day 70±2 ▪ Telephonic – follow-up 7: week 11, day 77±2 ➢ **Final visit/early termination visit (ETV)** ▪ Visit 7 (Final visit): week 12, day 84±2. In case of early discontinuation, discontinued subjects will undergo an early termination visit (ETV). 2) A colonoscopy was foreseen at the screening visit, i.e. from Day -14 to Day -8 in case of patients without colonoscopy in the last 5 years or 2 years, if patient's age >50.
Final version 2.0, 27FEB17	1) To complete all 14 days of treatment up to the evening of Day 14, the day of Visit 4 will be Day 15. To maintain 7-day intervals between subsequent visits, the day of Visits 3 to 7 and of all the Phone calls is rectified, as follows: ➢ **Treatment phase** ▪ Ambulatory – visit 2: week 0, day 1, baseline ▪ Ambulatory – visit 3: week 1, day **8**±1 ▪ Ambulatory – visit 4: week 2, day **15**±1 ➢ **Follow-up** ▪ Telephonic – follow-up 1: week 3, day **22**±2 ▪ Ambulatory – visit 5: week 4, day **29**±2 ▪ Telephonic – follow-up 2: week 5, day **36**±2 ▪ Telephonic – follow-up 3: week 6, day **43**±2 ▪ Telephonic – follow-up 4: week 7, day **50**±2 ▪ Ambulatory – visit 6: week 8, day **57**±2 ▪ Telephonic – follow-up 5: week 9, day **64**±2 ▪ Telephonic – follow-up 6: week 10, day **71**±2 ▪ Telephonic – follow-up 7: week 11, day **78**±2 ➢ **Final visit/early termination visit (ETV)** ▪ Visit 7 (Final visit): week 12, day **85**±2. In case of early discontinuation, discontinued subjects will undergo an early termination visit (ETV) 2) Since the bowel cleansing preparation and the colonoscopy may bias the baseline symptom data to be collected by the patients from Visit 1 to Day -1, the following changes are introduced to standardise the baseline data collection and to prevent any bias: ➢ the screening phase is extended to Day -21, ➢ the screening visit will be scheduled between Days -21 and -15, ➢ the colonoscopy, if needed, will be scheduled between Days -21 and -16, ➢ baseline data collected from Day -14 to Day -1 will be considered for the evaluation of inclusion criterion 4 at Visit 2 – Day 1. 3) Details of the co-ordinating site in Germany and of the CRO designated for monitoring and submissions in Belgium are added. 4) "Oral body temperature" is changed to "body temperature" to allow more measurement types since this parameter is not expected to vary critically during the study

Final version 3.0, 20FEB18	The aim of this substantial amendment is: 1) To replace the use of the electronic diary with the paper one. 2) To specify the permitted values of averages calculated for confirming the inclusion criterion 4 point b), when the values calculated is >4. 3) To specify the use the once-off medications related to the preparation or performance of the colonoscopy inside the study. 4) To amend the name of responsible person at ArisGlobal Ltd. (provider of EDC and randomisation system) 4) To integrate the Note to File 1 in the new protocol version. 5) To integrate the Amendment Nr. 01, Final version 1.0, 21JUL17 in the new protocol version. Sections affected: • Synopsis • Section 5.2, 5.3, 5.3.1 • Section 6.1.1 • Section 7.4.2, 7.4.3, 7.4.4, 7.4.5 • Section 7.6 • Section 8.1, 8.2 • Section 12.1 • Section 13.2 • Section 15.1 • Section 16.2.4.1 • Section 16.5

PROTOCOL APPROVAL

SPONSOR
Cosmo Technologies Ltd., Ireland

Sponsor Representative
Richard Jones

21 Feb 2018 — Date
Richard Jones — Signature

Medical Expert
Alessandro Mazzetti, MD, Chief Medical Officer

22 Feb 2018 — Date
A. Mazzetti — Signature

INTERNATIONAL STUDY CO-ORDINATOR

Principal Investigator Co-ordinator in Belgium

Jan Tack, MD, PhD
Professor of Medicine, Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven
Head, Department of Clinical and Experimental Medicine, University of Leuven
Head of Clinic, University Hospital Gasthuisberg, Department of Gastroenterology

21/2/18
Date

Signature

INVESTIGATORS

Principal Investigator
I have read this protocol and agree to conduct this study in accordance with all the stipulations of the protocol and in accordance with the Declaration of Helsinki.

Investigator's name and title: _____

Clinical Unit name and address: _____

_____

_____ _____

Date Signature

CRO for Co-ordination, Monitoring responsibility, Data Analysis and Reporting
CROSS Research S.A. and CROSS Metrics S.A. sister companies, Switzerland

Coordination
Diego Scanniffio, Clinical Project Leader

21 FEB 2018 _____ _____
Date Signature

Medical Writing
Andrea Di Stefano, Senior Medical Writer

26 FEB 2018 _____ _____
Date Signature

Biometry Unit Representative
Matteo Rossini, Biometry Manager, Unit Head

21 FEB 2018 _____ _____
Date Signature

Quality Assurance Unit Representative
Mario Corrado, Quality Assurance Manager, Unit Head

21 FEB 2018 _____ _____
Date Signature

Pharmacovigilance CRO
Zeincro Kft, Pharmacovigilance & Safety Department, Bulgaria, Sofia

Managing Director and Safety Manager
Sjoerd Miedema

23 Feb 2018 _____ _____
Date Signature

Provider of EDC and randomisation system
ArisGlobal Ltd., Dublin 2, Ireland

Project Manager
Eleni Vanden Eede

6 Mar 2018 _____ _____
Date Signature

STUDY SYNOPSIS

Title: A Phase II, multicentre, randomised, double-blind, placebo controlled, proof of concept study of efficacy and safety of Rifamycin SV-MMX® 600 mg tablets administered three or two times daily to patients with diarrhoea-predominant irritable bowel syndrome (IBS-D)

Protocol number: CRO-16-130 - Sponsor code CB-01-11/28 - EudraCT Number: 2016-004977-42

Clinical phase: Phase II

Study design: Multicentre, randomised, double-blind, proof of concept, dose finding study

Planned nr. of centres / countries: 25 centres/4 countries including Spain, Belgium, Italy and Germany.

International study co-ordinator: Jan Tack, MD, PhD, Professor of Medicine,
Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven
Head, Department of Clinical and Experimental Medicine, University of Leuven
Head of Clinic, University Hospital Gasthuisberg, Department of Gastroenterology
Herestraat 49, BE-3000 Leuven, Belgium

Investigational product:
TEST (T): Rifamycin SV-MMX® 600 mg modified release tablets, manufactured by Cosmo S.p.A., Italy
PLACEBO (P): Rifamycin SV-MMX® matching placebo tablets, manufactured by Cosmo S.p.A., Italy

Dose regimens:
The subjects will be randomly assigned (1:1:1) to a treatment group and will receive one of the following treatments for 14 consecutive days:

- Treatment group 1: dose regimen 1
  Rifamycin SV-MMX® 600 mg modified release tablets, three times daily (t.i.d.)
  Morning: one 600 mg tablet
  Afternoon: one 600 mg tablet
  Evening: one 600 mg tablet

- Treatment group 2: dose regimen 2
  Rifamycin SV-MMX® 600 mg modified release tablets, two times daily (b.i.d.) + matching placebo daily (q.d.)
  Morning. one 600 mg tablet
  Afternoon: one matching placebo tablet
  Evening: one 600 mg tablet

- Treatment group 3: matching placebo
  Rifamycin SV-MMX® matching placebo tablets, t.i.d.
  Morning. one matching placebo tablet
  Afternoon: one matching placebo tablet
  Evening: one matching placebo tablet

All the subjects will take the assigned tablets t.i.d., ideally at the following times: 07:30±2 h, 15:30±2 h and 23:30±2 h, for 14 days. The treatment weeks will be followed by ten (10) follow-up weeks.

Objectives:
- to compare two dose regimens of Rifamycin SV-MMX® 600 mg tablets versus matching placebo in terms of proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency;
- to evaluate the safety and tolerability of Rifamycin SV-MMX® 600 mg tablets, two and three times daily, in subjects with diarrhoea-predominant irritable bowel syndrome.

STUDY SYNOPSIS (cont.)

End-points:

Primary end-point:

- Proportion of weekly responders defined as subjects who weekly have relief of the composite of abdominal pain and stool consistency, on the basis of their daily assessments. Relief of abdominal pain is defined as a decrease in the weekly average of abdominal pain score of at least 30% compared with baseline and relief of stool consistency is defined as a 50% or greater reduction in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

Secondary end-points:

- Proportion of subjects with adequate relief of global IBS symptoms for at least 2 (consecutive or not) of the 10 weeks during the follow-up period (i.e., weeks 3 through 12). Adequate relief of global IBS symptoms is defined as a response of "yes" to the following question, which will be asked weekly (every 7 days):
  "In regard to all your symptoms of IBS, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptoms? [Yes/No]"
- Proportion of subjects with adequate relief of global IBS symptom during at least 2 weeks (consecutive or not) per month ("monthly response") during month 1, during month 1 through 2 and during month 1 through 3 will be assessed to identify the onset and duration of the therapeutic effect.
- Proportion of subjects with adequate relief of IBS-related bloating for at least 2 (consecutive or not) of the 10 weeks during the follow-up period (i.e., weeks 3 through 12). Adequate relief of bloating is defined as a response of "yes" to the following question, which will be asked weekly (every 7 days):
  "In regard to your symptom of bloating, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptom of bloating? [Yes/No]."
- Proportion of subjects with adequate relief of bloating during at least 2 weeks (consecutive or not) per month ("monthly response") during month 1, during month 1 through 2, and during month 1 through 3 will be assessed to identify the onset and duration of the therapeutic effect.
- Proportion of subjects with relief (weekly responders) determined from the subjects' daily assessments of IBS symptoms, bloating, and abdominal pain; relief of IBS symptoms and bloating is defined as a score of either 0 (not at all) or 1 (hardly) for at least 50% of the days in a given week or a score of 0 (not at all), 1 (hardly), or 2 (somewhat) for 100% of the days in a given week for at least 2 (consecutive or not) of the 4 weeks during a given month. Relief of abdominal pain is defined as a decrease by ≥30% from baseline in weekly mean rating of IBS-related abdominal pain.
- Number of weeks (consecutive or not) subjects achieve adequate relief of IBS symptoms during the follow up period.
- Number of weeks (consecutive or not) subjects achieve adequate relief of bloating during the follow up.
- Change from baseline to week 12 in daily IBS symptoms, bloating and abdominal pain.
- Proportion of monthly responders during month 1, during month 1 through 2 and during month 1 through 3 determined from the subjects' daily assessments of IBS symptoms, bloating, and abdominal pain; relief of IBS symptoms and bloating is defined as a score of either 0 (not at all) or 1 (hardly) for at least 50% of the days in a given month or a score of 0 (not at all), 1 (hardly), or 2 (somewhat) for 100% of the days in a given month. Relief of abdominal pain is defined as a decrease by ≥30% from baseline in weekly mean rating of IBS-related abdominal pain. Relief of stool consistency is defined as a 50% or greater reduction in the number of days per month with at least one stool that has a consistency of Type 6 or 7 compared with baseline.
- Change from baseline to each week during the 12 week follow up for daily IBS symptoms, bloating, abdominal pain, stool consistency and sense of urgency, asked as "Have you felt or experienced a sense of urgency today? [Yes/No]" and calculated as 100* (number of days with urgency/number of days with data), and daily number of stools.
- Change from baseline at weeks 4, 8 and 12 in quality of life inquired as IBS quality of life questionnaire (IBS-QoL)
- Monitoring of treatment emergent adverse events.
- Changes from baseline in physical examination, vital signs, and clinical laboratory tests and ECG.

Study variables:

Primary variable:

- Daily assessment of IBS related abdominal pain [11-point (i.e. 0 to 10) numeric rating scale]
- Daily assessment of stool consistency [7-point Bristol Stool Form Scale]

STUDY SYNOPSIS (cont.)

Secondary variables:
- Weekly assessment of global IBS symptoms [yes/no]
- Weekly assessment of IBS related bloating [yes/no]
- Daily assessment of global IBS symptoms (severity) [how bothersome?] [7-point scale]
- Daily assessment of IBS related bloating [7-point scale]
- Daily assessment of bowel movements [number]
- Daily assessment of stool urgency [yes/no]
- IBS quality of life questionnaire (IBS-QoL)

Safety and tolerability assessments: Treatment-emergent adverse events (TEAEs); vital signs (blood pressure, heart rate, body temperature, body weight), physical examinations; laboratory tests; electrocardiogram (ECG)

Sample size:
At least 342 subjects will be enrolled to have at least 106 subjects per treatment group included into the FAS.

Main selection criteria:
Inclusion criteria:
1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Sex and Age*: males/females, ≥18 year old
3. *IBS diagnosis*: confirmed IBS-D diagnosis per Rome IV criteria
4. *Symptoms*: active symptoms of IBS at baseline (day 1) as measured by average daily scores for at least 7 days before baseline during the period from Day -14 to Day -1:
   a. abdominal pain score ≥3 using an 11-point numeric rating scale and
   b. bloating score: 2-4* inclusive and
   c. stool consistency: score 6 or 7 (Bristol stool form scale) for at least 2 days from day -7 to day -1

   and by a negative response to the global IBS symptom assessment question and to the IBS-related bloating assessment question both given weekly during the screening phase up to day 1 before randomisation:
   d. "In the past 7 days, have you had adequate relief of your IBS symptoms?" [No] and
   e. "In the past 7 days, have you had adequate relief of your IBS symptom of bloating?"[No]
5. *Colonoscopy*: performed within 5 years; if patient's age >50, colonoscopy performed within 2 years
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the study
7. *Literacy:* sufficiently literate to comply with the study requirement of using and filling diaries
8. *Contraception and fertility*: females of childbearing potential and fertile males must be using at least one reliable method of contraception.

   Reliable methods of contraception for women include:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 2 months before the screening visit
   b. A non-hormonal intrauterine device [IUD] or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 2 months before the screening visit

   Reliable methods of contraception for men and male partners of female patients include:
   c. Male condoms with spermicide

   Reliable methods of contraception for both women and men include:
   d. A sterile sexual partner or sexual abstinence

   Women of non-childbearing potential or in post-menopausal status for at least 1 year and sterile or surgically sterilised men will be admitted.

   For women of childbearing potential, serum pregnancy test result must be negative at screening

**in case of an average value>4 , values ≤4.49 will be admitted*

Exclusion criteria:
1. *IBS*: symptoms of constipation at baseline evaluated during the period from Day -14 to Day -1:
   a. less than 3 bowel movements a week and
   b. stool consistency score ≤2 for ≥2 days in a week
2. *Screening phase:* failure to record the daily symptom assessments in the diary cards for at least 7 days before baseline

STUDY SYNOPSIS (cont.)

Main selection criteria (continued):

3. *Gastroenteric*: underlying gastrointestinal diseases including ulcerative colitis, Crohn's disease, pancreatitis, any active infectious, haemorrhagic or inflammatory disorder not related to IBS-D, gastrointestinal motility disorders such as ileus, gastroparesis or pseudoobstruction, gastroduodenal ulcer, gastrointestinal malignancy or potentially fatal diseases if not full in remission (5 years from diagnosis and without maintenance treatment), amyloidosis and cholelithiasis if cholecystectomy not performed
4. *Intolerance*: ascertained underlying lactose intolerance with response to diet or any other malabsorption syndrome with the exclusion of asymptomatic lactose malabsorption
5. *Coeliac disease*: ascertained or presumptive underlying coeliac disease
6. *Bile*: ascertained or presumptive bile acid malabsorption or bile acid induced diarrhoea
7. *Diabetes*: underlying diabetes type I or II
8. *Thyroid*: abnormal thyroid function not controlled by thyroid medications
9. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
10. *Renal function*: ascertained or presumptive clinically significant renal insufficiency or creatinine above twice the upper limit of normal (ULN) of the performing laboratory reference range
11. *Liver function*: chronic liver disease or clinically significant liver enzyme abnormality as evidenced by elevated AST, ALT or total bilirubin >1.5 times ULN
12. *AIDS/HIV*: ascertained or presumptive acquired immunodeficiency (AIDS) or known infection with human immunodeficiency virus (HIV)
13. *Diseases*: significant history of medical or surgical conditions excluding hysterectomy, caesarean section, appendectomy, cholecystectomy, benign polypectomy and inguinal hernia and including renal, hepatic, cardiovascular, haematological, endocrine, immune, psychiatric or neurological diseases that in the investigator's opinion may interfere with the aim of the study; malignant diseases not in remission for at least 5 years
14. *Medications*: alosetron, eluxadoline, ondansetron, tegaserod, lubiprostone, warfarin, antipsychotic, antispasmodic, prokinetic, antidiarrhoeal, laxative, probiotic, narcotic or antibiotic agents within 14 days before the screening visit; antidepressant agents of the selective serotonin-reuptake inhibitor and tricyclic classes unless taken at a stable dose for at least 6 weeks before the screening visit. Once-off medications related to the preparation or performance of the colonoscopy should be recorded as concomitant medications but are not exclusionary.
15. *Investigational drugs*: participation in the evaluation of any investigational product within 30 days before this study
16. *Drug and alcohol*: known history of drug or alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015] abuse
17. *Pregnancy (females only)*: pregnant or lactating women or wishing to become pregnant in the 3 months following this visit

STUDY SYNOPSIS (cont.)

Schedule:				
		Day	**Procedures/Assessments**	**Notes**
Screening phase	**Screening – visit 1**	*Between Day -21 and Day -15*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number ➢ Demographic data and life style recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (body weight, height, vital signs, physical abnormalities) ➢ ECG recording ➢ Laboratory analyses: haematology, blood chemistry, urinalysis and serum pregnancy test (females only) ➢ Rome IV classification ➢ Colonoscopy on days between -21 and -16 (only in case of patients without colonoscopy in the last 5 years or 2 years, if patient's age >50) ➢ Adverse event monitoring ➢ Inclusion/exclusion criteria evaluation ➢ Eligibility evaluation ➢ Subject's training on diary data entry ➢ Dispensation of the screening phase diary to the subject ➢ Supporting the subjects in filling in the diary	*Note: The subjects will use their diary cards to daily record their IBS symptoms and to report the occurrence of any change in physical or medical conditions as well as the intake of any concomitant treatment.*
	Baseline symptom data collection at home	*From Visit 1 to Day -1*	Throughout this period, the patients will daily record in their diary: ➢ Daily global IBS symptoms ➢ Daily IBS bloating ➢ Daily IBS abdominal pain ➢ Daily bowel movements ➢ Daily stool urgency ➢ Daily stool consistency ➢ Any change in physical or medical conditions ➢ Intake of any concomitant treatment Patients will weekly record in their diary: ➢ Relief of IBS symptoms ➢ Relief of IBS bloating	*Baseline data collected from Day -14 to Day -1 will be used to confirm the subject's eligibility on Day 1. Failure to daily record the IBS symptoms for at least 7 days before Visit 2, Day 1 will result in a screen failure*

STUDY SYNOPSIS (cont.)

Schedule (continued):				
		Day	**Procedures/Assessments**	**Notes**
Treatment phase	**Visit 2**	*Day 1*	➢ Collection of the screening phase patient diary ➢ AE and concomitant medications ➢ Physical examination ➢ Vital signs and body weight ➢ Inclusion/exclusion criteria evaluation; in particular inclusion criterion 4 ➢ Eligibility evaluation ➢ Enrolment and randomisation ➢ 1st study treatment supply ➢ 1st dose under supervision of the investigator or deputy ➢ Subject's retraining on diary data entry (if necessary) ➢ Dispensation of the treatment phase diary to the subject ➢ Patients will fill in the IBS-QOL in their diary	*Ambulatory visit.* *The investigator or his/her deputy will check that the subjects fill in correctly their diary and will check the data at each ambulatory visit.*
	Treatment phase procedures at home	*Weeks 1 and 2 (Days 1 to 15)*	Patients will daily record in their diary: ➢ Daily intake of the study treatment ➢ Daily global IBS symptoms ➢ Daily IBS bloating ➢ Daily IBS abdominal pain ➢ Daily bowel movements ➢ Daily stool urgency ➢ Daily stool consistency ➢ Any change in physical or medical conditions ➢ Intake of any concomitant treatment Patients will weekly record in their diary: ➢ Assessment of global IBS symptoms ➢ Assessment of IBS bloating	
	Visit 3	**Day 8±1**	➢ AE and concomitant medications ➢ Vital signs and body weight ➢ Drug accountability and compliance check ➢ Subjects' diary check ➢ 2nd study treatment supply ➢ Subject's retraining on diary data entry (if necessary)	*Ambulatory visit.* *The investigator or his/her deputy will check the diary data at each ambulatory visit.*
Treatment phase	**Visit 4**	**Day 15±1**	➢ Collection of the treatment phase patient diary ➢ AE and concomitant medications ➢ Drug accountability and compliance check ➢ Subjects' diary check ➢ Physical examination (physical abnormalities) ➢ Vital signs and body weight ➢ ECG recording ➢ Laboratory analyses: haematology, blood chemistry, urinalysis and serum pregnancy test (females only) ➢ Subject's retraining on diary data entry (if necessary) ➢ Dispensation of the follow-up phase diary to the subject ➢ Patients will fill in the IBS-QOL in their diary	*Ambulatory visit.* *The investigator or his/her deputy will check the diary data at each ambulatory visit.*

STUDY SYNOPSIS (cont.)

Schedule (continued):				
		Day	**Procedures/Assessments**	**Notes**
Follow up phase	**Follow-up phase procedures <u>at home</u>**	*Weeks 3 to 12 (Days 16 to 85)*	Patients will daily record in their diary: ➢ Daily global IBS symptoms ➢ Daily IBS bloating ➢ Daily IBS abdominal pain ➢ Daily bowel movements ➢ Daily stool urgency ➢ Daily stool consistency ➢ Any change in physical or medical conditions ➢ Intake of any concomitant treatment Patients will weekly record in their diary: ➢ Assessment of global IBS symptoms ➢ Assessment of IBS bloating	
	Phone call 1	*Day 22±2*	➢ Check that subjects correctly record the above mentioned data in their diary ➢ AE and concomitant medications	*The investigator or his/her deputy will call the subjects to verify that they are compliant and that the data are correctly recorded in the diary.*
	Visit 5	*Day 29±2*	➢ AE and concomitant medications ➢ Subjects' diary check ➢ Physical examination (physical abnormalities) ➢ Vital signs and body weight ➢ Subject's retraining on diary data entry (if necessary) ➢ Subjects will fill in the IBS-QOL in their diary	*Ambulatory visit.* *The investigator or his/her deputy will check the diary data at each ambulatory visit.*

STUDY SYNOPSIS (cont.)

Schedule (continued):				
		Day	**Procedures/Assessments**	**Notes**
Follow-up phase	**Phone calls 2-7**	*Days 36, 43, 50, 64, 71 and 78 (±2)*	➢ Check that subjects correctly record the data in their diary ➢ AE and concomitant medications	*The investigator or his/her deputy will call the subjects to verify that the data are correctly recorded in the diary and to check their response to the weekly global assessments.*
	Visit 6	*Day 57±2*	➢ As visit 5	*As visit 5*
	Visit 7 (Final Visit)/ETV	*Week 12, Day 85±2 / at ETV in case of discontinuation*	➢ Collection of the follow-up phase patient diary ➢ Subjects' diary check ➢ AE and concomitant medications ➢ Full physical examination (physical abnormalities) ➢ Vital signs and body weight ➢ ECG recording ➢ Laboratory analyses: haematology, blood chemistry, urinalysis and serum pregnancy test (females only) ➢ Subjects will fill in the IBS-QOL in their diary Subjects discontinuing from data collection or from both data collection and treatment (intervention) will be asked to undergo, as far as possible, an ETV. Subjects discontinuing the treatment (intervention) will be asked to continue the collection of the data according to the study schedule. In case of clinically significant results at the final visit, the subjects will be followed-up by the investigator until the normalisation of the concerned clinical parameter(s)	*Upon leaving, the subjects will be instructed to contact immediately the investigator in case of occurrence of any adverse reactions*

Data analysis:
All statistical processing will be performed using SAS® unless otherwise stated. Summary tables (descriptive statistics and/or frequency tables) will be provided for baseline variables, efficacy variables and safety variables. Continuous variables will be described by descriptive statistics (mean, SD, CV%, min, median and max). Frequency counts and percentage of subjects within each category will be provided for categorical data. Summaries will be provided for each treatment group.

Analysis Sets:
The intent-to-treat (ITT) set will include all randomized subjects. The full analysis set (FAS) will be a subset of the ITT set and will include all the subjects who take at least one dose of the study medication and have at least one post randomisation assessment of the primary endpoint. The per-protocol (PP) set will be a subset of the FAS and will include subjects who complete the study without any significant protocol deviations, have an acceptable compliance to the study medication and have all the evaluations of the primary endpoint. All subjects who receive at least one dose of the study medication will be used for the safety analyses and will be included in the Safety set. The analysis of efficacy will be conducted on the ITT set, FAS and PP set with the FAS considered as the primary set for statistical analysis. Missing data in the ITT set and FAS will be replaced using a multiple imputation (MI) under missing at random (MAR) assumption.

Efficacy Analyses:
Primary Efficacy Analyses
The proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects. Analysis centres will be defined by grouping the study centres according to geographic region in order to reach a minimum number of 12 subjects per treatment group per analysis centre included in the FAS.
Secondary Efficacy Analyses
All the proportions of subjects (see the definition of the secondary endpoints) will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects. Analysis centres will be defined by grouping the study centres according to geographic region in order to reach a minimum number of 12 subjects per treatment group per analysis centre included in the FAS.
Dosing Compliance
Descriptive statistics will be used to summarize test article compliance for the ITT set, FAS and PP set. Measures of test article compliance will include the duration of treatment, the total number of tablets taken and the number of days during which the subject was compliant.

Data analysis (continued):
Adverse Events
Adverse events will be coded using the MedDRA coding dictionary and summarized by relationship to test article and severity. The treatment groups will be compared with respect to the comparability of their AE profiles (severity and frequency).
Safety Laboratory Tests
Changes in haematology, clinical chemistry and urinalysis will be assessed using shift tables.
Physical Examination and Vital Signs
Descriptive statistics will be provided for the values and the change from Baseline of vital signs. Physical examination date and whether or not a physical exam was performed will be recorded.
Electrocardiography
ECGs will be evaluated for any material changes during the study period. HR, PR, QRS, QT data will be collected

Subjects starting to take antibiotics (other than the study medication) or taking more than two doses of a medication that was prohibited per the study protocol will be considered not to have had a response to treatment starting from the time the medication will be initiated, regardless of their response data.

Timing:
Submissions to ECs: MAY17; start of clinical phase: OCT17

STUDY SCHEDULE

Study period	Screening	Treatment			Follow-up									
Visit	**V1**	**V2**	**V3**	**V4**	**Phone1**	**V5**	**Phone2**	**Phone3**	**Phone4**	**V6**	**Phone5**	**Phone6**	**Phone7**	**V7 (Final visit) or ETV[6]**
Day	**Day -21/-15**	**Day 1**	**Day 8±1**	**Day 15±1**	**Day 22±2**	**Day 29±2**	**Day 36±2**	**Day 43±2**	**Day 50±2**	**Day 57±2**	**Day 64±2**	**Day 71±2**	**Day 78±2**	**Day 85±2**
Week	**-3**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Informed consent	x													
Demography	x													
Medical history and underlying disease[1]	x													
Physical abnormalities	x	x		x		x				x				x
Prior/concomitant treatments	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Body weight	x	x	x	x		x				x				x
Laboratory analysis[3]	x			x										x
Vital signs[4]	x	x	x	x		x				x				x
Pregnancy test [2]	x			x										x
12-lead ECG	x			x										x
Colonoscopy [5]	x													
Inclusion/Exclusion criteria	x	x												
Enrolment		x												
Randomisation		x												
IMP dispensation		x	x											
Study treatment		x	x											
Drug accountability			x	x										
Diary dispensation	x	x		x										
Collection of the previous patient diary		x		x										x
Stool consistency <u>at home</u>[7]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Weekly IBS symptoms[8] <u>at home</u>	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Daily IBS symptoms[9] <u>at home</u>	x	x	x	x	x	x	x	x	x	x	x	x	x	x
IBS-QOL[10]		x		x		x				x				x
AEs monitoring[11]	x	x	x	x	x	x	x	x	x	x	x	x	x	x

1. *IBS diagnosis per Rome IV criteria*
2. *Women of childbearing potential only - serum β-HCG test*
3. *Haematology, blood chemistry and urine analysis*
4. *Blood pressure, heart rate and body temperature*
5. *Only in case of patients without colonoscopy in the last 5 years or 2 years, if patient's age >50, to be performed between Day -21 and -16*
6. *Early termination visit (ETV) in case of premature discontinuation*
7. *Daily score in the patient's diary according to the 7-point Bristol stool form scale*
8. *Weekly in the diary*
9. *Daily in the diary*
10. *In the diary*
11. *AEs monitored starting at the screening visit, immediately after informed consent, up to the final visit/ETV*

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LIST OF ABBREVIATIONS

β-HCG	human chorionic gonadotropin β
γ-GT	γ-Glutamyl transpeptidase
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
AUC_{0-t}	Area under the concentration-time curve from time zero to time t
$AUC_{0-\infty}$	Area under the concentration vs. time curve up to infinity
BA	Bioavailability
BE	Bioequivalence
BLQL	Below Lower Quantification Limit
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
C_{max}	Peak drug concentration
CMS	Clinical Medical Service
CPL	Clinical Project Leader
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CSP	Clinical Study Protocol
CRS	Clinical Study Report
CS	Clinically Significant
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
ETV	Early Termination Visit
FDA	Food and Drug Administration
F_{rel}	Relative Bioavailability
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBs Ag	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibodies
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IB	Investigator's Brochure
IBS	Irritable bowel syndrome
IBS-C	Constipation predominant irritable bowel syndrome
IBS-D	Diarrhoea predominant irritable bowel syndrome
IBS QoL	Irritable bowel syndrome quality of life questionnaire
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
IV	Intravenous
LQL	Lower Quantification Limit
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
MCH	Mean Cell Haemoglobin

MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean Residence Time
MW	Molecular Weight
N	Normal
NA	Not Applicable
NC	Not calculated
NCS	Not clinically significant
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over The Counter
PD	Pharmacodynamics
PE	Point Estimate
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
R	Reference
RBC	Red Blood Cells
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Test
TEAE	Treatment-Emergent Adverse Event
THC	delta-9-tetrahydrocannabinol
TLUS	Time to last unformed stool
$t_{1/2}$	Half-life
T_{max}	Time to achieve C_{max}
USDA	United States Department of Agriculture
WBC	White Blood Cells
WHODDE	World Health Organisation Drug Dictionary Enhanced

1 INTRODUCTION

1.1 Background

Irritable bowel syndrome (IBS), initially described as "mucous colitis" due to the excretion of excess mucus (1, 2), is today defined as a functional gastrointestinal disorder characterised by abdominal pain and altered bowel habits in the absence of structural or biochemical abnormalities (3). Other clinical manifestations may include bloating, fatigue, psychological disorders including depression or anxiety and other chronic pain disorders, i.e. headache, back and low back pain, myalgia or soreness (4). IBS is associated with a disturbed bowel function: either constipation or diarrhoea (5, 6), then it is further classified in diarrhoea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), IBS alternating between constipation and diarrhoea (IBS-M) and unsubtyped IBD on the basis of stool consistency (6). IBS is one of most common functional bowel diseases affecting up to 15% of the population, with women accounting for 70–75% of this group. The criteria used to diagnose IBS are the presence of symptoms that meet the Rome IV criteria (7). Due to the lack of specific diagnostic tests, up to date, IBS is considered a diagnosis of exclusion (2).
An increasing amount of data suggests that IBS is commonly associated with small intestinal bacterial overgrowth (SIBO) and that a bacterial overgrowth may be part of IBS pathogenesis (8, 9, 10).

1.1.1 Small intestinal bacterial overgrowth (SIBO) and functional gastrointestinal disorders (FGID)

Under normal conditions, the bacterial concentration in the small intestine is not higher than 10^2 colony forming units (CFU) per mL (11). A SIBO is defined as a finding of coliform bacteria $\geq 1x10^3$ CFU/mL of proximal jejunal aspirate (8). This normal limit has been redefined after suspicion that the former limit ($\geq 1x10^5$) was too high (12, 13) and circumscribed by one pathological condition: the stagnant loop syndrome.
In SIBO, Gram-negative aerobes and anaerobic species that ferment carbohydrates into gas predominate (8, 14, 15). However, the small intestinal microbiota are relatively inaccessible to the standard culture techniques. Indeed, the culture technique studies are in their infancy. However, the diagnosis of SIBO by breath tests is not yet fully validated (13, 16, 17). Gram-positive aerobes are known to predominate proximally, while Gram-negative and Gram-positive anaerobes and facultative anaerobes predominate in the terminal ileum (16). Functional gastrointestinal disorders (FGID) are differentiated through their peculiar chronic or recurrent gastrointestinal symptoms which are used as diagnostic criteria. Among these, IBS commonly implies a poor health-related quality of life (16, 18, 19) and substantial costs to society (16, 20, 21, 22). Several evidences indicate that bacteria are involved in the pathogenesis of IBS through the metabolic capacity of the luminal microbiota and the potential of the mucosa-associated microbiota to influence the host via immune-microbial interactions (16, 23). Indeed, the prevalence of SIBO in IBS varies from 30% to 85% according to the source (13, 15, 24, 25, 26). The current hypothesis is that the expansion of bacteria into the small intestine from the large intestine leads to symptoms including bloating, abdominal discomfort and changes in stool form (15, 27). It is hypothesised that an intestinal stasis, following either mechanical or physiological or pathological reasons, gives rise to the proliferation of coliforms in the small intestine and, subsequently, to IBS. Hypothetical mechanisms of diarrhoea caused by SIBO are: 1) bacteria digest carbohydrates generating gas

and osmotically active by-products that promote osmotic diarrhoea, 2) bacteria and fatty acid by-products injure the mucosa, 3) mucosal injuries yield lactase deficiency and 4) bacterial deconjugation of bile salts interferes with fat absorption of fat soluble vitamins (15). The hypothesis matches the observation that many patients with IBS report symptom onset after an enteric infection (28). An infective gastroenteritis, which precedes IBS onset, produces a profound depletion of the commensal microbiota (29) whose production of short chain fatty acids and antibiotics normally inhibits pathogen colonisation (30). The frequency of gastroenteritis in UK is 19% a year (31). Commonest bacterial foci found in infectious gastroenteritis are *Campylobacter* (10%) and *Salmonella* (3%). The frequency of onset of IBS after an episode of gastroenteritis varies from 6% to 18% according to the authors (31, 32). The predominant IBS symptom after a gastroenteric infection is diarrhoea. A meta-analysis of the risk of acquiring IBS after a bacterial gastroenteritis shows that the syndrome develops one year after the gastroenteritis (34). Strongest risk factors of a post-infectious IBS are bacterial toxicity (35), prolonged diarrhoea (36), rectal bleeding (37) and fever (34). During an acute enteritis, changes between intestinal microbiota and host defence factors occur which may be important for IBS pathophysiology. Substantially, an acute gastroenteritis implies little changes in aerobes, a fall in anaerobes and a rise in pathogens (16). The fall in anaerobes contributes to the diarrhoeic phenotype of IBS.

1.1.2 Non-absorbable antibiotics and IBS-D treatments

The therapeutic agents currently approved in case of IBS-D include: alosetron, indicated only in women with severe, chronic IBS-D, eluxadoline and rifaximin. Ramosetron is approved for the treatment of men with IBS-D in Japan (38). If it is true that a previous antibiotic treatment may be related to the development of IBS (38, 40) and may increase long term digestive symptoms after bacterial gastroenteritis (41), poorly absorbable antibiotics may have therapeutic potential in IBS (24). Neomycin was the original choice, but interest is now focused on non-absorbable antibiotics like rifaximin (42). Three fully published, double blind, placebo controlled trials of rifaximin have proven its efficacy in the treatment of IBS (43, 44, 45). The therapeutic advantage over placebo was around 10% with doses ranging between 600 and 2400 mg daily for 7-14 days (46, 47, 48, 49, 50, 51). Meanwhile, rifaximin proved to be effective in eradicating bacterial overgrowth in up to 70-80% of SIBO patients (52, 53, 54). In a recent study which comprised 106 patients with IBS who were positive at the lactose breath test, treatment with rifaximin showed a significant improvement in bloating, flatulence, diarrhoea and pain (55). FDA approved rifaximin (Xifaxan) for the treatment for IBS-D in May 2015 after a prospective controlled clinical trial evaluating the efficacy of rifaximin for re-treatment of recurrent symptoms in IBS was concluded (38, 56).

The possibility to administer non-absorbable antibiotics in a new oral formulation designed to deliver the substances directly into the intestinal lumen, offers consistent advantages over the existing used formulations, improving the drug efficacy due to direct topical delivery.

1.2 The product: Rifamycin SV-MMX® 600 mg tablets or CB-01-11

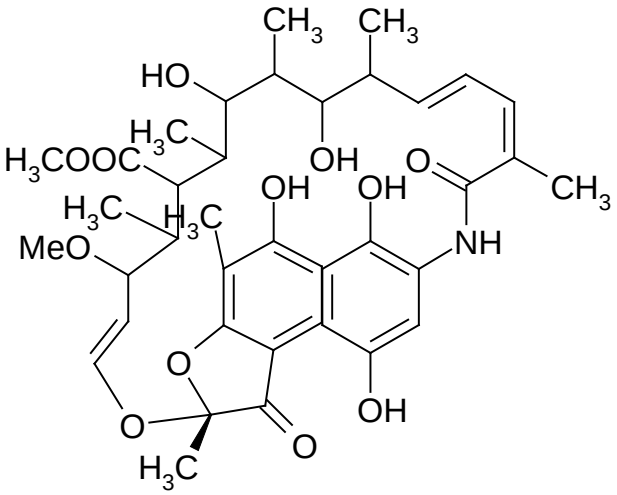
Rifamycin SV-MMX® 600 mg tablets codified as CB-01-11 are a new oral modified release formulation, in the form of coated tablets, containing sodium rifamycin SV. The tablets are formulated using a patented multimatrix structure (MMX®), which allows the delivery of the active ingredient when a pH of at least 6 is met in the gut lumen. With this technology, the

maximum local bioavailability of the active ingredient is achieved in the intestinal lumen and, consequently, the biological effect is optimised (56). Active pharmaceutical ingredient and excipients employed in the manufacturing of Rifamycin SV-MMX® 600 mg tablets are well known in the pharmaceutical field for their selective functionality and are described in compendia texts.

1.3 Rifamycin SV

Rifamycin SV is an antibiotic belonging to the class of ansamycins, obtained by chemical transformation of rifamycin B, which is produced in the fermentation broth of *Streptomyces mediterranei n. sp.* (57).

Figure 1.3.1 Rifamycin SV chemical structure



The substance is endowed with broad-spectrum of activity against Gram-positive, Gram-negative microorganisms, and mycobacteria (57). Its activity is predominantly bactericidal by interference with bacterial respiration and protein synthesis. Rifamycin SV inhibited 86.7% of *Clostridium difficile* strains and was the most active agent tested against this species (minimal inhibitory concentration MIC_{50}, 0.03 µg/mL; four-fold more potent than rifaximin and 16-fold more potent than vancomycin). Rifamycin SV inhibited all tested pathogenic strains of *Escherichia coli* (enterohaemorrhagic, enterotoxigenic ETEC, enteropathogenic and enteroaggregative EAEC strains) with $MIC_{50/90}$=32-120 µg/mL (58). No cross-resistance is observed between rifamycin SV and other antibiotics. Oral administration of rifamycin SV to animals and to normal subjects did not result in appreciable blood serum levels (57). This makes the drug an ideal agent for the topical treatment of diseases within the gastrointestinal tract.
Rifamycin SV, as sodium salt, is marketed in some countries, among which Italy, Belgium and Ukraine, as solution only for parenteral or local use.

1.4 Dosage form

Rifamycin SV-MMX® 600 mg is presented as an enteric coated, modified release tablet containing sodium rifamycin SV.

1.5 Clinical indications and dose regimen

Rifamycin SV-MMX® 600 mg tablets are proposed for the therapy of diarrhoea-predominant IBS. Considerations on the planned dose regimen of the product are given § 4.2.

1.6 Preclinical experience

Sodium rifamycin SV was investigated in many experiments *in vitro* and *in vivo* that demonstrated its efficacy against bacteria responsible for infections of skin, bone, respiratory tract, biliary tree, and septicaemia (57).

1.7 Human experience

1.7.1 Phase I trial

An oral bioavailability study of Rifamycin SV-MMX® 200 mg tablets after single (200 mg) and multiple (200 mg x 3) administration to male and female healthy volunteers was performed. Rifamycin SV was well tolerated and plasma concentrations were below the lower quantification limit (BLQL≤200 ng/mL), indicating the absence of or a negligible (<1%) systemic availability (59).

1.7.2 Phase I absolute bioavailability study

The pharmacokinetic (PK) profile of systemically available rifamycin SV was investigated in 24 healthy male and female subjects in plasma and in urine after single oral dose of two Rifamycin SV-MMX® 200 mg tablets and after a single i.v. injection of 250 mg of rifamycin SV (as Rifocine®). Faecal elimination of rifamycin SV was investigated after single oral dose of two Rifamycin SV-MMX® 200 mg tablets administered under fasting conditions.
Plasma concentrations >2 ng/mL were infrequently and randomly quantifiable after single oral dose under either conditions. The systemic exposure to rifamycin SV of subjects who received single oral doses of 400 mg of Rifamycin SV-MMX® under fasting and fed conditions were considered as negligible. The drug was confirmed to be systemically unabsorbed. The amount of systemically absorbed antibiotic, which is excreted by the renal route, was not higher than 0.0010% of the single oral dose administered under fasting conditions and not higher than 0.0004% when administered under fed conditions in the 24 h of observation. The total amount of elimination with faeces (ΣXf) was on average 331.6±6.9 mg corresponding to 82.9% of the administered dose.
No significant effect of Rifamycin SV-MMX® 200 mg tablets on vital signs, electrocardiograms (ECGs) or laboratory parameters was observed (60, 61).

1.7.3 Phase I multiple dose pharmacokinetic study

The PK profile of systemically available rifamycin SV was investigated in 24 healthy male and female subjects in plasma and in urine on Day 4 up to 12 h after the last of 12 doses of 200 mg of Rifamycin SV-MMX® 200 mg tablets q.i.d. or the last of 6 doses of 400 mg of Rifamycin SV-MMX® 200 mg tablets twice a day (b.i.d.) (62).
Plasma concentrations >2 ng/mL were infrequently and randomly quantifiable after the last dose of the 3-day treatment (Table 1.7.3.1).

Table 1.7.3.1 Mean rifamycin SV plasma concentration (ng/mL) measured on Day 4 after last of 12 doses of 200 mg Rifamycin SV-MMX® tablets administered q.i.d. and after last of 6 doses of 400 mg Rifamycin SV-MMX® tablets administered b.i.d.

Time	Mean rifamycin SV plasma concentrations (ng/mL) ±SD	
	200 mg Rifamycin SV-MMX® tablets q.i.d.	400 mg Rifamycin SV-MMX® tablets b.i.d.
	N=12	N=12
Pre-dose	BLQL	1.41±2.49**
1 h	BLQL	0.40±0.93°°
2 h	BLQL	BLQL
3 h	BLQL	BLQL
4 h	BLQL	0.25±0.87°
5 h	BLQL	0.80±1.20**
6 h	0.75±1.39*	1.57±2.71**
8 h	BLQL	0.77±1.94°°
10 h	BLQL	0.44±1.52°
12 h	BLQL	0.87±1.58*

BLQL: below the lower quantification limit (2 ng/mL)
°: one quantifiable value and 11 BLQL values;
°°: 2 quantifiable values and 10 BLQL values;
*: 3 quantifiable values and 9 BLQL values;
**: 4 quantifiable values and 8 BLQL values;

The systemic exposure to rifamycin SV in subjects treated for 3 days with a daily dose of 800 mg of Rifamycin SV-MMX® 200 mg tablets subdivided into the two dose regimens was negligible. With both oral regimens, the drug was confirmed to be systemically unabsorbed. The amount of systemically absorbed antibiotic, which is excreted by the renal route (Table 1.7.3.2), was not higher than 0.0036% of the administered dose in the 12 h of observation after the last dose.

Table 1.7.3.2 Mean ± SD of main urinary rifamycin SV PK parameters measured and calculated on Day 4 after last of 12 doses of 200 mg Rifamycin SV-MMX® tablets administered q.i.d. and after last of 6 doses of 400 mg Rifamycin SV-MMX® tablets administered b.i.d. (N=12)

Treatment		Xu (ng)	Xu (% of dose)	dXu/dt (ng/h)	ΣXu (ng)	ΣXu (% of dose)
200 mg Rifamycin SV-MMX® tablets q.i.d.	**0-3 h**	1873.9±1405.8	0.0009±0.0007	624.6±468.6	7172.7±4180.0	0.0036±0.0021
	3-6 h	1611.1±931.2	0.0008±0.0005	537.0±310.4		
	6-12 h	3687.7±2377.6	0.0018±0.0012	614.6±396.3		
400 mg Rifamycin SV-MMX® tablets b.i.d.	**0-3 h**	3216.9±2437.2	0.0008±0.0006	1072.3±812.4	10914.5±5309.3	0.0027±0.0013
	3-6 h	2713.3±1249.5	0.0007±0.0003	904.4±416.5		
	6-12 h	4984.3±3029.6	0.0012±0.0008	830.7±504.9		

No significant effect of Rifamycin SV-MMX® 200 mg tablets on vital signs, ECGs or laboratory parameters was observed (61, 62).

1.7.4 Phase I single and multiple dose pharmacokinetic study

The PK profile and the safety of systemically available rifamycin SV after single and multiple t.i.d. doses of Rifamycin SV-MMX® 600 mg tablets were investigated in 18 healthy male and female subjects (63). The subjects were exposed to a daily dose of 1800 mg of rifamycin SV for 14 consecutive days. Main PK parameters of plasma rifamycin SV on Days 1 and 7 of the treatment with the IMP are presented in the following table for both days.

Table 1.7.4.1 PK parameters of plasma rifamycin SV measured and calculated on Days 1 and 7 of the treatment with test IMP; mean±SD is reported (N=18)

Parameter	Unit	Day 1	Parameter	Unit	Day 7
$C_{max,0-6}$	ng/mL	2.19±1.94	$C_{max,ss12-18}$	ng/mL	2.90±1.73
$t_{max,0-6}$	h	6.00 (4.00-6.00)	$t_{max,ss12-18}$	h	12.00 (12.00-18.00)
AUC_{0-6}	ng/mLxh	3.26±2.85	$C_{min,ss12-18}$	ng/mL	1.55±0.39
$C_{max,0-24}$	ng/mL	5.79±4.24	$C_{average,12-18}$	ng/mL	2.02±0.66
$t_{max,0-24}$	h	9.00 (4.00-24.00)	$AUC_{ss,12-18}$	ng/mLxh	12.15±3.95
AUC_{0-24}	ng/mLxh	43.67±20.15	$PTF\%_{12-18}$	%	59.70±42.48
			$AUC_{ss,0-24}$	ng/mLxh	80.08±34.09

mean±SD is reported except for t_{max} for which median (range) is shown;

Main PK parameters of urine rifamycin SV on Days 1 and 7 of the treatment with the IMP are presented in the following table for both days.

Table 1.7.4.2 Total amount of rifamycin SV excreted in urine calculated on Days 1 and 7 of the treatment with test IMP; mean±SD is reported (N=18)

Parameter	Unit	Day 1	Parameter	Unit	Day 7
Ae_{0-24}	ng	23076±14328	Ae_{0-24}	ng	52036±22631
$\%Ae_{0-24}$	% of dose	0.0013±0.0008	$\%Ae_{0-24}$	% of dose	0.0029±0.0013

mean±SD is reported;

After 7 days of t.i.d. treatment, the absorption of rifamycin SV slightly increased in rate (C_{max}) and extent (AUC), while it did not show any remarkable sign of accumulation. The systemic exposure to rifamycin SV measured in the present study is to be considered as negligible, similarly to the conclusions of the previous studies. The amount of rifamycin SV excreted in urine at the steady state (approximately 52000 ng) corrected by the bioavailability data obtained in a previous trial after intravenous administration (60, 61) accounts for less than 0.15% of the oral 1800 mg daily dose.

1.7.5 Phase II dose finding trial

A phase II dose-finding study was performed in Mexico and Turkey in 40 patients, randomised in three parallel groups, affected by acute infectious diarrhoea. The patients were treated with Rifamycin SV-MMX® 200 mg tablets at the daily doses of 400 mg (one tablet in the morning and evening) or 800 mg (two tablets in the morning and evening) or 1200 mg (two tablets in the morning, afternoon and evening) for up to 7 days. Safety and clinical efficacy were evaluated. Primary clinical end-point was the time elapsed from the ingestion of the 1st dose of medication to the passage of the last unformed stool (TLUS). The intent-to-treat (ITT) population included 37 subjects, and the per protocol (PP) population included 34

subjects. The overall median TLUS was 46.8 (ITT) and 48.1 (PP) h; no significant differences were seen among the three dose groups, even though the regimen of 800 mg administered in two refracted doses was the most effective one (TLUS 36.7 h in ITT population, and 39.0 h in PP population). All tested doses were well tolerated (64).

1.7.6 Phase II efficacy trial

A phase II exploratory study to evaluate the efficacy and safety of Rifamycin SV-MMX® 200 mg tablets vs. the marketed reference rifaximin 200 mg tablets (Normix®, Alfa-Wassermann) in the treatment of infectious diarrhoea was performed in 8 clinical sites located in South Africa. Both treatments were administered four times a day (q.i.d.) for three consecutive days. Primary end-point was to demonstrate the non-inferiority (therapeutic bioequivalence) of Rifamycin SV-MMX® 200 mg tablets vs. Normix® based upon the TLUS in compliance with the relevant EMA guideline CPMP/EWP/482/99 (65). A total of 120 patients were screened. All 115 randomised subjects were grouped in the safety analysis set and were considered in the safety analysis. The ITT set included 99 subjects. The PP analysis set included 78 subjects. The efficacy analysis was performed on both ITT and PP sets.
Rifamycin SV-MMX® 200 mg tablets demonstrated an efficacy non-inferior to that of Normix® rifaximin 200 mg tablets. The median TLUS of the PP analysis set receiving Rifamycin SV-MMX® was 67.71 h and the median TLUS of the PP analysis set treated with rifaximin was 60.33 h. The two treatments did not differ in terms of rate and frequency of therapeutic success. The antimicrobial action was similar after both treatments as shown by the microbiological assays in stools.
Safety and tolerability of the two treatments did not differ significantly and laboratory assays did not show evidence of any untoward effect (66, 67).

1.7.7 Phase III efficacy studies in patients with travellers' diarrhoea

In a Phase III multicentre, randomised, double-blind, placebo-controlled efficacy and safety study patients who were travelling to developing regions with a known high incidence of travellers' diarrhoea were enrolled. A total of 264 patients were enrolled at eight sites in Mexico and Guatemala and randomized 3:1 to receive Rifamycin SV-MMX® (N=199) or placebo (N=65). Rifamycin SV-MMX® 400 mg tablets were administered b.i.d. for 3 days. Fifty-three (53) (81.5%) placebo recipients and 178 (89.4%) patients receiving Rifamycin SV-MMX® completed the study. The TLUS was significantly shortened in the overall group of patients treated with rifamycin SV (median TLUS of 46 h vs. 68 h; p-value=0.0008) and in the subgroups of patients who had isolates of *E. coli* (median TLUS of 49.3 h vs. 68.3 h; p-value=0.0035) or invasive pathogens identified in baseline stools. The overall frequency and severity of adverse events (AEs) did not increase in patients receiving Rifamycin SV-MMX® compared with placebo (68).

A second multi-centre, randomised, double-blind, non-inferiority Phase III study in travellers' diarrhoea (RIT-1/AID) investigated the efficacy and safety of Rifamycin SV MMX®, 400 mg tablets (two tablets of 200 mg each) taken b.i.d. (800 mg total daily dose) for three days in the treatment of subjects with travellers' diarrhoea in comparison with ciprofloxacin. More than 835 patients were enrolled and the recruitment has completed (69). The clinical study report is not yet available. The primary study end-point was achieved.

1.8 Safety considerations

1.8.1 Pre-clinical toxicity studies

Investigations on various animal species showed a very low toxicity of sodium rifamycin SV when administered by oral and parenteral route, so that its therapeutic index can be considered remarkably good (57). Also in pregnant animals the product did not produce abnormalities (70, 71). The acute toxicity is very low (oral toxicity: DL_{50} 2120 mg/kg in mouse, 2600 mg/kg in rat; subcutaneous toxicity: DL_{50} 1080 mg/kg in mouse, 1200 mg/kg in rat; intraperitoneal toxicity: DL_{50} 625 mg/kg in mouse, 480 mg/kg in rat). Chronic toxicity in rats treated orally for 168 days at daily doses up to 500 mg/kg did not show any significant changes in the body weight, weight of main internal organs, and haematology.
The potential to exert adverse effects is highly dependent on the systemic exposure to rifamycin SV. As could be expected from the toxicokinetic data demonstrating virtually no absorption, the study of Rifamycin SV-MMX® over 28 days in dogs did not result in changes of toxicological relevance up to the highest dose of 1800 mg. The NOAEL (164 mg/kg b.w.) was ≥5-fold above the daily doses intended to be used in patients (800-1800 mg corresponding to 13-30 mg/kg of body weight [BW]).

1.8.2 Safety in humans

In both Phase I pharmacokinetics studies (60, 61, 62), treatment emergent adverse events (TEAEs) occurred at a frequency of 16.7% of the subjects receiving Rifamycin SV-MMX® tablets. None of the reported events was related to the treatment.
TEAEs that occurred in the 2 Phase II studies (64, 66, 67) are summarised in the following table.

Table 1.8.2.1 Frequency of TEAEs which occurred to subjects receiving Rifamycin SV-MMX® (Studies CB-01-11/02 and CB-01-11/03) (N=94)

MedDRA Description	**Rifamycin SV-MMX® tablets**
	n (%)
Gastrointestinal Disorders	**12 (12.8)**
Diarrhoea	1 (1.0)
Diarrhoea aggravated	1 (1.0)
Constipation	3 (3.2)
Dry mouth	1 (1.0)
Abdominal distension	1 (1.0)
Abdominal cramps	1 (1.0)
Tenderness	1 (1.0)
Abdominal pain after medication	1 (1.0)
Flatulence	1 (1.0)
Gastritis	1 (1.0)
Investigations	**1 (1.0)**
ALT increased	1 (1.0)
General Disorders	**1 (1.0)**
Fever	1 (1.0)
Skin and subcutaneous tissue Disorders	**1 (1.0)**
Hyperaemia on the neck	1 (1.0)

Source: (64, 66, 67).

In the first Phase III study, TEAEs occurred at a frequency of 29.6% (59 out of 199 patients receiving Rifamycin SV-MMX® tablets). Most frequent TEAEs are summarised in the following table.

Table 1.8.2.2 TEAEs occurred at a frequency ≥2.0% in either treatment group by SOC and Preferred Term (Safety set of study C2009-0201)

MedDRA System Organ Class Preferred Term	Placebo (N=65) n (%)	Rifamycin MMX (N=199) n (%)	Total (N=264) n (%)
Infections and infestations	10 (15.4)	17 (8.5)	27 (10.2)
Amoebic dysentery	2 (3.1)	0 (0.0)	2 (0.8)
Diarrhoea infectious	5 (7.7)	10 (5.0)	15 (5.7)
Gastrointestinal infection	2 (3.1)	0 (0.0)	2 (0.8)
Nervous system disorders	7 (10.8)	19 (9.5)	26 (9.8)
Headache	6 (9.2)	17 (8.5)	23 (8.7)
Gastrointestinal disorders	9 (13.8)	19 (9.5)	28 (10.6)
Constipation	1 (1.5)	7 (3.5)	8 (3.0)
Diarrhoea	6 (9.2)	10 (5.0)	16 (6.1)

Source: (68)
MedDRA version 14.1. The denominator for calculating percentages is the number of patients in the Safety Set. The number and percentage of patients reporting at least one occurrence of an AE for each unique System Organ Class and Preferred Term are tabulated. At each level of summation (Overall, System Organ Class, Preferred Term) patients are counted only once.

In the second Phase III study, TEAEs had a frequency of 12.4% among the subjects receiving rifamycin SV. One subject discontinued the study due to an AE (0.2%) (69).

In the recent Phase I single and multiple dose pharmacokinetic study of Rifamycin SV-MMX® 600 mg tablets, the IMP showed an excellent safety profile (63). Overall, treatment emergent adverse events (TEAEs) occurred at a frequency of 33.3% during the 14 treatment days. The investigator judged 2 of the 9 reported TEAEs as related to treatment. The most frequent TEAE was headache (3 episodes), whose overall frequency was 16.7%. No significant change in vital signs, body weight, ECGs or laboratory parameters was observed after treatment with rifamycin SV for 14 consecutive days. No significant change in liver or kidney functions was observed during the 14 day treatment.

Other known adverse reactions reported for the parenteral use of sodium rifamycin SV include (70): hypersensitivity reactions (cutaneous rash, urticaria, itching, eosinophilia, shock, Quinke's oedema, asthma) and gastrointestinal and hepatic reactions (nausea, vomiting, jaundice, increase of transaminases and bilirubin).

1.9 Hypersensitivity

As for similar ansamycins a hypersensitivity to rifamycin SV cannot be excluded. Rifamycin SV should not be administered to patients with intestinal obstruction, or severe intestinal ulcerative lesions. Precautions should be taken in the presence of diarrhoea complicated by fever and/or blood in the stool, or in worsening diarrhoea persisting for greater than 24 to 48 h.

1.10 Rationale

The present trial aims at investigating the efficacy of Rifamycin SV-MMX® 600 mg modified release tablets administered t.i.d. or b.i.d to patients suffering from IBS-D for 14 consecutive days. The present proof of concept trial is designed to preliminarily investigate the efficacy of rifamycin SV in the indicated pathology versus matching placebo. Moreover, the efficacy will be also compared between two different rifamycin SV dose regimens.

1.11 Risk and benefits

Rifamycin SV is administered for the first time to patients suffering from IBS-D with the objective of investigating its efficacy and safety in this population. The participants in the current study may receive some benefit from the study treatment in as much as they may experience a relief of IBS-D symptoms during the study.

In the present study, the participants will take a maximum of 48 doses of sodium rifamycin SV in a window of 16 days. The risk of adverse drug reactions is expected to be very low on the basis of the data collected up to date (§ 1.8).

2 STUDY OBJECTIVES

The objective of the study is to preliminary investigate the efficacy and to compare two dose regimens of Rifamycin SV-MMX® 600 mg tablets (t.i.d. and b.i.d.) versus matching placebo in patients suffering from diarrhoea-predominant IBS. The safety and tolerability of Rifamycin SV-MMX® 600 mg tablets (t.i.d. and b.i.d.) will be also investigated as a secondary study objective.

2.1 Primary objective

- The objective of the study is to compare two dose regimens of Rifamycin SV-MMX® 600 mg tablets versus matching placebo in terms of proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency;

2.2 Secondary objective

- to evaluate the safety and tolerability of Rifamycin SV-MMX® 600 mg tablets, two and three times daily, in subjects with diarrhoea-predominant irritable bowel syndrome.

2.3 Study end-points

2.3.1 Primary end-point

- Proportion of weekly responders defined as subjects who weekly have relief of the composite of abdominal pain and stool consistency, on the basis of their daily assessments. Relief of abdominal pain is defined as a decrease in the weekly average of abdominal pain score of at least 30% compared with baseline and relief of stool consistency is defined as a 50% or greater reduction in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline.

2.3.2 Secondary end-points

2.3.2.1 Efficacy

- Proportion of subjects with adequate relief of global IBS symptoms for at least 2 (consecutive or not) of the 10 weeks during the follow-up period (i.e., weeks 3 through 12). Adequate relief of global IBS symptoms is defined as a response of "yes" to the following question, which will be asked weekly (every 7 days):
  "In regard to all your symptoms of IBS, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptoms? [Yes/No]"
- Proportion of subjects with adequate relief of global IBS symptoms during at least 2 weeks (consecutive or not) per month ("monthly response") during month 1, during month 1 through 2 and during month 1 through 3 will be assessed to identify the onset and duration of the therapeutic effect.
- Proportion of subjects with adequate relief of IBS-related bloating for at least 2 (consecutive or not) of the 10 weeks during the follow-up period (i.e., weeks 3 through

12). Adequate relief of bloating is defined as a response of "yes" to the following question, which will be asked weekly (every 7 days):
"In regard to your symptom of bloating, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptom of bloating? [Yes/No]."

- Proportion of subjects with adequate relief of bloating during at least 2 weeks (consecutive or not) per month ("monthly response") during month 1, during month 1 through 2 and during month 1 through 3 will be assessed to identify the onset and duration of the therapeutic effect.
- Proportion of subjects with relief (weekly responders) determined from the subjects' daily assessments of IBS symptoms, bloating, and abdominal pain; relief of IBS symptoms and bloating is defined as a score of either 0 (not at all) or 1 (hardly) for at least 50% of the days in a given week or a score of 0 (not at all), 1 (hardly), or 2 (somewhat) for 100% of the days in a given week for at least 2 (consecutive or not) of the 4 weeks during a given month. Relief of abdominal pain is defined as a decrease by ≥30% from baseline in weekly mean rating of IBS-related abdominal pain.
- Number of weeks (consecutive or not) subjects achieve adequate relief of IBS symptoms during the follow up period.
- Number of weeks (consecutive or not) subjects achieve adequate relief of bloating during the follow up period.
- Change from baseline to week 12 in daily IBS symptoms, bloating and abdominal pain.
- Proportion of monthly responders during month 1, during month 1 through 2 and during month 1 through 3 determined from the subjects' daily assessments of IBS symptoms, bloating, and abdominal pain; relief of IBS symptoms and bloating is defined as a score of either 0 (not at all) or 1 (hardly) for at least 50% of the days in a given month or a score of 0 (not at all), 1 (hardly), or 2 (somewhat) for 100% of the days in a given month. Relief of abdominal pain is defined as a decrease by ≥30% from baseline in weekly mean rating of IBS-related abdominal pain. Relief of stool consistency is defined as a 50% or greater reduction in the number of days per month with at least one stool that has a consistency of Type 6 or 7 compared with baseline.
- Change from baseline to each week during the 12 week follow up for daily IBS symptoms, bloating, abdominal pain, stool consistency and sense of urgency, asked as "Have you felt or experienced a sense of urgency today? [Yes/No]" and calculated as 100* (number of days with urgency/number of days with data), and daily number of stools.
- Change from baseline at weeks 4, 8 and 12 in quality of life inquired as IBS-QoL

2.3.2.2 Safety

- Monitoring of treatment emergent adverse events.
- Changes from baseline in physical examination, vital signs, and clinical laboratory tests and ECG.

3 CLINICAL SUPPLIES

3.1 Treatment

3.1.1 Description of products

The analytical certificates will be supplied with the investigational medicinal product (IMP). The Rifamycin SV-MMX® 600 mg tablets contain 600 mg of the drug substance rifamycin SV sodium and the following excipients: ascorbic acid, lecithin, stearic acid, carboxymethylcellulose sodium, mannitol, colloidal anhydrous silica, magnesium stearate, methacrylic acid copolymers, titanium dioxide, red ferric oxide, talc, triethyl citrate and polyethylene glycol 6000.

3.1.1.1 Test product

TEST (T)

IMP	Rifamycin SV-MMX® 600 mg modified release tablets
Active substance	Sodium rifamycin SV
Manufacturer (active substance)	CKD Bio Corporation, South Korea (GMP compliant)
Manufacturer (finished product)	Cosmo S.p.A., Italy (GMP compliant)
Pharmaceutical form	Modified release tablets
Dose	600 mg
Administration route	Oral

3.1.1.2 Placebo

PLACEBO (P)

	Rifamycin SV-MMX® matching placebo tablets
Manufacturer (finished product)	Cosmo S.p.A., Italy
Pharmaceutical form	Tablets
Administration route	Oral

3.1.2 Dose regimen

The subjects will be randomly assigned (1:1:1) to a treatment group and will receive one of the treatments shown in the table below for 14 consecutive days. The ideal dose timing of the 3 regimens are summarised in the following table.

Table 3.1.2.1 Dosing times in each one of the 3 study dose regimens

Dose regimen	Morning	Afternoon	Night
1	Rifamycin SV 600 mg (07:30 ± 2 h)	Rifamycin SV 600 mg (15:30 ± 2 h)	Rifamycin SV 600 mg (23:30 ± 2 h)
2	Rifamycin SV 600 mg (07:30 ± 2 h)	Placebo (15:30 ± 2 h)	Rifamycin SV 600 mg (23:30 ± 2 h)
3	Placebo (07:30 ± 2 h)	Placebo (15:30 ± 2 h)	Placebo (23:30 ± 2 h)

3.1.3 Route and method of administration

Each dose will be administered with half a glass (≈150 mL) of still water.

The study subjects will take each dose at the scheduled dosing time and will record each administration and its timing in their diaries.

3.1.4 Investigational product distribution

The test IMP and placebo will be dispensed by the investigator or by his/her deputy in excess of the amount sufficient for the time elapsing between 2 consecutive study visits. Test IMP and matching placebo will be dispensed at Visits 2 and 3. The IMP will be exclusively used for the present clinical study and will only be dispensed to the subjects enrolled in the study.

3.2 Packaging and labelling

The primary packaging of the IMP and placebo will be an aluminium/PA/PVC-aluminium blister contained in a carton-box secondary packaging.

The clinical supply for each subject will be packed in 2 distinct carton boxes: each box containing the amount of IMP necessary and sufficient for the doses of one week, to be dispensed to the subjects for the multiple dose treatment at Visits 2 and 3.
Each box will contain also reserve units corresponding to the dose for one extra day.

The formulation labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4; 72) as follows:

a. Name, address and telephone number of the sponsor, contract research organisation or investigator (the main contact for information on the product, clinical study and emergency unblinding)
b. Pharmaceutical dosage form, route of administration and the quantity of dosage units,
c. The batch and/or code number to identify the contents and packaging operation
d. A study reference code allowing identification of the study, site, investigator and sponsor if not given elsewhere
e. The study subject identification number/treatment number and where relevant, the visit number
f. The name of the investigator (if not included in (a) or (d))
g. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study subject or person administering the product)
h. “For clinical study use only” or similar wording
i. The storage conditions
j. Period of use (use-by date, expiry date or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

k. "Keep out of reach of children"

Labels will be in local language.

3.3 Storage conditions

The IMP at the site will be stored at 15-25° C in a dry locked place, sheltered from light. Study subjects will store their drug supply in a secure dry place, protected from light. The investigator, or designee, will explain these requirements to the patients.

3.4 Drug accountability

The test IMP will be provided directly to the investigator by the manufacturer, in excess of the amount necessary for the study (at least 25% excess).

After receipt of the IMP supply, the pharmacist will confirm in writing by signing and dating standard drug accountability forms. Drug inventory and accountability records will be kept by the investigator/pharmacist or their deputy.

At the end of the study, used, unused and partially used supplies of test IMP will returned to the manufacturer, after assessment of drug accountability.

4 INVESTIGATIONAL PLAN

4.1 Overall study design

Multicentre, randomised, double-blind, proof of concept, dose finding study. The planned number of clinical sites is 25 located in 4 countries including Spain, Belgium, Italy and Germany.
The present placebo-controlled proof of concept study will investigate the efficacy of rifamycin SV in IBS-D for the first time. Moreover, two different rifamycin SV dose regimens will be also compared.

4.2 Discussion of design

The present study aims at evaluating the efficacy of rifamycin SV in male and female in patients with IBS-D receiving the IMP according to 2 different dose regimens.
The study has been designed in accordance with the literature studies of rifaximin by Pimentel *et al*. (45). In particular, the study population, the end-points, the variables and the schedule have been planned basing upon the work by Pimentel *et al*. A more recent study of rifaximin by Lembo *et al*. (56) was also taken into account to design the present study with respect to the choice of the population and the evaluation of the variables. The FDA and EMA guidance documents on the clinical evaluation of drugs for the treatment of IBS were also considered to design the present Phase II proof of concept study and to define the primary study endpoint (73, 74). However, the sample size was calculated using the literature data of rifaximin by Pimentel *et al*. (45), who used a similar endpoint to evaluate the relief of the composite of abdominal pain and stool consistency.

The dose regimen planned for the present study is congruent with the recommended dose of the structurally similar product, rifaximin, which is indicated for IBS (rifaximin 550 mg tablets t.i.d. or 2 x rifaximin 200 mg tablets t.i.d.). The dose regimen foresees a daily intake of 1800 mg of sodium rifamycin SV which is similar to the maximal dose recommended for the injectable preparation of sodium rifamycin SV available in Europe (in Italy: Rifocin® recommended maximal dose is 1500 mg a day in case of severe infections; in Belgium: Rifocine® recommended maximal dose is 2000 mg a day by slow infusion; in Ukraine: Rifonat® recommended maximal dose is 1200 mg a day by drop infusion in case of tuberculosis for 12 months or longer).

The planned dose regimen shows several advantages. First of all, the intervals between the 3 daily doses (8 h+8 h+8 h; see § 3.1.2) aim at standardising the dosing conditions for each daily dose. The planned dose regimen may favour the compliance and the adherence to the treatment schedule because it fits better the usual daily life style.

5 STUDY POPULATION

5.1 Target population

Male and female patients suffering from IBS-D will be the target population.

5.2 Inclusion criteria

To be enrolled in this study, subjects must fulfil all these criteria:

1. *Informed consent*: signed written informed consent before inclusion in the study
2. *Sex and Age*: males/females, ≥18 year old
3. *IBS diagnosis*: confirmed IBS-D diagnosis per Rome IV criteria
4. *Symptoms*: active symptoms of IBS at baseline (day 1) as measured by average daily scores for at least 7 days before baseline during the period from Day -14 to Day -1:
   a. abdominal pain score ≥3 using an 11-point numeric rating scale and
   b. bloating score: 2-4* inclusive and
   c. stool consistency: score 6 or 7 (measured by the Bristol stool form scale) for at least 2 days from day -7 to day -1

   and by a negative response to both the global IBS symptom assessment question and to the IBS-related bloating assessment question given weekly during the screening phase up to day 1 before randomisation:
   d. "In the past 7 days, have you had adequate relief of your IBS symptoms?" [No] and
   e. "In the past 7 days, have you had adequate relief of your IBS symptom of bloating?" [No]
5. *Colonoscopy*: performed within 5 years; if patient's age >50, colonoscopy performed within 2 years
6. *Full comprehension*: ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to co-operate with the investigator and to comply with the requirements of the entire study
7. *Literacy:* sufficiently literate to comply with the study requirement of using and filling diaries
8. *Contraception and fertility*: females of childbearing potential and fertile males must be using at least one reliable method of contraception.
   Reliable methods of contraception for women include:
   a. Hormonal oral, implantable, transdermal, or injectable contraceptives for at least 2 months before the screening visit
   b. A non-hormonal intrauterine device [IUD] or female condom with spermicide or contraceptive sponge with spermicide or diaphragm with spermicide or cervical cap with spermicide for at least 2 months before the screening visit

   Reliable methods of contraception for men and male partners of female patients include:

c. Male condoms with spermicide

Reliable methods of contraception for both women and men include:

d. A sterile sexual partner or sexual abstinence

Women of non-childbearing potential or in post-menopausal status for at least 1 year and sterile or surgically sterilised men will be admitted.

For women of childbearing potential, serum pregnancy test result must be negative at screening

**in case of an average value >4, values ≤4.49 will be admitted.*

5.3 Exclusion criteria

Subjects meeting any of these criteria will not be enrolled in the study:

1. *IBS*: symptoms of constipation at baseline evaluated during the period from Day -14 to Day -1:
   a. less than 3 bowel movements a week and
   b. stool consistency score ≤2 for ≥2 days in a week
2. *Screening phase:* failure to record the daily symptom assessments in the diary cards for at least 7 days before baseline
3. *Gastroenteric*: underlying gastrointestinal diseases including ulcerative colitis, Crohn's disease, pancreatitis, any active infectious or inflammatory disorder not related to IBS-D, gastrointestinal motility disorders such as ileus, gastroparesis or pseudoobstruction, gastroduodenal ulcer, gastrointestinal malignancy or potential fatal diseases if not full in remission (5 years from diagnosis and without maintenance treatment) amyloidosis and cholelithiasis if cholecystectomy not performed
4. *Intolerance*: ascertained underlying lactose intolerance with response to diet or any other malabsorption syndrome with the exclusion of asymptomatic lactose malabsorption
5. *Coeliac disease*: ascertained or presumptive underlying coeliac disease
6. *Bile*: ascertained or presumptive bile acid malabsorption or bile acid induced diarrhoea
7. *Diabetes*: underlying diabetes type I or II
8. *Thyroid*: abnormal thyroid function not controlled by thyroid medications
9. *Allergy*: ascertained or presumptive hypersensitivity to the active principle and/or formulations' ingredients; history of anaphylaxis to drugs or allergic reactions in general, which the investigator considers may affect the outcome of the study
10. *Renal function*: ascertained or presumptive clinically significant renal insufficiency or creatinine above twice the upper limit of normal (ULN) of the performing laboratory reference range
11. *Liver function*: chronic liver disease or clinically significant liver enzyme abnormality as evidenced by elevated AST, ALT or total bilirubin >1.5 times ULN

12. *AIDS/HIV*: ascertained or presumptive acquired immunodeficiency (AIDS) or known infection with human immunodeficiency virus (HIV)
13. *Diseases*: significant history of medical or surgical conditions excluding hysterectomy, caesarean section, appendectomy, cholecystectomy, benign polypectomy and inguinal hernia and including renal, hepatic, cardiovascular, haematological, endocrine, immune, psychiatric or neurological diseases that in the investigator's opinion may interfere with the aim of the study; malignant diseases not in remission for at least 5 years
14. *Medications*: alosetron, eluxadoline, ondansetron, tegaserod, lubiprostone, warfarin, antipsychotic, antispasmodic, prokinetic, antidiarrhoeal, laxative, probiotic, narcotic or antibiotic agents within 14 days before the screening visit; antidepressant agents of the selective serotonin-reuptake inhibitor and tricyclic classes unless taken at a stable dose for at least 6 weeks before the screening visit. Once-off medications related to the preparation or performance of the colonoscopy should be recorded as concomitant medications but are not exclusionary.
15. *Investigational drugs*: participation in the evaluation of any investigational product within 30 days before this study
16. *Drug and alcohol*: known history of drug or alcohol [>1 drink/day for females and >2 drinks/day for males, defined according to the USDA Dietary Guidelines 2015 (76)] abuse
17. *Pregnancy (females only)*: pregnant or lactating women or wishing to become pregnant in the 3 months following this visit.

5.3.1 Disallowed treatments

Alosetron, eluxadoline, ondansetron, tegaserod, lubiprostone, warfarin, antidiarrhoeal, probiotic, antipsychotic, antispasmodic, prokinetic, laxative, narcotic or antibiotic agents will be forbidden within 14 days before the screening visit and during the whole study. Antidepressant agents of the selective serotonin-reuptake inhibitor and tricyclic classes will be forbidden unless taken at a stable dose for at least 6 weeks before the screening visit. Non-steroidal anti-inflammatory agents will be prohibited if used for the treatment of IBS. Once-off medications related to the preparation or performance of the colonoscopy should be recorded as concomitant medications but are not exclusionary.

Subjects who start to take antibiotics or who take more than two doses of any disallowed medication between the screening visit and Day 43 will be considered not to have had a response to treatment starting from the time the disallowed medication initiates, regardless of their response data.

Patients will be instructed not to change their dietary habits or not to start a diet during the entire study period. Probiotic agents and other overt-the-counter IBS therapies are not allowed. Yogurt or food containing yogurt will be allowed.

6 STUDY SCHEDULE

The schedule of the study is summarised at page 19. The table of activities and procedures by visit is available in the protocol synopsis.

6.1 Study visits and procedures

Each study subject will undergo 7 ambulatory visits and 7 telephonic follow-ups.
The study protocol foresees a screening phase including a baseline symptom evaluation period (from the screening visit to the baseline, i.e. on Day 1 before randomisation), followed by a treatment period of 2 weeks (visits 2, 3 and 4) and by a follow-up period of 10 weeks (visits 5 to 7, telephonic follow-ups 1 to 7).
The first subject first visit (FSFV) is defined as the 1st visit performed at any clinical site by the 1st screened subject. The last subject last visit (LSLV) is defined as the last visit performed at any clinical site by the last subject, i.e. the last visit foreseen by the study protocol, independently of the fact that the subject is a completer or a withdrawn subject. Additional safety follow-up visits (if needed) may be performed after the LSLV. The following phases, visits and procedures will be performed:

- **Screening phase**
  - Screening – visit 1: between day -21 and day -15
  - Baseline symptoms – daily collection of symptoms between screening visit and day -1
- **Treatment phase**
  - Ambulatory – visit 2: week 0, day 1, baseline
  - Ambulatory – visit 3: week 1, day 8±1
  - Ambulatory – visit 4: week 2, day 15±1
- **Follow-up**
  - Telephonic – follow-up 1: week 3, day 22±2
  - Ambulatory – visit 5: week 4, day 29±2
  - Telephonic – follow-up 2: week 5, day 36±2
  - Telephonic – follow-up 3: week 6, day 43±2
  - Telephonic – follow-up 4: week 7, day 50±2
  - Ambulatory – visit 6: week 8, day 57±2
  - Telephonic – follow-up 5: week 9, day 64±2
  - Telephonic – follow-up 6: week 10, day 71±2
  - Telephonic – follow-up 7: week 11, day 78±2

- **Final visit/early termination visit (ETV)**
  - Visit 7 (Final visit): week 12, day 85±2. In case of early discontinuation, discontinued subjects will undergo an early termination visit (ETV). Subjects discontinuing from data collection or from both data collection and treatment (intervention) (see § 14.4.1) will be asked to undergo, as far as possible, an ETV. Subjects discontinuing the treatment (intervention) will be asked to continue the collection of the data according to the study schedule.

6.1.1 Data collection and patient's diary

From the screening visit (Visit 1) to the end of the study (Final visit/ETV), the patients will daily record in their diary:

- Daily global IBS symptoms
- Daily IBS bloating
- Daily IBS abdominal pain
- Daily bowel movements
- Daily stool urgency
- Daily stool consistency
- Any change in physical or medical conditions
- Intake of any concomitant treatment

Patients will weekly record in their diary:

- Relief of IBS symptoms
- Relief of IBS bloating

From Day 1 to Visit 4, the patients will also record in their diary:

- Daily intake of the study treatment

Details about the study variables and procedures are given in § 7.

6.2 Diet and lifestyle

Patients will continue their usual diet and lifestyle as before screening. Prescribed diet, if any, may not be modified after inclusion in the study. The investigator will ask the subjects about changes in their dietary and smoking habits at each visit. Any significant change will be reported in the subjects' source documentation.

7 DESCRIPTION OF SPECIFIC PROCEDURES

7.1 Demography and medical history

Information on demography, lifestyle, the past and present significant medical conditions and surgeries, the intake of previous and concomitant medications will be collected at the screening visit and reported in the CRF.

7.2 Physical examination

A physical examination will be performed at:

- Screening visit
- Visit 2 (Day 1 - Baseline)
- Visit 4 (Day 15 ± 1)
- Visit 5 (Day 29 ± 2)
- Visit 6 (Day 57 ± 2)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

Information about the physical examination will be recorded by the investigator. Any abnormalities will be recorded. If a new clinically significant (CS) finding (i.e., not noticed at screening) or change in intensity occurs after screening, the finding will be recorded in the CRF as an AE.

7.2.1 Vital signs, height and body weight

Vital signs (blood pressure [BP], heart rate [HR], body temperature) and body weight will be recorded at:

- Screening visit
- Visit 2 (Day 1 - Baseline)
- Visit 3 (Day 8 ± 1)
- Visit 4 (Day 15 ± 1)
- Visit 5 (Day 29 ± 2)
- Visit 6 (Day 57 ± 2)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

Subjects' vital signs will be measured by the investigator or his/her deputy after 5 min at rest (in sitting position). Subjects will be weighed (kg) lightly clothed without shoes. Height will be measured at screening only and BMI will be recorded. BMI will be calculated as weight [kg]/(height [m] x height [m]).

The following normal ranges will be used:
- Systolic blood pressure (SBP): 100-139 mmHg
- Diastolic blood pressure (DBP): 50-89 mmHg
- HR: 50-90 beats/min
- Body temperature: 36.0 - 37.5 °C

7.2.2 ECGs

12-Lead ECGs will be performed (in supine position) at:

- Screening visit
- Visit 4 (Day 15 ± 1)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

The following normal ranges will be used:
- Heart rate: 50-90 beats/min
- PR interval: 100-220 msec
- QRS duration: 80-120 msec
- QT interval: ≤ 460 msec

7.2.3 Colonoscopy

The patient will undergo a colonoscopy in the screening study phase (not later than Day -16) in case no previous colonoscopy is available. Previous colonoscopy must not be older than 5 years. If the screened patient's age is >50, colonoscopy must not be older than 2 years.

7.3 Clinical laboratory assays

Samples of blood and urine will be collected. The following laboratory analyses will be performed at the site laboratory at the screening visit:

Haematology
Leukocytes and leukocyte differential count (percentage values and absolute values), erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, thrombocytes.

Blood chemistry
Electrolytes: sodium, potassium, calcium, chloride, inorganic phosphorus
Enzymes: alkaline phosphatase, γ-GT, AST, ALT
Substrates/metabolites: total bilirubin, creatinine, glucose, urea, uric acid, total cholesterol, triglycerides
Proteins: total proteins

Serum pregnancy test (women).

Urine analysis
Urine chemical analysis: pH, specific weight, appearance, colour, nitrites, proteins, glucose, urobilinogen, bilirubin, ketones, haematic pigments, leukocytes
Urine sediment (analysis performed only if positive): leukocytes, erythrocytes, flat cells, round cells, crystals, cylinders, mucus, bacteria

The same analyses will be performed also at Visit 4 (Day 15 ± 1) and at the final visit (Day 85 ± 2)/ETV.

7.4 IBS symptoms

7.4.1 IBS diagnosis

Patients with a confirmed IBS-D diagnosis per Rome IV criteria will be enrolled in this study.

7.4.2 Patients' diary

The patients will receive paper diary cards at the clinical centre at the screening visit (V1), randomisation visit (V2) and at the end of the treatment (V4).

The investigator will instruct and train the patients on diary entry.

The subjects will use their diary to daily record their IBS symptoms, each treatment intake and to report the occurrence of any change in physical or medical conditions as well as the intake of any concomitant treatment.
At the screening visit, the investigator will remind the subjects that the data recorded daily from Day -14 to Day -1 will be used to confirm their inclusion and randomisation in the study and that failure to daily record the IBS symptoms for at least 7 days before the baseline visit (Visit 2, Day 1) will result in a screen failure.

The investigator will check at each ambulatory visit that all the required information is entered in the subject's diary and will reinstruct the patients on daily recording whenever necessary.
At visit 2, visit 4 and at the end of the study (V7 or ETV), the Investigator will collect the paper diary cards filled in by the patient during the previous period.

7.4.3 Weekly assessment of global IBS symptoms/IBS related bloating relief during the screening phase [yes/no]

The patients will weekly respond "yes" or "no" to the following questions in their diary:

"In the past 7 days, have you had adequate relief of your IBS symptoms?"

"In the past 7 days, have you had adequate relief of your IBS symptom of bloating?"

This weekly assessment of relief of IBS symptoms will be performed by the patients, only during the screening phase, i.e. from Visit 1 - screening visit to Visit 2 (Day 1 - Baseline).

7.4.4 Weekly assessment of global IBS symptoms [yes/no] after randomisation

The patients will weekly respond "yes" or "no" to the following question in their diary:

"In regard to all your symptoms of IBS, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptoms?"

The weekly assessment of global IBS symptoms will be performed by the patients each study week after randomisation, i.e. at the following ambulatory visits:

- Visit 3 (Day 8 ± 1)
- Visit 4 (Day 15 ± 1)
- Visit 5 (Day 29 ± 2)
- Visit 6 (Day 57 ± 2)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

and at weeks 3 (Day 22 ± 2), 5 (Day 36 ± 2), 6 (Day 43 ± 2), 7 (Day 50 ± 2), 9 (Day 64 ± 2), 10 (Day 71 ± 2) and 11 (Day 78 ± 2) when the investigator or his/her deputy will contact the patients over the phone.

7.4.5 Weekly assessment of IBS related bloating [yes/no]

The patients will weekly respond “yes” or “no” to the following question using their diary:

"In regard to your symptom of bloating, as compared to the way you felt before you started study medication, have you, in the past 7 days, had adequate relief of your IBS symptom of bloating?"

The weekly assessment of IBS related bloating will be performed by the patients each study week after randomisation, i.e. at the following ambulatory visits:

- Visit 3 (Day 8 ± 1)
- Visit 4 (Day 15 ± 1)
- Visit 5 (Day 29 ± 2)
- Visit 6 (Day 57 ± 2)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

and at weeks 3 (Day 22 ± 2), 5 (Day 36 ± 2), 6 (Day 43 ± 2), 7 (Day 50 ± 2), 9 (Day 64 ± 2), 10 (Day 71 ± 2) and 11 (Day 78 ± 2) when the investigator or his/her deputy will contact the patients over the phone.

7.4.6 Daily assessment of global IBS symptoms

Throughout the whole study, the subjects will daily score in their diary the severity of their global IBS symptoms in response to the question:

"In regard to all your symptoms of IBS on a scale of 0-6, how bothersome were your symptoms of IBS today?"

according to the following 7-point scale:

- 0 = not at all
- 1 = hardly
- 2 = somewhat
- 3 = moderately
- 4 = a good deal
- 5 = a great deal
- 6 = a very great deal.

➢

7.4.7 Daily assessment of IBS related bloating

The patients will daily score in their diary the severity of their IBS related bloating in response to the question:
"In regard to your specific IBS symptom of bloating on a scale of 0-6, how bothersome was your IBS-related bloating today?"

according to the following 7-point scale:
- 0 = not at all
- 1 = hardly
- 2 = somewhat
- 3 = moderately
- 4 = a good deal
- 5 = a great deal
- 6 = a very great deal.

7.4.8 Daily assessment of IBS related abdominal pain

The patients will daily score in their diary the severity of their IBS related abdominal pain in response to the question:

"In regard to your specific IBS symptom of abdominal pain on a scale of 0-10, how bothersome was your IBS-related abdominal pain today?"

using a 11-point (i.e. 0 to 10) numeric rating scale, where 0 corresponds to “no pain” and 10 corresponds to “pain as bad as it could be”.

7.4.9 Daily assessment of bowel movements and daily stool urgency

The patients will daily respond to the following questions using their diary:

"How many bowel movements did you have today?"

"Have you felt or experienced a sense of urgency today with any of your bowel movements? [yes/no]"

7.5 Bristol stool form scale

The patients will daily score in their diary the consistency of their stool according to the 7-point Bristol stool scale answering the question: “On the below scale from 1 to 7, what was the overall stool form of your bowel movements today?”.

The Bristol stool scale is a graded visual scale of stool density (82). It is validated as a proxy for gastrointestinal transit times (83, 84) and used to define “diarrhoea” by the European Society for Clinical Microbiology and Infectious Disease for *Clostridium difficile* infection (CDI) (85).

Table 7.5.1 Bristol stool form scale

Score	Description
1	Separate hard lumps, like nuts
2	Sausage-shaped but lumpy
3	Like a sausage but with cracks on the surface
4	Like a sausage or snake, smooth and soft
5	Soft blobs with clear-cut edges
6	Fluffy pieces with ragged edges, a mushy stool
7	Watery, no solid pieces, entirely liquid

7.6 IBS quality of life questionnaire

The patients will complete their IBS-QoL questionnaire using their diary at the following ambulatory visits:

- Visit 2 (Day 1 - Baseline)
- Visit 4 (Day 15 ± 1)
- Visit 5 (Day 29 ± 2)
- Visit 6 (Day 57 ± 2)
- Visit 7 (Day 85 ± 2 – Final visit)/ETV

The IBS-QoL is a quality of life questionnaire administered to IBS subjects for their subjective evaluation. The questionnaire consists of 34 items:

1. I feel helpless because of my bowel problems.
2. I am embarrassed by the smell caused by my bowel problems
3. I am bothered by how much time I spend on the toilet.
4. I feel vulnerable to other illnesses because of my bowel problems.
5. I feel fat because of my bowel problems.
6. I feel like I'm losing control of my life because of my bowel problems.
7. I feel my life is less enjoyable because of my bowel problems.
8. I feel uncomfortable when I talk about my bowel problems.
9. I feel depressed about my bowel problems.
10. I feel isolated from others because of my bowel problems.
11. I have to watch the amount of food I eat because of my bowel problems.
12. Because of my bowel problems, sexual activity is difficult for me.
13. I feel angry that I have bowel problems.
14. I feel like I irritate others because of my bowel problems
15. I worry that my bowel problems will get worse.
16. I feel irritable because of my bowel problems
17. I worry that people think I exaggerate my bowel problems.
18. I feel I get less done because of my bowel problems.
19. I have to avoid stressful situations because of my bowel problems
20. My bowel problems reduce my sexual desire.
21. My bowel problems limit what I can wear.
22. I have to avoid strenuous activity because of my bowel problems.
23. I have to watch the kind of food I eat because of my bowel problems.

24. Because of my bowel problems, I have difficulty being around people I do not know well.
25. I feel sluggish because of my bowel problems.
26. I feel unclean because of my bowel problems.
27. Long trips are difficult for me because of my bowel problems.
28. I feel frustrated that I cannot eat when I want because of my bowel problems.
29. It is important to be near a toilet because of my bowel problems.
30. My life revolves around my bowel problems.
31. I worry about losing control of my bowels
32. I fear that I won't be able to have a bowel movement.
33. My bowel problems are affecting my closest relationships
34. I feel that no one understands my bowel problems.

Responses to items 1, 2, 4, 8-10, 12, 13, 16, 25-29, 34 are on the following 5-point scale:

- 1=not at all
- 2=slightly
- 3=moderately
- 4=quite a bit
- 5=extremely

Responses to items 3, 5-7, 11, 14-15, 17-24, 30-33 are on the following 5-point scale:

- 1=not at all
- 2=slightly
- 3=moderately
- 4=quite a bit
- 5=a great deal

Item responses are used to compute an overall score and 8 subscale scores listed below. Each score is on a 0 to 100 scale with higher scores indicating a better quality of life.

- Dysphoria: items 1, 6, 7, 9, 10, 13, 16, 30
- Interference with activity: items 3, 18, 19, 22, 27, 29, 31
- Body image: items 5, 21, 25, 26
- Health worry: items 4, 15, 32
- Food avoidance: items 11, 23, 28
- Social reaction: items 2, 14, 17, 34
- Sexual: items 12, 20
- Relationship: items 8, 24, 33.

The overall score and subscale scores will be calculated using the following formula:

$$\mathbf{100}\frac{(\mathbf{5}*\boldsymbol{N})-\sum_{j=1}^{N}\boldsymbol{Score_j}}{(\mathbf{5}*\boldsymbol{N})-\boldsymbol{N}}$$

where $\boldsymbol{N}$ is the number of items included in the calculation. If the response to an item needed to compute a score is missing, then the score will not be calculated.

8 ASSIGNMENT OF STUDY TREATMENT

8.1 Randomisation

The randomisation list was generated by ArisGlobal, the provider of the EDC. The randomisation list is blocked and stratified by centre and by sex. The allocation ratio among treatment groups is 1:1:1.

The kit list was generated by the Biometry unit of CROSS Research.

The randomisation number includes the 3-digit site code (e.g. 101, 102, 103, 104 …) and a unique progressive 3-digit number within each site (i.e. 001, 002, 003 …). For example, according to this structure, the randomisation numbers of site 101 will be 101001, 101002, 101003, etc. The kit number will be a unique progressive 3-digit number with the capital letter "K" as prefix (i.e. K001, K002, K003 …).

8.2 Treatment allocation

The subjects will be assigned to one of the treatments according to the randomisation list. Within each study site, the randomisation numbers will be assigned to the subjects using an IWRS in a progressive order (e.g. 101001, 101002, 101003, 101004 … for study site 101). The IWRS system will assign the kit numbers to the subjects on the basis of their treatment arm (derived from the assigned randomisation number) and of the kits available at the clinical centre starting from the smallest available number (based on the unique progressive 3-digit kit number).

8.3 Blinding

Both the investigators and the subjects will not be aware of the treatment administered. Neither the members of the clinical staffs nor the CPL or the CRA/monitors will have access to the randomisation code.

8.3.1 Emergency code and unblinding procedures

Unblinding of the code for specific subjects will be fully documented in the source documents, in the eCRF and in the clinical study report.
Breaking of an individual randomisation code by the investigator during the study is allowed only when knowledge of the code is essential for the subject's health. In these cases, the investigator will access the individual code of the concerned subject in the integrated eCRF system, where the unblinding action will be audit trailed. The date and the reason for breaking the code will be recorded in the system.
The system will automatically email the monitor, the Sponsor and the pharmacovigilance CRO any code breaking.

9 EVALUATION PARAMETERS

9.1 Study variables

9.1.1 Primary variables

- Daily assessment of IBS related abdominal pain [11-point numeric rating scale]
- Daily assessment of stool consistency [7-point Bristol Stool Form scale]

9.1.2 Secondary variables

- Weekly assessment of global IBS symptoms [yes/no]
- Weekly assessment of IBS related bloating [yes/no]
- Daily assessment of global IBS symptoms (severity) [how bothersome?] [7-point scale]
- Daily assessment of IBS related bloating [7-point scale]
- Daily assessment of bowel movements [number]
- Daily assessment of stool urgency [yes/no]
- IBS-QoL (quality of life questionnaire)
- TEAEs, vital signs (BP, HR, body temperature), body weight, ECG, laboratory parameters

9.2 Efficacy assessments

The efficacy assessments planned for collecting the efficacy variables are illustrated in § 7.4, 7.5 and 7.6.

9.3 Safety assessments

Safety and general tolerability of the IMP will be based on TEAEs, physical examinations including body weight, vital signs, ECG and routine haematology, blood chemistry and urinalysis laboratory tests.

10 STATISTICAL METHODS

The data documented in this study and the parameters measured will be evaluated and compared using classic descriptive statistics, i.e. geometric mean, arithmetic mean, SD, CV (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables.

Not available data will be evaluated as "missing values". The statistical analysis of demographic and safety and efficacy data will be performed using SAS® version 9.3 (TS1M1) (78) or higher (the actual versions will be stated in the final report).

10.1 Analysis Sets

10.1.1 Definitions

A subject will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A subject will be defined as eligible if he/she meets all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A subject will be defined as enrolled in the study if he/she is included into the interventional phase of the study. The enrolment can be performed through randomised allocation to a treatment arm.

A subject will be defined as randomised in the study when he/she is assigned to a randomised treatment arm.

- Enrolled Set: all enrolled subjects. This analysis set will be used for demographic, baseline and background characteristics
- Intention-To-Treat Set (ITT): all randomised subjects. This analysis set will be used for sensitivity analyses
- Full Analysis Set (FAS): all randomised subjects, who receive at least one dose of the investigational medicinal product and have at least one post randomisation assessment of the primary efficacy data. This analysis set will be used for the primary efficacy analysis
- Per Protocol Set (PP): all randomised subjects who fulfil the study protocol requirements in terms of IMP intake and collection of primary efficacy data and with no major deviations that may affect study results. This analysis set will be used for sensitivity analyses
- Safety Set: all subjects who receive at least one dose of the IMP. This analysis set will be used for the safety analyses

Each subject will be coded by the CRO Biometry Unit as valid or not valid for the Enrolled set, ITT set, FAS, PP set and Safety set. Subjects will be evaluated according to the treatment they actually receive (Safety set) and according to the treatment they are assigned to (Enrolled set, ITT set, FAS, PP set).

10.1.2 Reasons for exclusion from the Full Analysis Set

Reasons for the exclusion of subjects from the FAS are the following:

- failure to take at least one dose of the IMP
- lack of any primary efficacy data post randomisation
- failure to satisfy major inclusion/exclusion criteria (eligibility deviations). Subjects who fail to satisfy an inclusion/exclusion criterion may be excluded from the analysis without the possibility of introducing bias only under the following circumstances:
  - the inclusion/exclusion criterion was measured prior to enrolment
  - the detection of the relevant eligibility deviations can be made completely objectively
  - all subjects receive equal scrutiny for eligibility deviations (blind review)
  - all detected deviations of the particular inclusion/exclusion criterion are excluded

10.1.3 Reasons for exclusion from the Per Protocol set

Reasons for the exclusion of subjects from the PP set include (but are not limited to) the following:

- lack of compliance to the IMP
- exposure to an IMP different from the one assigned to the subject
- missing primary efficacy data
- failure to satisfy any inclusion/exclusion criteria (eligibility deviations)
- intake of prohibited medications

10.2 Sample size and power considerations

No formal hypothesis test will be performed for the primary efficacy endpoint and the sample size is based on the precision estimate for the odds ratio of the proportions of weekly responders defined as subjects who weekly have relief of the composite of abdominal pain and stool consistency, on the basis of their daily assessments (§ 2.3.1).

When the sample size in each treatment group is 106, the placebo proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency, π_P, is expected to be 0.374, the rifamycin proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency, π_T, is expected to be 0.466 for both dose regimens and the odds ratio $\pi_T(1-\pi_P)/\pi_P(1-\pi_T)$ is expected to be 1.464 for both dose regimens. A two-sided 95.0% CI for a ln(odds ratio) expected to be 0.381 for both dose regimens will extend 0.550 from the observed ln(odds ratio) corresponding to confidence limits of 0.845 and 2.537 for an odds ratio of 1.464. The threshold of 0.550 on the ln-scale corresponds to 42.5% of the expected odds ratio of the original scale.

At least 342 subjects will be enrolled in order to have at least 318 subjects (at least 106 per treatment group) included into the FAS.

10.3 Compliance to the IMP

The assessment of subjects' compliance to the study treatment will be made by determining the amount of study medication dispensed to the subject at Visit 2 and the amount of unused medication returned at Visit 3 and the amount of study medication dispensed to the subject at Visit 3 and the amount of unused medication returned at Visit 4. Compliance will be evaluated according to the following formula:

$$\textbf{Compliance} = \frac{\textbf{Number of tablets dispensed - Number of unused tablets returned}}{\textbf{Number of scheduled intakes}}$$

Non-compliance will be defined as compliance lower than 80% or greater than 120% and in case of non-compliance the subject will be excluded from the PP Set (see § 10.1.3).

10.4 Demographic, baseline and background characteristics

Critical demographic characteristics will be examined according to qualitative or quantitative data. Qualitative data will be summarised in contingency tables. Quantitative data will be summarised using classic descriptive statistics.

10.5 Analysis of efficacy parameters

10.5.1 Primary efficacy analysis

The number and proportion of subjects with and without adequate relief of the composite of abdominal pain and stool consistency (defined in § 2.3.1) will be summarised by treatment group in the FAS, ITT and PP sets using tables of frequency.

No formal hypothesis test will be performed for the primary efficacy endpoint.

The primary efficacy analysis will be conducted on the subjects included in the FAS.

The proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects. Analysis centres will be defined by grouping the study centres according to geographic region in order to reach a minimum number of 12 subjects per treatment group per centre included in the FAS.

Missing values of abdominal pain and stool consistency are filled in by the multiple imputation method which involves the following distinct phases:

- the missing data of abdominal pain score and stool consistency are filled in to generate complete data sets using SAS procedure PROC MI. Ten (10) imputations are foreseen
- the proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency are derived from the complete data sets
- the proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects

- the results from the complete data sets are combined for statistical interference using SAS procedure PROC MIANALYZE

Subjects starting to take antibiotics (other than the study medication) or taking more than two doses of a medication that was prohibited per the study protocol between the screening visit and Day 42 will be considered not to have had a response to treatment starting from the time the medication will be initiated, regardless of their response data.

Subject discontinuing the study due to adverse events related to the study IMP will be considered not to have had a response to treatment starting from the time of study discontinuation, regardless of their response data.

10.5.2 Sensitivity analyses

Two sensitivity analyses will be conducted to address the impact of missing data on the primary efficacy endpoint.

The first sensitivity analysis will be conducted on the subjects included in the ITT set where missing values of are filled in by the multiple imputation method which involves the following distinct phases:

- the missing data of abdominal pain score and stool consistency are filled in to generate complete data sets using SAS procedure PROC MI. Ten (10) imputations are foreseen
- the proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency are derived from the complete data sets
- the proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects
- the results from the complete data sets are combined for statistical interference using SAS procedure PROC MIANALYZE

The second sensitivity analysis will be conducted on the subjects included in the PP set. The proportion of subjects with adequate relief of the composite of abdominal pain and stool consistency will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects.

10.5.3 Secondary efficacy analyses

No formal hypothesis test will be performed for the secondary efficacy endpoints.

All the proportions of subjects (see § 2.3.2.1) will be summarised by treatment group in the FAS, ITT and PP sets using tables of frequency and will be analysed using a logistic regression model with treatment group, analysis centre and sex as fixed effects. Analysis centres will be defined by grouping the study centres according to geographic region in order to reach a minimum number of 12 subjects per treatment group per analysis centre included in the FAS.

The number of weeks subjects achieve adequate relief of IBS symptoms during the follow up period and the number of weeks subjects achieve adequate relief of bloating during the follow up period (see § 2.3.2.1) will be summarised by treatment using descriptive statistics and will be analysed using a proportional odds model for ordinal outcome with treatment group, analysis centre and sex as fixed effects. Analysis centres will be defined by grouping the study centres according to geographic region in order to reach a minimum number of 12 subjects per treatment group per analysis centre included in the FAS.

The value at week 6 and change from baseline to week 6 in daily IBS symptoms, bloating and abdominal pain (see § 2.3.2.1) will be summarised by treatment using descriptive statistics.

The value at each week during the 12 week follow up and the change from baseline to each week during the 12 week follow up for daily IBS symptoms, bloating, abdominal pain, stool consistency, sense of urgency and daily number of stools (see § 2.3.2.1) will be summarised by treatment using descriptive statistics.

The value at weeks 4, 6, 9 and 12 and the change from baseline at weeks 4, 6, 9 and 12 in quality of life inquired as IBS-QoL (see § 2.3.2.1) will be summarised by treatment using descriptive statistics.

10.6 Safety and tolerability evaluation

- **AEs**

AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs will be classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarised by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

The treatment groups will be compared in frequency and severity of TEAEs.

- **Physical examination**

Date of the physical examination, overall investigator's interpretation (as normal [N], abnormal not clinically significant [NCS] or abnormal clinically significant [CS]) and CS abnormalities (if any) will be listed.

- **Laboratory data**

Parameter values will be listed and summarised by presenting a table of subjects with abnormalities, the descriptive statistics of parameter values and their changes from baseline (n, mean, SD, CV%, min, median and max) for quantitative parameters or tables of frequencies for qualitative parameters and shift tables.

- **Vital signs**

Vital signs values will be listed and summarised by presenting a table of subjects with abnormalities and the descriptive statistics of vital signs values and their changes from baseline (n, mean, SD, CV%, min, median and max).

- **Body weight**

Body weight values will be listed and summarised by presenting the descriptive statistics of body weight values and their changes from baseline (n, mean, SD, CV%, min, median and max).

- **ECG**

Parameter values will be listed and summarised by presenting a table of subjects with abnormalities and the descriptive statistics of parameter values and their changes from baseline (n, mean, SD, CV%, min, median and max).

11 DEFINITION AND HANDLING OF AEs AND SAEs

11.1 Applicable SOPs

AEs definition, classification and management will follow the SOP of CROSS Research S.A., based upon applicable local and international regulations. The full SOP or an operative summary will be made available to the clinical centre.

A brief summary of AE definition, classification and management is reported below.

11.2 Definitions

- **Adverse event (AE)**

Any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with treatment.

- **Adverse Drug Reaction (ADR)**

Any noxious and unintended response to a medicinal product (i.e. if a causal relationship between a medicinal product and an AE is at least reasonably possible in the investigator's or sponsor's opinion, the relationship cannot be ruled out). An ADR may result not only from the authorised use of a medicinal product at normal doses, but also from medication errors and uses outside the terms of the marketing authorisation, including the misuse and abuse of the medicinal product.

- **Pre-treatment AE (PTAE)**

Any AE occurring before the first dose of the study treatment and not worsening after the first dose. The following medical occurrences and clinical investigations are the only clinically significant events which, according to the investigator judgement, can be defined and recorded as PTAEs:

- trauma (fractures, sprains, strains, falls, domestic accidents, car accidents, etc.) which occurred after the signature of the informed consent and before the first study treatment administration
- new measurements (vital signs, ECG, laboratory parameters, etc.) performed after the signature of the informed consent and before the first study treatment administration, which show a clinically significant worsening in comparison with a previous measurement performed after the signature of the informed consent
- any disease diagnosed after the anamnesis recorded at visit 1 and before the first study treatment administration
- physical and mental status changes (pre-syncope, anxiety, dizziness, fainting, etc.) occurred after the signature of the informed consent and before the first study treatment administration

- **Treatment-emergent AE (TEAE)**

Any AE occurring or worsening after the first dose of a medicinal product

- **Serious Adverse Event (SAE)**

Any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event that may jeopardize the subject's health status or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are cancer, 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

- **Unexpected ADR**: an ADR the nature or severity of which is not consistent with the Reference Safety Information (RSI)

- **Reference Safety Information (RSI)**: in order to assess whether an adverse reaction is expected, the Investigator's Brochure (IB) for the test formulation will be used.

- **Suspected Unexpected Serious Adverse Reaction (SUSAR)**

An ADR that is both unexpected (not consistent with the RSI) and also meets the definition of a SAE.

11.3 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: last follow-up visit/ETV

An AE occurring after the last follow-up visit/ETV and coming to knowledge of the investigator (e.g. by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

11.4 AEs recording

All AEs derived by spontaneous, unsolicited reports of the subjects, by observation and by routine open questioning should be collected and reported.
The following minimal information will be recorded for an AE (detailed explanation for each element is available in the SOP or in the operative summary made available to the clinical centre) in the source documents and transcribed into the eCRF as soon as possible:

1. Adverse Event: progressive number of the adverse event

2. Description: verbatim description of the adverse event or
Follow-up: progressive number of follow-up of the adverse event

3. Acknowledgment Date/Time: acknowledgment date/time of the adverse event or
Follow-up Date/Time: follow-up date/time of the adverse event

4. Start Date/Time: start date/time of the adverse event

5. End Date/Time: end date/time of the adverse event

6. Affected Body Area: anatomical location relevant for the event

7. Whether the adverse event starts before or after the first intake of the study drug or whether the adverse event has worsened or not after the first intake of the study drug

8. Last Study Drug Administration Date/Time Before Onset: if the adverse event started after the start of the treatment, the date/time of last administration of the study drug before the onset of the adverse event or
Last Study Drug Administration Date/Time Before Worsening: In case of treatment emergent adverse event, the date/time of the last administration of the study drug(s) before the worsening of the adverse event.

9. Investigator's opinion about the reasonable possibility of a causal relationship with the study drug.

10. Investigator's opinion about other causal relationship (e.g. non study drug, concomitant therapy, study device, etc.).

11. Severity: the severity or intensity of the event

- 1 Mild –The AE causes minimal discomfort and does not interfere in a significant manner with the subject's normal activities. Clinical or diagnostic observations only; intervention not indicated.
- 2 Moderate – The AE is sufficiently uncomfortable to produce some impairment of the subject's normal activities. Minimal, local or non-invasive intervention indicated.
- 3 Severe – The AE is incapacitating, preventing the subject from participating in their normal activities. Intervention required, seriousness of the event to be considered.

12. Pattern: Used to indicate the pattern of the event over time

- 1 Single Event
- 2 Continuous
- 3 Intermittent

13. Serious Adverse Event

- 1 Yes
- 2 No

If "yes", reason for seriousness must be one of the following:

1. results in death
2. is life-threatening
3. requires inpatient hospitalisation or prolongation of existing hospitalisation
4. results in persistent or significant disability/incapacity
5. is a congenital anomaly/birth defect
6. is an important medical event;

14. Action Taken with Study Drug: describes changes to the study drug as a result of the event. It is specifically for actions taken with the study drug

- 1 Dose Not Changed

- 2 Dose Increased
- 3 Dose Reduced
- 4 Drug Interrupted (i.e. temporary stop)
- 5 Drug Withdrawn (i.e. definitive stop)
- 6 Not Applicable (e.g. drug administration not started yet or completed)
- 7 Unknown

15. Concomitant Therapy: if a concomitant therapy is given, it must be reported in the specific CRF forms

16. Study Discontinuation: if the adverse event cause the subject to be discontinued from the study

17. Other Action Taken: other actions taken as a result of the event that are unrelated to dose adjustments of study drug. This must include any steps taken to mitigate or treat the AE, if any.

18. Outcome: Outcome of the event

- 1 Recovered/Resolved
- 2 Recovered/Resolved With Sequelae
- 3 Recovering/Resolving
- 4 Not Recovered/Not Resolved
- 5 Fatal
- 6 Unknown

11.5 SAEs reporting

IMPORTANT: National regulations and requirements for SAE reporting **prevail**, however, in general, the following indications should be followed.

The investigator must report in the eCRF any SAE within 24 h of becoming aware of the event. The Pharmacovigilance CRO will report all SAEs to the Sponsor, to CROSS Research SA and to the local CROs.

The investigator shall notify the competent Ethics Committee (EC) within 7 days of any SAE with lethal outcome occurred during a study. If the investigator is initially unable to obtain all the necessary details for completing the form, he/she should in any case transmit all the available information. The investigator should provide an appropriate follow-up of SAEs to all concerned parties.
Seriousness and causality must be assessed by the investigator. Expectedness is usually assessed by the sponsor.
If the investigator is unable to assess the causality it is recommended to adopt a conservative approach and treat the event as a suspected adverse reaction until follow-up information is available.
The sponsor may also make an assessment of causality, independent of that of the investigator. The most conservative approach should be taken when it comes to regulatory reporting. Under no circumstances should the sponsor downgrade the investigator's opinion or

put the investigator under pressure to change his/her assessment. In case of disagreement, both the opinion of the investigator and the sponsor should be provided in the report.
The sponsor will evaluate the SAE expectedness on the basis of the RSI.

The Pharmacovigilance CRO should track the SAE occurred during a study, for example, using a spreadsheet to ensure that missing SAE reports are requested in a timely manner. It is also recommended that a record of follow-up attempts is maintained as, in the event of the information not being received, this would demonstrate due Pharmacovigilance CRO diligence.

11.6 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the sponsor (day 0).
For fatal and life-threatening SUSARs the EC and Competent Authority (CA) should be informed as soon as possible and in any case within 7 days.

If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days.

SUSARs which are not fatal and not life-threatening are to be reported within 15 days.

The minimum information to be reported includes:

- Valid EudraCT number (where applicable)
- Sponsor study number
- One identifiable coded subject
- One identifiable reporter
- One SUSAR
- One suspect IMP (including active substance name, code)
- A causality assessment (a reasonable possibility of a causal relationship with the study drug can be excluded only if there is information supporting this decision, otherwise it cannot be excluded).

11.7 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g.: a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical trial and are reported to the investigator by the subject.
- new events relating to the conduct of the trial or the development of the medicinal product likely to affect the safety of the subjects, such as :

- a SAE which could be associated with the trial procedures and which could modify the conduct of the trial
- a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
- a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical trials.

11.8 SAEs: contacts

Zeincro Kft, Pharmacovigilance & Safety Department, Bul. Totleben 53-55, Sofia 1606, Bulgaria
Phone: +359.2.805.7193
Fax: +359.2.805.7001
Mobile: +359.876.150.797
Email: smiedema@zeincro.com

12 DATA MANAGEMENT PROCEDURES

12.1 Data collection – CRFs

The investigator must ensure that the clinical data required by the study protocol are carefully reported in the eCRF. He must also check that the data reported in the eCRF correspond to those in the subject's source documents.
The eCRF will be filled out in English. Any correction to the eCRFs' entries must be carried out by the investigator or a designated member of staff. In the interest of completeness of data acquisition, the questions which are repeated in each section of the eCRF should be answered in full, even if there are no changes from a previous examination. The investigator must provide a reasonable explanation for all missing data.
The eCRF will be completed and signed by the investigator. Afterwards, the Biometry Unit of CROSS Research will use it for data management procedures and it will be finally archived by the sponsor in the format agreed with the eCRF provider.
The primary and secondary efficacy measures will be recorded directly by the patients in their diary cards.

12.2 Unique subject identifier

All the subjects who sign the informed consent form for the present study will be coded with "unique subject identifiers" when data are extracted from the study database into the domains of the CDISC SDTM model. The unique subject identifier consists of the sponsor study code (i.e. CB-01-11/28), the 3-digit site number (e.g. 101, 102, 103…) and the 4-digit screening number (e.g. S001, S002, etc.). Study code, site number and screening number are separated by slashes ("/"). The last 8 digits of the unique subject identifier, corresponding to the site number and the subject screening number separated by a slash, will appear as subject identifier in the individual listings and figures of the clinical study report.

12.3 SDTM and ADaM

The Biometry Unit of CROSS Metrics will extract data from the eCRF and will provide tabulation datasets according the SDTM model of CDISC and analysis datasets according to the ADAM model of CDISC using a double programming approach with comparison reconciliation instead of single programming approach with quality check (QC). SDTM and ADaM domains will be validated using the Pinnacle 21 Community (former OpenCDISC) validator. The final data file will be transferred to the sponsor in the agreed format with all the other study documentation.

12.3.1 Coding dictionaries

Medical/surgical history and underlying diseases and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).
Previous and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). The version of the coding dictionaries will be stated in the study report.

13 STUDY MONITORING, QUALITY CONTROL and QUALITY ASSURANCE

13.1 Monitoring

The monitoring visits will be conducted by appropriate staff of the selected CROs (§ 16.4).

Monitoring activities, including monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports will comply with ICH-GCP chapter 5.18 requirements.

Adequate time and availability for monitoring activities should be ensured by the investigators and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the investigator will assure support to the monitor at all times.

The investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorised individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the sponsor could perform some quality control activities to verify the compliance with the study procedures and the ICH-GCP guidelines.

13.2 Quality Control and Quality Assurance

CROSS Research, CROSS Metrics, ArisGlobal, Zeincro and the local CROs have implemented and maintain a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirements and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOAC principles (Attributable-Legible-Contemporaneous-Original-Accurate-Complete).

The clinical sites are responsible for implementing and maintaining quality assurance and a quality control system to ensure that the study is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH-GCP, and the applicable regulatory requirements.

The CROs and the sponsor will be responsible for their respective activities.

The sponsor may transfer any or all of the sponsor’s trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor.

13.3 Applicable SOPs

The sponsor, the clinical sites and the CROs will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. SOPs will be made available for review, if required. For definition, handling and reporting of TEAEs, the SOP of CROSS Research will be applied. SOPs of CROSS Research will be followed in the conduct of monitoring activities.

13.4 Data access

The investigator and the CROs will ensure that all raw data records, medical records, CRFs and all other documentation that is relevant to this study will be made accessible for monitoring activities, audits, IEC review, and regulatory inspections.

13.5 Audits and inspections

The sponsors, independent bodies acting on behalf of the sponsor and the CROs have the right to perform audits according to ICH-GCP responsibilities.

The study may also be inspected by regulatory authorities.

The investigators and the CROs agree, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorised individuals to have access to all the study documentation.

14 ETHICAL CONSIDERATIONS

14.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.
The approval and/or the acknowledgment of the study protocol, the Investigator's brochure and all other relevant documentation by the National Competent Authorities, central Ethics Committees and local Ethics Committees competent for each study site will be obtained before the start of the study, according to the current regulations.

The present clinical study will be carried out according to the general principles of "Integrated Addendum to ICH E6(R1): Guideline For Good Clinical Practice E6(R2)", 9 November 2016.

14.2 Informed consent

Before being enrolled into the clinical study, the subjects must have expressed their consent to participate, after the investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP recommendations.
In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment (information on the IMP and treatment procedures, as applicable)
- any potential negative effects attributable to the study product or treatment
- the freedom to ask for further information at any time
- the subjects' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of a subject insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the subjects and the time will be recorded.

The investigators will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the investigator and the subject.
A copy of the signed form will be given to the subject.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the investigator's study file according to the regulatory requirements (see § 15.3). The investigators will allow inspection of the forms by authorised representatives of the sponsor, EC members and regulatory authorities. They will confirm, by signing and dating the forms, that informed consent has been obtained.

14.3 Insurance policy

An insurance cover has been issued in favour of the subjects participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

14.4 Withdrawal of subjects

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the type of discontinuation and the primary reason for discontinuation will be recorded.

14.4.1 Discontinuation type

- **Discontinuation from interventions**: the subject discontinues from the intake of the IMP but agrees to continue the collection of primary and secondary end-points (or at least of the primary end-point) according to the study schedule
- **discontinuation from data collection**: the subject discontinues from the collection of primary and secondary end-points
- **discontinuation from interventions and data collection**: the subject discontinues from the intake of the IMP and from the collection of primary and secondary end-points

14.4.2 Primary reason for discontinuation

- **Adverse event**: Any (significant) adverse event that in the opinion of the investigator or concerned subject is not compatible with study continuation. For the definition of AE, please refer to § 11.2.
- **death**: the absence of life or state of being dead
- **lack of efficacy**: the lack of expected or desired effect related to a therapy
- **lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **non-compliance with study drug**: an indication that a subject has not agreed with or followed the instructions related to the study medication
- **physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the subject
- **pregnancy**: pregnancy is the state or condition of having a developing embryo or foetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth
- **progressive disease**: a disease process that is increasing in scope or severity
- **protocol deviation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by sponsor**: an indication that a clinical study was stopped by its sponsor

- **site terminated by sponsor**: an indication that a clinical site was stopped by the study sponsor
- **technical problems**: a problem with some technical aspect of a clinical study, usually related to an instrument
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified

14.4.3 Discontinuation procedures

For any subject discontinuing from interventions only, the investigator will:

- ask the subject to continue the collection of the primary and secondary end-points (or at least the primary end-points) according to the study schedule.
- arrange for alternative medical care of the withdrawn subject, if necessary
- continue the collection of safety and tolerability data
- report in the eCRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the eCRF any follow-up, if the subject is withdrawn for an AE

For any subject discontinuing from data collection only, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- report in the eCRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the eCRF any follow-up, if the subject is withdrawn for an AE

For any subject discontinuing from interventions and data collection, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions and perform the required blood sampling for the laboratory assays. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e. not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, if necessary
- report in the eCRF date and time of the last dose administration, and date and primary reason of study discontinuation
- record in the eCRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will not be replaced. Subjects withdrawn from the study will retain their randomisation number.

14.5 Study termination

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol. The investigator and the sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately.

15 ADMINISTRATIVE PROCEDURES

15.1 Material supplied to the clinical centre

Beside IMP, the following study material will be supplied to the clinical centre:

- final version of the study protocol
- final version of the diary cards
- eCRF
- copy of the investigator's brochure (IB) relative to the IMP
- informed consent forms

Moreover, before the start of the study, the investigators will be provided with the following documents: ICH guidelines, confidentiality agreement (if applicable), protocol amendments (if any), declaration of Helsinki, insurance statement, SAE forms, financial agreement (if applicable), confidential subject identification code list form, drug accountability forms, investigator and study staff list form.

15.2 Protocol amendments

In order to obtain interpretable results, neither the investigator nor the sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the investigator and the sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

All amendments will be sent to the EC and concerned Competent Authorities.

15.3 Study documentation and record keeping

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the eCRF and in all required reports.

The investigator must keep source documents for each subject in the study. All information on the eCRF must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ECGs, etc., and the original signed informed consent forms.

Data reported on the eCRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests and ECG tracing, insurance contracts, certificate of analysis of the IMP, drug accountability records, signed informed consent forms, confidential subjects identification code, eCRF, patients' diaries, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the investigator and the sponsor as long as needed to comply with ICH-GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

15.4 Study subjects' recruitment

Study participants will be enrolled at the recruited clinical sites in a competitive manner. No ceiling will be established on the number of subjects to be enrolled per site.

15.5 Confidentiality and data protection

By signing this protocol, all the parties agree to keep all the information provided by the sponsor in strict confidentiality and to request the same confidentiality from their staff. Study documents provided by the sponsor (protocols, IB, CRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the sponsor to the investigators, to the CROs and to the other providers cannot be disclosed to others without direct written authorisation from the sponsor, except for the extent necessary to obtain the informed consent from the subjects wishing to participate in the study.

Data on subjects collected in the eCRF during the study will be documented in an anonymous way (see § 12.2). If, as an exception, for safety or regulatory reasons identification of a subject becomes necessary, the monitor, the sponsor and the investigator will be bound to keep this information confidential.

15.6 Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.
Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report.

As the sponsor agrees that the study results can be published by the investigators, the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.
This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigators will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).
On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

16 STUDY RESPONSIBLE PERSONS

16.1 Sponsor

Cosmo Technologies Ltd., Riverside II, Sir John Rogerson's Quay, Dublin 2, Ireland
Phone: +353.1.8170370
Fax: +353.1.8230718
Email: rjones@cosmopharma.com

Sponsor representative
Richard Jones, Research and Development Manager

Medical Expert
Alessandro Mazzetti, MD, Chief Medical Officer

16.2 Institutes performing the study

16.2.1 Belgium

16.2.1.1 Co-ordinating site

Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven, University Hospital Gasthuisberg, Department of Gastroenterology, Herestraat 49, BE-3000 Leuven, Belgium
Phone: +32.16.344225
Fax: +32.16.344419
Email: jan.tack@med.kuleuven.be

Principal investigator and National Co-ordinator
Jan Tack, MD, PhD, Univ. Prof.,
Head, Department of Clinical and Experimental Medicine, University of Leuven
Head of Clinic,

16.2.2 Spain

16.2.2.1 Co-ordinating site

Vall d'Hebron Research Institute (VHIR) at Vall d'Hebron Hospital, Gastroenterology dept., Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona, Spain
Phone: +34.93.4894035
Email: javier.santos@vhir.org

Principal investigator and National Co-ordinator
Javier Santos, MD

16.2.3 Italy

16.2.3.1 Co-ordinating site

Centre for Research and Care of Intestinal Diseases, Department of Gastroenterology,
Istituto di Ricovero e Cura a Carattere Scientifico IRCCS, Istituto Clinico Humanitas,
I-20089 Rozzano, Italy
Phone: +39.02.822.44771
Fax: +39.02.822.45101
Email: silvio.danese@humanitas.it

Principal Investigator and National Co-ordinator
Silvio Danese, MD

16.2.4 Germany

16.2.4.1 Co-ordinating site

Internistenzentrum, Bahnhofstr. 30, D-82131 Gauting, Germany
Phone: +49.8151.97.10.96
Email: m.storr@internistenzentrum.de

Principal Investigator and National Co-ordinator
Martin Storr, MD, Univ. Prof.

16.3 Co-ordination, data analysis & reporting

CROSS Research S.A. and CROSS Metrics S.A., Switzerland, sister companies, sharing the same standard operating procedures and quality assurance system

CROSS Research S.A.
Via F.A. Giorgioli 14, CH-6864 Arzo, Switzerland
Phone: +41.91.6300510
Fax: +41.91.6300511

Coordination
Diego Scanniffio, Clinical Project Leader
Email: diego.scanniffio@croalliance.com

Medical Writing
Andrea Di Stefano, Senior Medical Writer
Email: medicalwriting@croalliance.com

Biometry Unit Representative
Matteo Rossini, Biometry Manager, Unit Head
Email: statistics@croalliance.com

Quality Assurance Unit Representative
Mario Corrado, Quality Assurance Manager, Unit Head
Email: qau@croalliance.com

16.4 Monitoring and submissions

16.4.1 Spain

Adknoma Health Research S.L.
Madrid office: Capitán Haya, 1 Planta 15, 28020 Madrid, Spain
Phone: +34.91.417.70.90
Fax: +34.93.206.66.67
Barcelona office: Martí i Julià 6-8 Entlo. 3ª Dcha., 08034 Barcelona
Phone: +34.93.206.66.66
Fax: +34.93.206.66.67

Contact Person
Miguel Campo
Business development manager
Email: miguelcampo@adknoma.com

16.4.2 Belgium

AML Clinical Services, Tempelzicht 8, B-3210 Linden, Belgium
Phone: +32.16.782170
Fax: +32.16.788672

Contact person:
Anne-Mie Lenaers
Clinical Research Consultant
Email: alenaers@aml-research.be

16.4.3 Italy

CROSS Research S.A., Via F.A. Giorgioli 14, CH-6864 Arzo, Switzerland
Phone: +41.91.6300510
Fax: +41.91.6300511

Monitoring activities will be coordinated by CROSS Research and performed by monitors engaged in subcontracts.

16.4.4 Germany

SSS International Clinical Research GmbH, Landsberger Str. 23/25, D-82110 Germering, Germany
Phone: +49.89.800.650.0
Fax: +49.89.800.650.555
E-mail: info@cro-sss.de

Contact person
Dr. Michael Sigmund,
Chief Executive Officer
Email: michael.sigmund@cro-sss.de

16.5 Provider of EDC and randomisation system

ArisGlobal Ltd., 16a Lincoln Place, Dublin 2, Ireland
Phone: +44.750.2255770

Responsible person
Eleni Vanden Eede
Project Manager
Email: EVandenEede@arisglobal.com

16.6 Pharmacovigilance

Zeincro Kft, Pharmacovigilance & Safety Department, Bul. Totleben 53-55, Sofia 1606, Bulgaria
Phone: +359.2.805.7193
Fax: +359.2.805.7001
Email: safety@zeincro.com

Main Contact
Sjoerd Miedema
Email: smiedema@zeincro.com

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