Statistical Analysis Plan[1]

Section 1: Administrative Information

Date	27/11/2023
Study Title	ProPILA-rifax
Study Registration Number	N/2016/71 / EudraCT 2017-000291-28 / NCT03069131
SAP Version Number	V1
Protocol version and date	V10 dated 05/02/2021
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Revision Control

Protocol version	Updated SAP version number	Section number changed	Description of change	Date changed

SAP Signatures

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[1] D'après Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017; 318(23):2337–2343. doi:10.1001/jama.2017.18556

Roles and responsibilities

Name	Role	Institution
Grégory Tio	Statistician	Besançon University Hospital
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Contributions

Maxime Desmarets developed the statistical analysis plan (SAP) based on the analyses provided for in the study protocol. Grégory Tio is the statistician for the study and helped answer questions related to the data and study management relevant to the development of the SAP. Thierry Thévenot has read and approved the PAS.

Abbreviations and Definitions

AAH, acute alcoholic hepatitis
CRP, C-reactive protein
HE, Hepatic encephalopathy
HR, Hazard ratio
HRS, Hepatorenal syndrome
SBP, Spontaneous bacterial peritonitis
IL-6, Interleukin 6
LPS, lipopolysaccharide
mITT, modified intention to treat
TIPS, Transjugular intrahepatic portosystemic shunt
vWF, von Willebrand Factor

Section 2: Introduction

Background and Rationale

The natural history of cirrhosis is characterized by the frequent occurrence of infections. Spontaneous bacterial peritonitis (SBP), one of the most common infections, leads to a heavy-hospital mortality rate (20-30%) despite appropriate treatment. It is now well-acknowledged that intestinal bacterial translocation is a major mechanism in the development of SBP. Prevention of SBP remains challenging in severe cirrhotic patients with ascites. The recommendation of HAS of poorly absorbable antibiotics to decrease bacterial translocation, such as norfloxacin, is well-accepted for the secondary prophylaxis of SBP, but only one third of practitioners working in French hospitals routinely prescribe norfloxacin for the primary prophylaxis of SBP (Thevenot T, et al. Liver Int 2013;33:389-97.). Although recent guidelines (EASL CPG. J Hepatol 2018;69:406-60. Biggins SW, et al. Hepatology 2021;74:1014-48) recommends a primary prophylaxis of SBP to cirrhotic patients with a low protein level in ascites (< 15 g/L) and a poor liver function or a fragile hemodynamic status (Fernández J, et al. Gastroenterology 2007;133:818-24), most French practitioners do not adhere to these guidelines probably because they are concerned with bacterial resistance associated with long-term use of norfloxacin. Moreover, the unique randomized trial assessing norfloxacin for the primary prophylaxis of SBP in severe cirrhotic patients had a small sample size (only 68 patients included) and the primary outcome (survival at 1 year) was at the limit of significance (p=0,05) mainly due to a loss of effectiveness of norfloxacin during the study period. A new strategy using a very poorly absorbed antibiotic without known major resistance (like rifaximin) could advantageously replace norfloxacin. The main purpose of this multicenter, double-blind, placebo-controlled randomized trial is to reduce the one-year

mortality rate by comparing two treatment strategies in “severe” cirrhotic patients with low protein level in ascites (< 15 g/L). Criteria of "severe" cirrhosis are the followings: 1) impaired renal function (serum creatinine ≥ 106 µmol/L, uremia ≥ 9 mmol/L or serum sodium ≤ 130 mmol/L) or 2) severe liver impairment (Child-Pugh score ≥ 9 with serum total bilirubin levels ≥ 51 µmol/L). The two proposed strategies will be to administer active or placebo rifaximin 550 mg x 2 / day for 12 months. Considering the probability of a noticeable survival benefit in the rifaximin arm, one interim analysis will be conducted, the significance boundaries being determined by a flexible alpha spending function. A total of 160 patients (80 per group) will permit to detect a 20% difference in the one-year mortality rate (60% in group with placebo versus 40% in the active rifaximin group), with a β error of 15% and a α nominal inferior to 5% (2-tailed assumption) and considering that 10% of the patients may drop-out of the study follow-up. The inclusion period will be 48 months and 17 centers will participate.

Objectives

Primary objective: To demonstrate the efficacy of rifaximin compared to placebo in reducing the risk of death at 12 months by primary prophylaxis of spontaneous ascites fluid in "severe" cirrhotic patients with high ascites containing low protein levels in ascites (< 15 g/L).

Secondary objectives:

Demonstrate the effectiveness of rifaximin compared to placebo in reducing:

- Hospital mortality rate and mortality rate at 3 and 6 months
- Incidence of spontaneous ascites fluid infection at 12 months
- Incidence of other complications of cirrhosis (hepatorenal syndrome, gastrointestinal hemorrhage, hepatic encephalopathy) at 12 months
- Number of days spent in hospital during the follow-up period
- Analyze the safety of the drug under study
- Analyze the composition of the gut microbiota in 25 patients in each group (day 1, months 3, 6, 12 and 18).
- To show that rifaximin significantly decreases (compared to placebo) serum levels of IL-6, lipopolysaccharides (LPS), copeptin, von Willebrand factor antigen and CRP between J1 and J30.

Section 3: Study Methods

3.1 Trial Design

Multicenter, double-blind, placebo-controlled randomized clinical trial,

Randomization in **two parallel groups** (group A: “active rifaximin” and group B: “rifaximin placebo”) in a 1:1 ratio and stratification on the center.

One interim analysis according to an alpha spending function approach

Investigational product

- Tablets for oral administration, containing either 550 mg of active rifaximin or rifaximin placebo
- Treatment phase [from Day 1 (post-randomization) to Study End Date]:

Group A (rifaximin 550 mg): twice daily administration of one tablet containing 550 mg of active rifaximin

Group B (rifaximin placebo): twice daily administration of one rifaximin placebo tablet

3.2 Randomization

All consecutive severe cirrhotic patients with large ascites who will be admitted to the participating centers will be screened for eligibility (see inclusion and exclusion criteria). After the patient signs the informed consent form, and after eligibility is documented based on inclusion / exclusion criteria mentioned above, the Investigator will connect to the CleanWeb web site (chrub.tentelemed.com/). For each new patient, the investigator will use the eCRF to include the patient into the study and the Interactive Web Response System

(IWRS) will affect a randomization arm to the patient. The IWRS center (INSERM CIC 1431, Besançon, France) will allocate treatment based on a pre- specified randomization list, generated by the data manager (F. Leroux) of the study, using study center as a stratification parameter. A list of treatment kit numbers corresponding to each treatment arm will be centrally generated by LC2 Pharma Company (ZA du Charpenay, 10 rue de l'Aqueduc, 69210 Lentilly, France. Contact: Anna-Rita Cherchi) and the treatment kits will be prepared in accordance with this list. Numbers will not be reused regardless of the status of the use of the corresponding study drug. The randomization will be performed in a 1:1 ratio, the number of subjects per block, block sequences will only be known by the methodologist (Pr Monnet E. and Dr Desmarets M.) and the data manager of the study. The IWRS (INSERM CIC 1431, Besançon, France) randomizes the patient into one of the two study arms and affects a randomization number as well as a treatment kit number to the patient. The IWRS developed by INSERM CIC 1431 ensures that the randomization is confirmed by email to the investigator and to the sponsor. The IWRS enables the randomization of patients at any time of the day throughout the whole study period. A technical helpdesk will provide support to investigators by phone or email. A web demonstration will be carried out in each participating center. In addition, an instruction manual describing the randomization process performed by the IWRS will be provided by the Clinical Research Assistant of the sponsor of the study to each participating center.
All patients randomized by the IWRS are irrevocably enrolled in the study, whether or not they are subsequently found to be eligible or actually receive the allocated treatment. As a consequence, patients should be followed until their last visit, until stopping rules apply or death.

3.3 Sample size

The sample size is estimated based on a projected 40% survival rate at 12 months in the placebo group and 60% in the rifaximin group and a dropout rate of 10% at 12 months. In order to be able to detect any noticeable survival benefit in the rifaximin arm early in the trial, one interim analysis will be conducted. The significance boundaries of the interim and the final analyses were determined using a flexible alpha spending function (specifically alpha time (t2) spending function). This method ensures an alpha nominal risk inferior to 0.05 for the whole analysis.
We calculated that 160 patients (80 per group) would yield an 85% power in a two-sided test of hypothesis. The interim analysis will be performed when about 70% of the expected information is available (112 patients with complete follow-up), with a significance boundary p-value of 0.01. Consequently, the final analysis will have to be performed with a significance boundary p-value of 0.043. The estimated alpha nominal risk using these critical values will be 0.048. All sample size calculations were performed using PASS 14 software.

3.4 Statistical Interim analyses and stopping guidance

One interim analysis was performed when 70% of the expected information will be available (112 patients with complete follow-up). The critical p-value was set at 0.01.

3.5 Timing of final analysis

The trial is scheduled to end with the final 12-month follow-up visit (expected around the end of June 2023). The data will be cleaned, verified, and locked. The final analysis will begin once the final gel has been confirmed by the Principal Investigator.

Section 4: Statistical Principles

4.1 Confidence Intervals and P-values

The interim analysis was performed when about 70% of the expected information is available (112 patients with complete follow-up), with a significance boundary p-value of 0.01. Consequently, the final analysis will have to be performed with a significance boundary **p-value of 0.043.**
All other tests will be two-sided, with p values of 0.05 or less denoting statistical significance. 95% confidence interval will be used to report effect estimates of secondary endpoints.

4.2 Adherence and protocol deviations

Treatment compliance for each patient will be calculated as the number of days that study drug was actually taken (i.e., number of days on treatment minus days of temporary discontinuation) divided by the number of days of follow-up. The percentage of patients at least 75% compliant will also be reported (as proposed by Israelsen M, et al. Lancet Gastroenterol Hepatol 2023;8:604).
Study drug discontinuation is defined as a minimum of 14 consecutive days without study drug intake.

4.3 Analysis populations

Modified intention to treat population (mITT)
This population will include all randomized patients who received at least 1 dose of the study drug. All patients will be analyzed in the arm defined by the random allocation. Patients wrongly included (ineligible patients that were erroneously randomized) will be excluded from the analysis.

Per protocol population
This population will exclude from the analysis:
- patients treated with the to the wrong study drug according to the arm defined by the random allocation, if applicable.
- patients who did not reach 75% compliance as defined in 4.2.
- patients who discontinued the study drug for more than 14 consecutive days

Safety population
For each study drug, the safety population will include all patients exposed to at least one dose of said study drug.

Section 5: Trial Population

5.1 Eligibility

Provide a list of eligibility criteria. Note if any changes to the eligibility criteria occurred at any time during the study (e.g., after randomization of the 1st patient).

Inclusion criteria
- Man or woman aged ≥18 and ≤ 80 years
- Cirrhosis diagnosed on clinical, radiological and/or histological findings
- Patients with large ascites (i.e., grade 3 ascites requiring paracentesis). Patients with grade 2 ascites are also authorized.
- Ascites with a low protein level in ascitic fluid (< 15 g/L) with one of the following three conditions:1) impaired renal function defined by serum creatinine ≥ 106 µmol/L, uremia ≥ 9 mmol/L or serum sodium

≤ 130 mmol/L), **or** 2) severe liver impairment defined by Child-Pugh C or Child-Pugh B ≥ 9 with serum total bilirubin levels ≥ 51 µmol/L.
- Patient who signed an informed consent form
- Patient with a social security system
- Woman must be surgically sterile or be postmenopausal, or must agree to use effective contraception during the study if childbearing potential
- Patients who need a TIPS if the procedure is performed 3 months or more following the randomization
- Patients with severe alcoholic hepatitis can be considered for inclusion if the MELD score < 30 and the Maddrey Discriminant Function < 60 providing that the patient is not infected (and consequently, will not receive antibiotics) and has not yet received corticosteroid (if necessary). Patients not responding to corticosteroid at day 7 (according to the Lille score) could be include after a wash-out of steroids for a period of 7 days.

Exclusion criteria
- Pregnant or breastfeeding woman
- Vulnerable person under French law
- Individual under legal protection measure
- Individual unable to express their consent
- Person under 18 years of age and over 80 years of age
- Transplanted patients, HIV infection (or patients who deny HIV serology) or immunosuppressive therapy (including patients under corticosteroids for severe alcoholic hepatitis if the patients respond to steroids with improvement of liver function)
- Patients with a liver transplant project and an estimated waiting time for liver transplantation of 3 months or less
- History of SBP
- Any present bacterial infection
- Patient who have received a TIPS procedure before the randomization
- Patients with an alfapump® system **(added in protocol V5)**
- Patient receiving antibiotics (including rifaximin) in the 7 days preceding the inclusion in this study except for patients participating to the microbiota study (15 days).
- Hypersensitivity to rifaximin, derivatives of rifamycin or one of the constituents of the preparation
- Hepatocellular carcinoma outside the Milan criteria, other cancer at a palliative stage
- Any clinical situation with a very low short-term prognosis (other than cirrhosis), i.e. a survival estimated lower than one month **(added in protocol V5)**
- Gastrointestinal bleeding within 7 days
- Intestinal obstruction
- Grade 3 hepatic encephalopathy (HE) during the previous 6 months before randomization
- Chronic heart failure (stage III or IV of the New York Heart Association [NYHA] Functional Classification)
- Patient judged as noncompliant
- Patients who cannot receive a clear information and who have no trusted relatives
- Patient who refuses the participation agreement by signing the information form and consent as defined in the protocol.
- Exclusion period from another biomedical study

5.2 Withdrawal/Follow-up

Criteria for stopping the study are:
- withdrawal of consent,

- liver transplantation,
- loss to follow up
- enrollment of ineligible subjects.

Patients withdrawn from the study for withdrawal of consent, liver transplantation or loss to follow up will be censored at the date of withdrawal. Ineligible subjects will be excluded from the mITT analyses.

For all other patients, monitoring of the patient will continue, follow-up will focus on collecting the endpoints of the trial:

- vital status,
- date of death,
- complications of liver cirrhosis (HE, gastrointestinal bleeding and HRS),
- dates and length of hospital stays during the 12 months following inclusion in the trial

5.3 Baseline Patient Characteristics

The description and initial comparison of randomized groups will focus on the demographic characteristics**,** clinical features of liver disease, previous complications of cirrhosis, and pre-existing treatment. Past medical history, concomitant treatments and biological results will also be compared.
Quantitative variables will be in the form of a table showing the number of values and the number of missing data, the mean and median, standard deviation, coefficient of variation, the minimum and maximum for each group and for the overall population.
Categorical variables will be in the form of a table revealing the number of values and the number of missing data, the percentage of each category and the confidence intervals for each group and for the overall population.
Continuous variables will be compared using Student's t-test or the Mann-Whitney test, as appropriate.
Categorical variables will be compared using the Chi-squared test or Fisher's exact test, as appropriate.

The description will include the following variables:
Age, years
Sex
Weight, kg
BMI, kg/m²
Comorbidities

- Type 2 diabetes
- Hypertension
- Severe acute alcoholic hepatitis (Maddrey score ≥ 32)
- Hepatocellular carcinoma within Milan criteria

Alcohol consumption

- Daily alcohol consumption if not abstinent, g

Cause of liver cirrhosis

- Alcohol
- Hepatitis B / B Delta
- Hepatitis C
- MAFLD (=NASH)
- Other

Child Pugh score
MELD score
Liver parameters

- Alanine aminotransferase, UI/L
- Aspartate aminotransferase, UI/L
- Gamma glutamyl transferase, UI/L

- Alkaline phosphatase, UI/L
- Bilirubin, µmol/L
- Platelet count, G/L
- International normalized ratio
- Prothrombin index
- Albumin, g/L
- Creatinine, µmol/L
- Baseline CRP
- CRP Day 30
- Difference between baseline and Day 30 CRP

Section 6: Analysis

6.1 Outcome Definition

Primary endpoint

12-month survival. Death, whatever the recorded cause, will be considered as the primary endpoints. The date and, whenever possible, the causes of death will be recorded.

Secondary endpoints

- Hospital survival and, 3- and 6-month survival
- Incidence of SBP during follow-up with date of diagnosis
- Incidence of the other complications of liver cirrhosis during follow-up (HE, gastrointestinal bleeding and HRS) with dates of diagnosis
- Number of days that patients spend in hospital during follow-up
- Safety analysis of the study drug
- The quantitative variations of IL-6, LPS, copeptin, CRP, vWF antigen in serum between day 1 and day 30
- The composition of the intestinal microbiota (quantity and quality of main bacterial strains) in 25 patients from groups A and B at day 1, and at months 3, 6, 12 and 18.

Definitions:

Spontaneous bacterial peritonitis: Diagnosis of SBP is based on the neutrophils cell count in ascitic fluid (> 250/mm^3) regardless the result of the ascitic fluid culture.

Hepatic encephalopathy (HE): HE is a brain dysfunction caused by liver insufficiency and portosystemic shunting. HE produces a wide spectrum of nonspecific neurological and psychiatric manifestations. Clinical HE is grading according to the West-Haven criteria.

Hepatorenal syndrome (HRS) is essentially a diagnosis of exclusion (i.e., there is an absence of other identifiable causes of renal failure). The hepatorenal syndrome is characterized by the following features in a patient who has established or clinically evident chronic liver disease:

i. Serum creatinine > 133 µM (15 mg/L),
ii. Absence of hypovolemia as defined by no sustained improvement of renal function (creatinine decreasing to <133µmol/L) following at least 2 days of diuretic withdrawal (if on diuretics), and volume expansion with albumin at 1g/kg/day up to a maximum of 100g/day.
iii. Absence of shock,
iv. No current or recent treatment with nephrotoxic drugs,
v. Absence of parenchymal renal disease as defined by proteinuria <0.5 g/day, no microhaematuria (<50 red cells/high powered field), and normal renal ultrasonography

6.2 Analysis Methods

Primary end-point analysis

The Kaplan Meier method will be used to estimate cumulative mortality rate over the 12-month follow-up. The survival rate will be compared using the log-rank test.

Hazard ratio for death (rifaximin group vs. control group) and its 95% confidence interval will be determined using a Cox proportional hazard model. In this model, a center effect will be tested as well as interaction between treatment and center. The assumption of proportional hazards will be tested using Schoenfeld residuals and if violated, the model will be fitted with a treatment- time interaction or a time-varying covariate, as appropriate.

The mortality rate at 12 months will be described according to age, sex, and specific liver scores (Child-Pugh and MELD) to examine their potential effects. Each of these factors will be analyzed statistically using a Cox proportional hazard model incorporating terms for treatment, the covariate and the treatment-by-covariate interaction.

The number of dead patients, estimated treatment effect hazard ratios, and associated 95% confidence intervals will be calculated within each of the subgroups generated by these analyses.

Covariate	**n**	**No. Deaths**	**Treatment effect HR - 95%CI**	**P value**
Intervention				
Placebo			Ref	
Rifaxim			Treatment effect	
Subgroup analyses				
Age				
<median			<median effect	—
>median			>median effect	—
Sex				
Male			Effect in men	—
Female			Effect in women	—
Child Pugh score				
B			Effect in B patients	—
C			Effect in C patients	—
Child Pugh score				
<median			<median effect	—
>median			>median effect	—

Sensitivity analyses

Analysis of the primary endpoint will be repeated after exclusion of patients with a suspicion of AAH and a Maddrey score ≥32 points.

Secondary end-points analysis

Hospital survival and, 3- and 6-month survival

The hospital survival will be estimated from the proportion of patients discharged alive. Proportions will be compared between the two groups using Chi-square test. Odds ratios for hospital death and 95% confidence intervals will be estimated by using logistic regression.

The 3-month and 6-month survival rates will be compared with the same methods as described above for mortality rate at 12 months.

Incidence of SBP during follow-up
The cumulative risk of SBP during follow-up in each group will be estimated by calculating the cumulative incidence function (CIF) of SBP at 12 months. The association of treatment group with SBP hazard function will be tested using univariate and multivariate Fine-Gray models. In this approach, death free of SBP during follow-up will be considered as a competing event.

Incidence of the other complications of liver cirrhosis during follow-up
The other complications (HE, gastrointestinal bleeding and HRS) will be analyzed as a composite event (the date of occurrence being the one of the first diagnosed complication) as well as separate events. The cumulative risk of other complications during follow-up in each group will be estimated by calculating the CIF at 12 months. The association of treatment group with the hazard function of other complications will be tested using univariate and multivariate Fine-Gray models. In this approach, death free of complications during follow-up will be considered as a competing event.

Number of days that patients spend in hospital during follow-up
Comparison between treatment groups will be performed with Student's t-test or the Mann- Whitney test, as appropriate.

Variations of IL-6, LPS, copeptin, CRP, vWF antigen in serum between day 1 and day 30.
Differences in IL-6, LPS, copeptin, CRP, vWF antigen serum values between day 1 and day 30 will be calculated and difference means in each treatment group will be compared with Student's t-test or the Mann-Whitney test, as appropriate.

Composition of the intestinal microbiota
To monitor changes in gut flora, 5 stool samples (day 1, M3, M6, M12, M18) will be collected. The composition of the intestinal microbiota (quantity and quality of main bacterial strains) will be analyzed and compared among 25 patients of each group. The patients' selection for this analysis will be performed as follows: 1) in the rifaximin arm, the 25 patients with the longer follow-up period will be retrospectively selected, 2) thereafter, 25 patients in the placebo arm will be selected by matching the follow-up period of the 25 patients selected in the rifaximin arm. In case of ties, a random drawing will be performed.
These analyses, with their sample size estimation, have been placed under the responsibility of Pr Didier Hocquet, expert bacteriologist.

6.3 Missing Data

In general, missing values will remain as missing, i.e., no attempt will be made to impute missing values and only observed values will be used in data analyses and presentations.

Survival analysis
All patients not reaching the primary endpoint at the end of follow-up or before their last assessment (for patients lost to follow-up) will be censored at the date of their last assessment visit. Patients receiving a liver transplant will be censored at the time of liver transplantation.

6.4 Harms

Safety analysis of the study drug
Safety analyses will be conducted in the safety population using the same evaluation period as for efficacy analyses (i.e. from randomization until study end date).
The number of adverse events and SAE observed in each treatment group will be described and compared by using Chi-squared test or Fisher's exact test, as appropriate. Averse events will be described in a table in order of decreasing frequency.