Official Title: Prevention of Mortality With Long-Term Administration of Human Albumin in Subjects With Decompensated Cirrhosis and Ascites

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Clinical Study Protocol

Protocol Title:	Prevention of Mortality with Long-Term Administration of Human Albumin in Subjects with Decompensated Cirrhosis and Ascites
Investigational Product:	Albutein® 20%
Sponsor's Name and Address:	Instituto Grifols, S.A. Can Guasch, 2 08150, Parets del Vallès Barcelona Spain
Additional Identifier:	PRECIOSA
EUDRACT Number:	2016-001789-28
IND/CTA Number:	17549
Development Phase:	3
Sponsor Signatories:	[redacted] MD [redacted] Grifols Therapeutics LLC Email address: [redacted] Telephone number: [redacted] [redacted] Grifols Bioscience Industrial Group Email address: [redacted] Telephone number: [redacted] See Sponsor Signatures on the cover page of the protocol

Summary of Changes for Amendment 5

Protocol Version	Date of Approval/Effective Date
6 Amendment 5 + Integrated Protocol	See Left Margin
5 Amendment 4 + Integrated Protocol	11 Mar 2020
4.0 Amendment 3 + Integrated Protocol	19 Dec 2018
3.0 Amendment 2 + Integrated Protocol	05 Jul 2018
2.0 Amendment 1 + Integrated Protocol	13 Oct 2017
1.0 Original	07 Jul 2017

Amendment 4

The protocol for IG1601 (Version 5, dated 11 Mar 2020) has been amended and reissued as Protocol Amendment 5, Version 6. See Appendix 11 for a summary of changes for Amendment 5.

Investigator Signature Page

The undersigned confirms that he/she agrees to conduct the study under the conditions described in this protocol and comply with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and all applicable regulatory requirements:

_____ _____

INVESTIGATOR NAME (Please Print) LOCATION

_____ _____

INVESTIGATOR SIGNATURE DATE

Title of the Investigator:

_____ _____

Address of the Site:

_____ _____

Telephone Number:

_____ _____

PROTOCOL SYNOPSIS

Title of Study:
Prevention of Mortality with Long-Term Administration of Human Albumin in Subjects with Decompensated Cirrhosis and Ascites

Study Number: IG1601

Phase: 3

Study Objectives:

Primary Efficacy Objective

- To evaluate the effect of standard medical treatment (SMT) plus long-term Albutein 20% (SMT + Albutein 20%) administration on 1-year transplant-free survival versus SMT alone.

Secondary Efficacy Objectives

- To evaluate the effects of SMT + Albutein 20% administration on 3- and 6-month transplant-free and overall survival versus SMT alone
- To evaluate the effects of SMT + Albutein 20% administration on 1-year overall survival versus SMT alone
- To evaluate the total number of paracenteses and the incidence of refractory ascites according the International Club of Ascites (ICA) criteria

Exploratory Objectives

The effects of SMT + Albutein 20% administration versus SMT alone on the following parameters will be evaluated:

- 3-, 6-, and 12-month incidence of acute-on-chronic liver failure (ACLF) according to the European Association for the Study of Liver- Chronic Liver Failure (EASL-CLIF) Consortium definition
- Incidence of individual organ failures: liver, renal, cerebral, coagulation, respiration, and circulation as defined by the CLIF-Consortium Organ Failure (CLIF-C OF) score and the total CLIf-C OF score
- Readmissions to the hospital due to an acute complication of cirrhosis
- Admissions to intensive care units (ICU)
- Incidence of disease-related complications (see Section 8.3.10)
- Serum albumin concentration and albumin functional capacity in serum (albumin binding capacity)
- Incidence of transjugular intrahepatic portosystemic shunt (TIPS) insertion for refractory ascites

- CLIF-C Acute-on-chronic liver failure (CLIF-C ACLF) score, CLIF-C Acute Decompensation (CLIF-C AD) score, Model for End-stage Liver Disease (MELD) score, and Child-Pugh score
- Subject's quality of life according to the EuroQol 5 Dimensions 5 Levels (EQ-5D-5L) health questionnaire.

Safety Objective

- To determine the safety and tolerability profile of SMT + Albutein 20% administration in subjects with decompensated cirrhosis and ascites

Overall Study Design and Description:

Approximately 410 subjects with decompensated cirrhosis and ascites will be included in this study after obtaining written informed consent. The study population will consist of subjects who have been hospitalized for acute decompensation of liver cirrhosis with ascites (or with prior history of ascites requiring diuretic therapy) with or without ACLF at admission or during hospitalization, but without ACLF at Screening.

There will be a screening period for enrollment of each hospitalized subject in the study. Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administred on the same day as randomization.

Randomization of subjects will be stratified by region (Europe or North America [NA]) and by whether or not subjects have been previously hospitalized for acute decompensation of liver cirrhosis prior to the most recent hospitalization for acute decompensation of liver cirrhosis with ascites as required by Inclusion Criterion #3v4 (below). Within each stratum (ie, each unique combination of region and history of hospitalization for acute decompensation of liver cirrhosis), eligible subjects will be randomized in a 1:1 ratio into 1 of 2 treatment groups:

- SMT + Albutein 20%
- SMT (control group)

SMT + Albutein 20% Treatment Group

Approximately 205 subjects will be randomized to the SMT + Albutein 20% treatment group. Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administred on the same day as randomization at the dose of 1.5 g/kg body weight (maximum 100 grams per subject). Thereafter, subjects will receive Albutein 20% infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for the rest of the study (up to a maximum of 12 months). Subjects in this treatment group will also receive SMT.

SMT Group

Approximately 205 subjects will be randomized to the control group and receive SMT. Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. Following randomization, subjects randomized to this group will receive SMT, which will be administered per institution standards.

The treatment period in the study will be up to 12 months. Subjects in both treatment groups (SMT + Albutein 20% and SMT alone) will be followed for up to 12 months. During the entire study, the safety of both treatment groups will be monitored by a Data Safety Monitoring Board.

Primary Endpoint and Secondary Endpoints:

The primary efficacy endpoint is to compare the 1-year transplant-free survival in the intent-to-treat (ITT) population between the SMT + Albutein 20% treatment group and the SMT alone group.

The secondary efficacy endpoints are to assess the effects of SMT + Albutein 20% treatment versus SMT alone on: (1) 3- and 6-month transplant-free and overall survival, (2) 1-year overall survival, and (3) total number of paracenteses and the incidence of refractory ascites.

Number of Subjects Planned:

Approximately 410 subjects will be enrolled in this study. These subjects will be randomized in a 1:1 ratio into the SMT + Albutein 20% treatment group or the SMT control group.

Study Centers Planned:

Approximately 40 study centers in the United States and Europe will participate in this study.

Diagnosis and Main Criteria for Inclusion:

Note that any criterion number containing v5 was modified in the protocol (eg, 2v5 indicates that the original criterion #2 was last modified in Protocol Version 5).

Inclusion Criteria:

A subject must meet all the following inclusion criteria to be eligible for participation in this study.

1. Male or female subject ≥18 years of age.

2v5. Subjects with diagnosis of liver cirrhosis (based on clinical, laboratory, endoscopic, and ultrasonographic features or on histology).

3v5. Subjects who have been hospitalized for acute decompensation of liver cirrhosis with ascites (or with prior history of ascites requiring diuretic therapy) with or without ACLF at admission or during hospitalization but without ACLF at Screening.

4v5. This criterion has been removed in protocol Version 5.

5. In subjects with cirrhosis due to hepatitis B virus, decompensation must occur in the setting of continuous (no less than 3 months) appropriate antiviral therapy.
6. In subjects with cirrhosis due to hepatitis C virus, only decompensated patients who will not receive antiviral therapy during the study period will be included (Subjects receiving antiviral therapy within 14 days prior to enrollment cannot be included in the study).
7. In subjects with cirrhosis due to autoimmune hepatitis, decompensation must occur in the setting of continuous immunosuppressive therapy.
8. Subjects must be willing and able to provide written informed consent or have an authorized representative able to provide written informed consent on behalf of the subject in accordance with local law and institutional policy.
9. CLIF-C AD score > 50 points at screening.

Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1v5. Subjects with ACLF at Screening
2v5. Subjects with type 1 HRS currently on treatment with vasoconstrictors or hemodialysis.
3. Subjects with TIPS or other surgical porto-caval shunts.
4v5. Subjects with refractory ascites as defined by ICA criteria without any other event of acute decompensation.
5v5. This criterion has been removed in protocol Version 5.
6v5. Subjects receiving dual anti-platelet therapy or anti-coagulant therapy (exception: DVT prophylaxis).
7v5. Subjects with ongoing endoscopic eradication of esophageal varices with ≤ 2 endoscopic sessions completed before screening.
8. This criterion has been removed from protocol Version 5
9. Subjects with evidence of current locally advanced or metastatic malignancy. Subjects with hepatocellular carcinoma within the Milan criteria (1 nodule ≤5 cm or 3 nodules ≤3 cm), non-melanocytic skin cancer, or controlled breast or prostate cancer can be included.
10v4. Subjects with acute or chronic heart failure (New York Heart Association [NYHA]).
11. Subjects with severe (grade III or IV) pulmonary disease (Global Obstructive Lung Disease [GOLD]).
12v5. Subjects with nephropathy with renal failure with serum creatinine ≥2 mg/dL or systemic hypertension.
13v5. This criterion has been removed from protocol Version 5.
14. Subjects with severe psychiatric disorders.

15. Subjects with a known infection with human immunodeficiency virus (HIV) or have clinical signs and symptoms consistent with current HIV infection.
16. Females who are pregnant, breastfeeding, or if of childbearing potential, unwilling to practice effective methods of contraception (oral, injectable, or implanted hormonal methods of contraception, placement of an intrauterine device or intrauterine system, condom, or occlusive cap with spermicidal foam/gel/cream/suppository, male sterilization, or true abstinence*) throughout the study.

    * True abstinence: When this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of the clinical study, and withdrawal are not acceptable methods of contraception).

17. Subjects with previous liver transplantation.
18. Subjects with known or suspected hypersensitivity to albumin.
19. Subjects participating in another clinical study within 3 months prior to screening.
20v4. Subjects with active drug addiction (exceptions: active alcoholism or marijuana).
21. In the opinion of the investigator, the subject may have compliance problems with the protocol and the procedures of the protocol.
22. Subjects with ongoing or recent variceal bleeding (subjects can be included 2 weeks after hemorrhagic episode).
23. Subjects with septic shock at screening.
24. Subjects with ongoing SBP infection (subjects can be included upon resolution).
25. Subjects with current infection of COVID19, those who are less than 14 days post recovery, or those who have clinical signs and symptoms consistent with COVID19 infection.

Investigational Product, Dose and Mode of Administration

Albutein 20% is the investigational product (IP).

Following randomization, subjects will receive the first Albutein 20% infusion at the dose of 1.5 g/kg body weight (maximum 100 grams per subject). Thereafter, subjects will receive Albutein 20% infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for the rest of the study (up to a maximum of 12 months). Subjects in this treatment group will also receive SMT.

Duration of Treatment:

Subject participation is up to 12 months after the screening period.

Reference Therapy, Dose, and Mode of Administration

Control Group: SMT per institution standards

Key Study Variables:

Primary Efficacy Variable:

- Time to liver transplantation or death through 1 year after randomization.

Secondary Efficacy Variables:

- Time to liver transplantation or death through 3 and 6 months after randomization, and time to death through 3 and 6 months after randomization
- Time to death through 1 year after randomization
- Total number of paracenteses and incidence of refractory ascites according the ICA criteria

Exploratory Efficacy Variables

- Incidence of ACLF (3, 6, and 12 months)
- Organ function subscores (liver, renal, cerebral, coagulation, circulation, and respiration), according to the CLIF-C OF score and the total CLIF-C OF score
- Number and length of hospital readmission for an acute complication of cirrhosis
- Number and length of ICU admission
- Incidence of disease-related complications (see Section 8.3.10)
- Serum albumin concentration and albumin functional capacity in serum (albumin binding capacity)
- Incidence of insertion of TIPS for refractory ascites
- CLIF-C ACLF score, CLIF-C AD score, MELD score, and Child Pugh score
- Quality of life: EQ-5D-5L health questionnaire

Safety Variables:

- The number of suspected adverse drug reactions (ADRs), including adverse reactions, and incidence rate of subjects with suspected ADRs during the treatment period and within 72 hours after Albutein 20% infusion completion (or after Albutein 20% infusion stops) will be considered as the main safety variables. The number and incidence rate of overall AEs and serious adverse events, as well as deaths and discontinuations due to AEs will be also collected and analyzed.
- Vital signs will be recorded at each scheduled visit and in particular before and after each Albutein 20% infusion.
- Clinical laboratory test parameters (blood cell counts, platelet count, international normalized ratio, fibrinogen, etc.) will be assessed according to the study schedule.
- Physical examinations will be assessed according to the study schedule.
- According to the investigator's assessment, all clinically relevant changes in vital function and laboratory testing parameters findings will be considered AEs.

Study Assessments and Procedures:

Screening Visit:

Following signature of the informed consent form (ICF), subjects will be screened while hospitalized. Local laboratory testing will be used for screening to determine inclusion and exclusion criteria have been met, prior to initiating treatment. Local laboratory tests performed for screening purpose only will be kept in subjects' source documents but will not be entered into the eCRF. In addition, Central Laboratory testing will be collected for the purpose of safety assessment and data analyses.

- Review of the inclusion and exclusion criteria to determine subject's eligibility
- Documentation of demographics: date of birth, gender, race, and ethnic origin
- Documentation of medical history, including etiology of cirrhosis, history, and significant concomitant disease conditions
- Urine pregnancy test (human chorionic gonadotrophin-based urine assay) for women of childbearing potential (to be performed locally at the investigative study center)
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria
- Hepatic encephalopathy evaluation
- Hemodynamics assessment (mean arterial pressure [MAP])
- Peripheral capillary oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score.
- Record endoscopic findings (if performed in the last 6 months)
- Record abdominal ultrasound (if performed in the last 6 months)
- Record ACLF grade
- Record prior (within the last 30 days) concomitant medications
- Record AEs

Treatment Period Day 1

Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administred on the same day as randomization. All assessments and activities will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Randomization (after confirmation of subject eligibility and prior to treatment)

- Record vital signs (systolic and diastolic blood pressure [BP], heart rate [HR], respiratory rate [RR], temperature [T]) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Ascites assessment according to the ICA criteria
- Record weight
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group
  - Systemic inflammatory assessment
  - Albumin functional capacity assessment (albumin binding capacity)
  - Blood biomarker retains (all clinical blood samples will need to be drawn first)
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire)
- Record AEs
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

<u>Treatment Period Day 11 and Monthly Visits (Month 1 through Month 11)</u>

Subjects will have assessments on Day 11 (±2 days), Month 1 (±2 days), and then monthly (±2 days) for up to 11 months: Days 31, 61, 91, 121, 151, 181, 211, 241, 271, 301, 331.

All assessments and activities will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Ascites assessment according to the ICA criteria
- Hepatic encephalopathy evaluation
- Hemodynamics assessment (MAP)
- Peripheral capillary oxygen saturation (SpO_2) assessment
- Assessment of TIPS insertion
- Record weight
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group
  - Systemic inflammatory assessment

- Albumin functional capacity assessment (albumin binding capacity [Day 11, Months 1, 3, 6, and 9 only])
- Blood biomarker retains (optional, all clinical blood samples will need to be drawn first) (Months 1, 3, 6, and 9 only)

- Record ACLF grade
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score
- Physical examination (Month 6 only)
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire) (Months 1, 3, 6, and 9 only)
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death (Months 3 and 6 only)
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

<u>SMT + Albutein 20% Treatment Period Days 21, 41, 51, 71, 81, 101, 111, 131, 141, 161, 171, 191, 201, 221, 231, 251, 261, 281, 291, 311, 321, 341, 351</u>

Subjects in the SMT + Albutein 20% treatment group will receive infusions every 10 (± 2) days for up to 12 months in the study. Study assessments and activities will include:

All treatment day assessments and activities will be performed prior to treatment in the SMT + Albutein 20% treatment group only. Vital signs will also be recorded in the SMT + Albutein 20% treatment group only after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Record weight
- Record concomitant medications
- Record AEs
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

<u>Month 12 Visit/ Early Discontinuation Visit</u>

All assessments and activities at the Month 12 (Day 361 ±2 days)/Early Discontinuation Visit will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)

- Ascites assessment according to the ICA criteria
- Hepatic encephalopathy evaluation
- Hemodynamics assessment (MAP)
- Peripheral capillary oxygen saturation (SpO_2) assessment
- Assessment of TIPS insertion
- Record weight
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group [Month 12 Visit only])
  - Systemic inflammatory assessment
  - Albumin functional capacity assessment (albumin binding capacity)
  - Blood biomarker retains (optional, all clinical blood samples will need to be drawn first)
- Record ACLF grade
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score
- Physical examination
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire)
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death
- Albutein 20% infusion (SMT + Albutein 20% treatment group only [Month 12 Visit only])

<u>End of Study Visit for Subjects who Complete Study Treatment</u>

Subjects will return to the study center within 1 week of study completion. End of study assessments in subjects who complete study treatment (up to 12 months) will include:

- Record concomitant medications
- Record AEs

<u>Follow-Up for Early Discontinued Subjects (Months 3, 6, 12)</u>

In subjects who discontinue from the study early, the following assessments will be performed at Months 3, 6, and 12 with a +/- 5 day window each visit:

- Record data on liver transplantation, subject survival, and cause of death

Statistical Methods:

Study Population

There will be 3 analysis populations in this study; 2 populations for efficacy assessment and 1 population for safety evaluation.

The ITT population is defined as all subjects who are randomized. The ITT population will be used for all efficacy analyses. Subjects will be grouped according to the treatment to which they are randomized in all efficacy analyses.

The Per-Protocol (PP) population is defined as the subset of subjects included in the ITT population who do not present major protocol violations which might have an impact on the primary efficacy analysis, complete at least 80% of the scheduled treatments and are not excluded from the study for interrupting the Albutein 20% treatment for more than 4 consecutive weeks (SMT + Albutein 20% treatment group subjects). The primary efficacy analyses will be carried out using the PP population in order to confirm the results based on the ITT population.

The Safety population is defined as the subset of patients who receive at least one SMT + Albutein 20% administration or SMT alone. Safety analyses will be based on this population. Subjects will be grouped according to the treatment they actually received in all safety analyses.

Primary Efficacy Analyses

This is a study to demonstrate superiority of SMT + Albutein 20% to SMT alone on 1-year transplant-free survival. The null hypothesis is that the hazard functions for liver transplantation or death are the same for the 2 treatment groups, or equivalently, the hazard ratio is equal to 1. The alternative hypothesis is that the hazard functions for liver transplantation or death are not the same for the 2 treatment groups, or equivalently, the hazard ratio is not equal to 1. The effect of SMT + Albutein 20% administration on the transplant-free survival at 1 year after randomization versus SMT alone will be summarized by means of Kaplan-Meier survival estimates and curves and compared between treatment groups by means of the Log-rank test stratified by region (Europe or NA) and history of hospitalization for acute decompensation of liver cirrhosis (yes or no). An un-stratified analysis will be performed as a sensitivity analysis.

In the event that clinically and statistically significant imbalances are observed between treatment groups in any of subjects' baseline characteristics, sensitivity analyses will be performed in which treatment effects will be adjusted by those characteristics by fitting an appropriate Cox Proportional-Hazards (PH) model. In addition, subgroup analyses by age group, sex, race, region (Europe vs NA), and history of hospitalization for acute decompensation of liver cirrhosis (yes vs no) will be carried out for the primary endpoint.

The primary efficacy analysis will be carried out on the ITT population and repeated on the PP population.

Secondary Efficacy Analyses

The analysis of the secondary endpoints will be performed using the ITT population.

The effects of SMT + Albutein 20% administration on the overall survival at 3, 6, and 12 months and transplant-free survival at 3 and 6 months after randomization versus SMT alone will be assessed by carrying out the same analyses for the primary efficacy variable described above.

Total number of paracenteses and incidence of refractory ascites will be listed for each subject, summarized by treatment group, and compared between treatment groups by means of analysis of covariance (ANCOVA) or Student's t test (for normally-distributed variables), Poisson regression model (for count variables), and Fisher's exact test or Chi-square test (for binomial variables).

The fixed-sequence testing method will be used to adjust for multiplicity in the analyses of the secondary efficacy endpoints.

Exploratory Analyses

As appropriate to the data, the original values or changes from baseline (last measurement taken prior to the start of study treatment on Day 1) of the following exploratory endpoints will be listed for each subject, summarized by treatment group, and compared between treatment groups by means of ANCOVA or Student's t tests (for normally-distributed variables), non-parametric Mann-Whitney U test (for non-parametric variables), Kaplan-Meier estimates and Log-rank test (for time-to-event variables), Poisson regression model (for count variables), and Fisher's exact test or Chi-square test (for binomial variables):

Incidence of ACLF, CLIF-C OF score, CLIF-C ACLF score, CLIF-C AD score, MELD score, Child Pugh score, number and length of hospital readmission for an acute complication of cirrhosis, number and length of ICU admission, incidence of disease-related complications, serum albumin concentration, albumin functional capacity (albumin binding capacity), incidence of insertion of TIPS for refractory ascites, and the EQ-5D-5L health questionnaire.

For the normally-distributed variables, the ANCOVA model will include change from baseline as the dependent variable, treatment as a fixed factor, and baseline value as a covariate. If any potential confounders are identified among subjects' baseline characteristics, they will be included as additional covariates in the ANCOVA models to adjust treatment effects. In addition, for the longitudinal measurements taken repeatedly over time, the treatment effects may be explored by using the mixed-effect model repeated measures analysis.

Safety Analysis

The safety analyses will be addressed by listing and tabulation of AEs (including suspected ADRs), vital signs, physical assessments, and clinical laboratory tests. Data will be described using descriptive analyses and treatment comparisons will be based on review of descriptive statistics. The safety analyses will be based on the Safety population.

Determination of Sample Size

Based on the CANONIC study, the overall 1-year transplant-free mortality rate in the current study population is assumed to be about 46%. Assuming a potential drop-out rate of 20% and 5% type-1 error for a 2-sided Chi-square test, a global sample size of 410 patients (205 per treatment group) will allow an 80% statistical power to detect an absolute reduction of 15% for the 1-year mortality rate in subjects treated with SMT + Albutein 20%.

Alternatively, the sample size was calculated based on the log-rank test as follows. Assuming the 1-year transplant or mortality rate is 46% for the control group, or equivalently, the transplant-free survival rate is 54%, which translates to a hazard rate of 0.0017 assuming an exponential distribution. Using a log-rank test at the 2-sided significant level of 5%, a total of 125 events would provide at least 80% power to detect an absolute reduction of 15% in transplant or mortality rate (a 31% 1-year transplant or mortality rate, or equivalently, a 69% transplant-free survival rate) in subjects treated with SMT + Albutein 20%. This absolute difference in transplant or mortality rates translates to a hazard ratio of 0.6022, or an approximate 40% risk reduction. With all subjects followed for the entire 1-year duration of the study, a total of 322 subjects would be required to obtain 125 events. Assuming a dropout rate of 20%, a global sample size of 404 subjects (202 per treatment group) is required. This sample size calculation was performed using Proc Power with the Twosamplesurvival statement in SAS version 9.4.

Taking a conservative approach, the larger of the 2 sample sizes (ie, the one based on the Chi-square test) will be used to ensure the study has sufficient power.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

GLOSSARY AND ABBREVIATIONS

ACLF	Acute-on-chronic liver failure
ADL	Activities of Daily Living
ADR	Adverse drug reaction
AE	Adverse event
AIFA	Agenzia Italiana del Farmaco
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AR	Adverse reaction
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
BP	Blood pressure
CI	Confidence interval
CJD	Creutzfeldt-Jakob disease
CLIF-C ACLF	Chronic Liver Failure-Consortium Acute-on-chronic liver failure
CLIF-C AD	Chronic Liver Failure-Consortium Acute Decompensation
CLIF-C OF	Chronic Liver Failure-Consortium Organ Failure
COVID-19	Coronavirus disease of 2019
CRP	C-reactive protein
DSMB	Data Safety Monitoring Board
EASL-CLIF	European Association for the Study of Liver- Chronic Liver Failure
EC	Ethics Committee
eCRF	Electronic case report form
EQ-5D-5L	EuroQol 5 Dimensions 5 Levels
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transpeptidase
GI	Gastrointestinal
GOLD	Global Obstructive Lung Disease
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HE	Hepatic encephalopathy
HIV	Human immunodeficiency virus
HNA2	Human nonmercaptalbumin-2
HR	Heart rate
HRS	Hepatorenal Syndrome
IB	Investigator's Brochure
ICA	International Club of Ascites
ICF	Informed consent form
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive care unit
INR	international normalized ratio

IP	Investigational product
IRB	Institutional Review Board
ITT	Intent-to-treat
IUD	Intrauterine device
IV	Intravenous
LDH	Lactate dehydrogenase
MAP	Mean arterial pressure
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-stage Liver Disease
NA	North America
NYHA	New York Heart Association
OPTN	Organ Procurement and Transplantation Network
PH	Proportional-Hazards
PP	Per-protocol
PRA	Plasma renin activity
RR	Respiratory rate
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Spontaneous bacterial peritonitis
SD	Standard deviation
SMT	Standard medical treatment
SOP	Standard operating procedures
SPO_2	Peripheral capillary oxygen saturation
T	Temperature
TIPS	Transjugular intrahepatic portosystemic shunt
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
WBC	White blood cells

1 GENERAL INFORMATION

Protocol title and other key study information are provided on the title page. Information regarding additional key personnel and organizations involved in the conduct of the study, including names and contact details of participating investigators, monitors, clinical laboratories, technical departments and/or institutions, as well as information on members of additional study committees, will be found in the study files of the sponsor and at the investigator study centers within the study reference manual/file.

Investigators and staff will receive training either via an investigators meeting or other appropriate individual study center training session(s).

Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be established for this clinical study to monitor the progress and the safety of the clinical study participants. The DSMB Charter will outline the DSMB composition, responsibilities, schedule of the DSMB meetings, and study stopping rules.

2 BACKGROUND INFORMATION

In addition to the information provided below, please also refer to the Investigator's Brochure (IB) (1) and any additional data supplied by the sponsor.

2.1 Name and Description of the Investigational Product

The investigational product (IP) in this study is Albumin (human) USP, 20% (Albutein® 20%), which will be administered as a treatment for decompensated cirrhosis and ascites. Albutein 20% is a solution containing 200 g/L of total protein of which at least 95% is albumin. See Section 4.4 for study treatments details.

Standard medical treatment (SMT) will be administered per institution standards. There are several published guidelines for the treatment and management of patients with decompensated cirrhosis (2-5).

2.2 Relevant Findings from Nonclinical and Clinical Studies

Effects of Long-term Human Albumin Treatment in Subjects with Decompensated Cirrhosis

Several studies assessing the effect of long-term human albumin treatment in subjects with decompensated cirrhosis have been reported (6-15). In one study, the effect of intravascular volume expansion with human albumin treatment in subjects with cirrhosis and ascites was evaluated. Hospitalized subjects were randomized to receive diuretics alone or diuretics + human albumin (12.5 g/day during hospitalization followed by 25 g/week after discharge) and were followed for a median duration of 20 months. Overall, human albumin treatment was more effective than diuretics alone in resolving ascites and reducing the length of the hospital stay; however, these results were only achieved in a subset of subjects receiving a low dose of diuretics (13).

In a second study, the effects of long-term human albumin administration on survival, recurrence of ascites, and onset of other complications were examined. Subjects hospitalized for first-onset ascites were randomized to receive diuretics alone or diuretics + human albumin (25 g/week for the first year, then 25 g every 2 weeks) and were followed for a median time of 84 months (range 2 to 120 months). In the study, subjects receiving human albumin had a significantly reduced recurrence rate of moderate to severe ascites and reduced rate of mortality (14).

A third study is a non-profit open-label, multicenter, randomized clinical study, sponsored by the Agenzia Italiana del Farmaco (AIFA) (Long-Term Use of Human Albumin for the Treatment of Ascites in Patients with Hepatic Cirrhosis: a Multicenter, Open-label Randomized Clinical Trial: The Interim Analysis of the ANSWER Study). In this study, the effectiveness of long-term human albumin administration on mortality, quality of life, and onset of other complications is being examined in subjects with cirrhosis and uncomplicated ascites (no renal failure or refractory ascites) receiving at least 200 mg per day of an anti-mineralocorticoid drug and 25 furosemide per day. Subjects are randomized to receive diuretics alone or diuretics + human albumin (40 g twice a week for 2 weeks, then 40 g/week for up to 24 months) (15).

Interim analysis of data from 386 out of a planned 420 subjects in the ANSWER study suggests that long-term human albumin administration significantly improves the management of ascites and is also able to reduce the incidence of other severe clinical complications of the disease to prolong survival in subjects with decompensated cirrhosis (15).

2.3 Known and Potential Risks and Benefits to Human Subjects

Administration of albumin has shown efficacy in prevention and treatment of circulatory dysfunction in cirrhosis (16). Paracentesis is the most used treatment in cirrhotic patients with ascites but can cause a worsening of circulatory dysfunction. Administration of albumin after paracentesis can prevent this circulatory dysfunction (17). In patients with spontaneous bacterial peritonitis (SBP), the administration of intravenous (IV) albumin improves their circulatory function, reduces the presence of hepatorenal syndrome (HRS) from approximately 30 to 10% (9), and improves patient survival (14). The administration of albumin together with vasoconstrictors reverses the circulatory dysfunction and normalizes the serum concentration of creatinine to 40 to 60% in patients with HRS type 1 and improves their survival (18,19). There are also data that suggest that long term albumin administration (25 g weekly) improves the control of ascites and reduces the incidence and intensity of the muscle cramps (20).

Based on these studies, long-term administration of human albumin has potential beneficial effects on circulatory dysfunction, inflammatory response, oxidative stress, organ dysfunction, and organ failure. Long-term treatment with human albumin was initially proposed for the management of patients with decompensated cirrhosis with ascites based on its oncotic properties. In patients with cirrhosis and portal hypertension, ascites formation is the consequence of effective hypovolemia, which promotes renal retention of sodium and water. The preservation of the central blood volume with human albumin represents a logical

approach for the management of cirrhotic patients with ascites since it would improve renal function and the response to diuretics (21).

Long-term human albumin administration may improve circulatory and organ function not only by directly acting as plasma-expander, but also indirectly modulating the deleterious effects of immune dysfunction and chronic or acute inflammation on the systemic circulation and organ function. Intravenous human albumin administration in decompensated cirrhosis increases cardiac contractility and systemic vascular resistances through the effects related to its non-oncotic properties. Maintaining an effective blood volume may support adequate organ perfusion and avoid/delay the occurrence of organ impairment/failure (21).

In addition, long-term human albumin administration may also modulate the deleterious effects of inflammatory mediators on mitochondrial and cell function and on organ microcirculation, thus protecting target organs against tissue damage and microcirculatory disturbances. These effects could potentially reduce the incidence of decompensation episodes (ie, ascites, renal or liver failure, hepatic encephalopathy (HE), severe sepsis, septic shock, and acute-on-chronic liver failure [ACLF]) and increase survival rates (21).

Albutein is made from pooled human plasma. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases, including a theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD). Although no cases of transmission of viral diseases or CJD have ever been identified for albumin, the risk of infectious agents cannot be totally eliminated (1).

Albutein is contraindicated in patients with severe anemia or cardiac failure in the presence of normal or increased intravascular volume. The use of Albutein is contraindicated in patients with a history of allergic reactions to this product. The most serious adverse reactions (ARs) are anaphylactic shock, heart failure, and pulmonary edema. The most common ARs are anaphylactoid type reactions. For additional information on potential risks and adverse events (AEs), please also refer to the IB (1) and any additional data supplied by the sponsor.

2.4 Description of and Justification for the Route of Administration, Dosage, Dosage Regimen, and Treatment Periods

2.4.1 Administration of Investigational Products

Albutein 20% will be infused at a dose of 1.5 g/kg body weight (maximum 100 g per subject) every 10 ± 2 days for the duration of study participation (up to 12 months).

2.4.2 Justification for Selection of Doses/Timing of Investigational Products

Preliminary Results of Human Albutein 20% Dose-Finding Study

The current albumin dosage in cirrhosis is based on empiric assumptions on the concept that it mainly acts as a plasma expander. However, albumin has other biological effects unrelated to its oncotic capacity (22).

A Grifols-sponsored clinical study (IG0802) was designed to test the hypothesis that normalization of serum albumin concentration by long-term exogenous albumin administration can be a target to achieve in order to potentiate the beneficial effects related to the oncotic and non-oncotic properties (23). In this open-label study, subjects with decompensated cirrhosis who were prone to develop severe circulatory dysfunction and HRS within a short period of time were treated with a low human albumin dose (1 g/kg body weight every 2 weeks for 12 weeks). Following interim analysis results, which did not demonstrate a change in plasma renin activity (PRA), human albumin was administered at a higher dose with an increased frequency (1.5 g/kg body weight every week for 12 weeks).

To assess dose effectiveness, serum human albumin levels were measured at the end of treatment. Treatment with low-dose human albumin was not sufficient to increase the serum albumin levels above 34 g/L (lower limit of normal). In this treatment group, 2 out of 7 subjects with hypoalbuminemia at the baseline visit had serum human albumin levels within the normal range at end of treatment. In contrast, treatment with high-dose human albumin was sufficient to increase serum human albumin levels to within the normal range. Eight of 8 subjects with hypoalbuminemia at the baseline visit had serum human albumin levels within the normal range at end of treatment.

In clinical study IG0802, an increase in a subject's PRA >30% with respect to baseline served to demonstrate a sustained impairment of effective blood volume. Overall, a sustained impairment of effective blood volume was observed in 29% of subjects (4 low-dose and 2 high-dose subjects). However, extreme circulatory instability (as manifested by acute, reversible increases in PRA) was documented in subjects receiving low-dose human albumin, but not in subjects receiving high-dose human albumin. Mean changes in PRA and serum human albumin concentrations correlated inversely ($r = -0.459$; $p = 0.039$), while inotropic cardiac function improved significantly and portal pressure did not change following long-term human albumin administration.

In the high-dose treatment group, the serum human albumin concentration normalized very rapidly in subjects with hypoalbuminemia and, once normalized, remained within the normal range throughout the study. No hyperalbuminemia was observed in any subject in the high-dose treatment group. The mean change of serum human albumin concentration during treatment correlated inversely with the baseline serum albumin concentration; subjects with the most severe hypoalbuminemia had the highest increases in serum human albumin.

In summary, clinical study IG0802 demonstrated that a weekly dose of 1.5 g/kg body weight was effective and tolerable in subjects with decompensated cirrhosis. In addition, the dose

appeared to be tolerable as no increase of portal pressure was documented and no cases of hyperalbuminemia developed.

2.5 Compliance Statement

This study will be conducted under the conditions described in this protocol and in compliance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), and all applicable regulatory requirements.

2.6 Study Population

The study population will consist of subjects who have been hospitalized for acute decompensation of liver cirrhosis and ascites (or with prior history of ascites requiring diuretic therapy) with or without ACLF at admission or during hospitalization, but without ACLF at Screening. Subjects may also present other acute complications (HE, gastrointestinal [GI] bleeding, and/or bacterial infections) at admission or during hospitalization.

Subjects will be screened while hospitalized for acute decompensation of liver cirrhosis due to:

1. Development of ascites alone or associated with other complications (GI, HE, acute bacterial infection).
2. Development of a non-ascitic complication in patients with prior history of ascites requiring diuretic treatment for prevention of ascites recurrence.

2.7 Relevant Data and Literature Review

Decompensated Cirrhosis

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe disruption of the intrahepatic venous flow, portal hypertension, and liver failure. Epidemiological studies indicate that there is an increasing prevalence of liver cirrhosis related to chronic infection by hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol consumption, and non-alcoholic steatohepatitis worldwide (24). The natural course of cirrhosis is divided into 2 stages. The first stage is compensated cirrhosis, which is defined as the period between the onset of cirrhosis and the appearance of the first major complication of the disease. During this period, which is relatively long in most patients (>10 years), symptoms are absent or minor, but liver lesions steadily progress. The term decompensated cirrhosis defines the period following the development of ascites, variceal bleeding, HE and bacterial infection. This period is associated with short-term survival (3 to 5 years). It is increasingly evident that patients rarely die as a consequence of an end-stage irreversible

destruction of the liver. Rather, in most patients the cause of death is an acute deterioration in their clinical condition promoted by a precipitating event, a syndrome termed ACLF.

The syndrome of ACLF had not been well characterized until recently when the CANONIC study was completed (25). The aim of the CANONIC study was to define ACLF, to assess the prevalence and clinical course of the syndrome and to assess the prognosis. The CANONIC study was an initiative of the European Association for the Study of Liver - Chronic Liver Failure (EASL-CLIF) Consortium. The EASL-CLIF Consortium defined ACLF as a syndrome that develops in patients with cirrhosis characterized by acute decompensation (AD), organ failure(s), and high short-term mortality.

Based on the CANONIC study, ACLF severity was characterized by grades based on the level of organ failure, ranging from grade 1 (single organ failure) to grade 3b (presence of ≥4 organ failures [25]). Table 2-1 lists the criteria for ACLF grades.

Table 2-1 Grades of Acute-on-Chronic Liver Failure

No ACLF	- No organ failure - One organ failure (liver failure, coagulation, circulatory or respiratory failure) with creatinine <1.5 mg/dL and no HE
ACLF grade 1a	- Single kidney failure without mild or moderate HE
ACLF grade 1b	- Single organ failure with serum creatinine ranging from 1.5 mg/dL to 1.9 mg/dL and/or mild-to-moderate HE
ACLF grade 2	- Presence of 2 organ failures
ACLF grade 3a	- Presence of 3 organ failures
ACLF grade 3b	- Presence of ≥ 4 organ failures

Source: (25)

Among patients with ACLF in the CANONIC study, 51% had ACLF-1, 35% had ACLF-2, and 13% had ACLF-3. The 90-day mortality rate ranged from 40 to 80%, with higher mortality associated with more severe ACLF (25,26). Mortality rates at Day 28 and Day 90 are shown in Table 2-2.

Table 2-2 Mortality Rates and Grades of ACLF in the CANONIC Study

	ACLF Grades				
Mortality Rates	No ACLF (n=928)	Grade 1 (n=148)	Grade 2 (n=108)	Grade 3 (n=47)	All Patients (n=1231)
28-Day	1.9%	22.1%	32.0%	76.7%	33%
90-Day	9.7%	40.7%	52.3%	79.1%	51%

Source: (25,26)

Current treatment of decompensated cirrhosis

Diuretics, antibiotics, and human albumin are the most frequently used treatments for the management of patients with cirrhosis. Based on the CANONIC study, human albumin was

indicated in 60% of subjects during hospital stay (25). Albumin is a critical determinant of the plasma oncotic pressure and therefore of circulatory homeostasis (6,7). It is also an important vehicle for the transport of water insoluble substances in plasma (ie, bilirubin, bile salts, steroids, thyroid hormones, fatty acids, drugs) (6). Perhaps the most outstanding function of albumin is that it also captures many inflammatory inducers and mediators and by this mechanism it modulates systemic inflammation and oxidative stress (14).

Serum albumin in cirrhosis is saturated by a myriad of endogenous ligands resulting in severe impairment of its transport capacity (scavenger). Albumin is also markedly oxidized in patients with decompensated cirrhosis as a consequence of the increased systemic oxidative stress in these patients (27,28). Oxidation of albumin to human nonmercaptalbumin-2 (HNA2) is irreversible and causes intense modifications of the protein structure and function and rapid clearance from the extracellular compartment (29,30).

Approximately 50% of circulating albumin in decompensated cirrhosis is oxidized and 10% is irreversible oxidized to HNA2 (31). The corresponding percentages in healthy subjects are 21% and 1%, respectively. The percentage of oxidized albumin is significantly higher in patients with ACLF than in those without ACLF. These data indicate there is a significant reduction in the scavenger capacity of serum albumin for inflammatory inducers and mediators. This reduction may participate in the exaggerated response to the pro-inflammatory precipitating events observed in these patients (21).

Therefore, substitution of the saturated and highly oxidized endogenous albumin molecules by exogenous albumin with higher functionality is therefore another potentially important beneficial effect of Albutein 20% in patients with decompensated cirrhosis.

2.7.1 Rationale for Selection of Study Population

An analysis of subjects in the CANONIC study (25) was performed to determine if the subject population was distinct from the ANSWER study population (15) with respect to mid-term mortality. The CANONIC study data base was composed of 867 subjects who were hospitalized with decompensated cirrhosis and ascites (with or without ACLF) and discharged from hospital without ACLF. The 1-year transplant-free mortality rate in these subjects was 44.5%. This finding indicates that subjects hospitalized by acute decompensation with or without ACLF at admission or during hospitalization, but without ACLF at discharge represent a distinct cohort of subjects who have decompensated cirrhosis with an increased fatality rate within 1 year after discharge. Based on these findings, the PRECIOSA study will be performed in this cohort of patients.

3 STUDY OBJECTIVES AND PURPOSE

The current study aims to assess the effectiveness of long-term Albutein 20% administration in the treatment of subjects with decompensated cirrhosis and ascites.

3.1 Efficacy Objectives

3.1.1 Primary Efficacy Objective

- To evaluate the effect of SMT plus long-term Albutein 20% (SMT + Albutein 20%) administration on 1-year transplant-free survival versus SMT alone.

3.1.2 Secondary Efficacy Objectives

- To evaluate the effects of SMT + Albutein 20% administration on 3- and 6-month transplant-free and overall survival versus SMT alone
- To evaluate the effects of SMT + Albutein 20% administration on 1-year overall survival versus SMT alone
- To evaluate the total number of paracenteses and the incidence of refractory ascites according the International Club of Ascites (ICA) criteria (Appendix 2)

3.1.3 Exploratory Efficacy Objectives

The effects of SMT + Albutein 20% administration versus SMT alone on the following parameters will be evaluated:

- 3-, 6-, and 12-month incidence of ACLF according to the EASL-CLIF Consortium (CLIF-C) definition (24)
- Incidence of individual organ failures: liver, renal, cerebral, coagulation, respiration, and circulation as defined by the CLIF-C Organ Failure (CLIF-C OF) score and the total CLIF-C OF score (Appendix 3)
- Readmissions to the hospital due to an acute complication of cirrhosis
- Admissions to intensive care units (ICU)
- Incidence of disease-related complications (see Section 8.3.10)
- Serum albumin concentration and albumin functional capacity in serum (albumin binding capacity [Table 7-1])
- Incidence of transjugular intrahepatic portosystemic shunt (TIPS) insertion for refractory ascites
- CLIF-C Acute-on-chronic liver failure (CLIF-C ACLF) score,CLIF-C Acute Decompensation (CLIF-C AD) score, Model for End-stage Liver Disease (MELD) score, and Child Pugh score
- Subject's quality of life according to the EuroQol 5 Dimensions 5 Levels (EQ-5D-5L) health questionnaire (Appendix 5).

3.2 Safety Objectives

- To determine the safety and tolerability profile of SMT + Albutein 20% administration in subjects with decompensated cirrhosis and ascites

4 STUDY DESIGN

4.1 Primary Endpoint and Secondary Endpoints

The primary efficacy endpoint is to compare the 1-year transplant-free survival in the intent-to-treat (ITT) population (see Section 9.7 for definition) between the SMT + Albutein 20% treatment group and the SMT alone group.

The secondary efficacy endpoints are to assess the effects of SMT + Albutein 20% treatment versus SMT alone on: (1) 3- and 6-month transplant-free and overall survival, (2) 1-year overall survival, and (3) total number of paracenteses and the incidence of refractory ascites.

4.2 Study Design and Plan

This is a phase 3, multicenter, randomized, controlled, parallel-group, and open-label clinical study to evaluate the efficacy of SMT + Albutein 20% administration versus SMT alone in subjects with decompensated cirrhosis and ascites. This clinical study is planned to be performed in approximately 40 study centers in the United States and Europe. The protocol must be approved by regulatory authorities and the Institutional Review Board (IRB) or Ethics Committee (EC) of each of the participating centers.

Approximately 410 subjects with decompensated cirrhosis and ascites will be included in this study after obtaining written informed consent. The study population will consist of subjects who have been hospitalized for acute decompensation of liver cirrhosis with ascites (or with prior history of ascites requiring diuretic therapy) with or without ACLF at admission or during hospitalization, but without ACLF at Screening.

There will be a screening period for enrollment of each hospitalized subject in the study. Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administred on the same day as randomization.

Randomization of subjects will be stratified by region (Europe or North America [NA]) and by whether or not subjects have been previously hospitalized for acute decompensation of liver cirrhosis prior to the most recent hospitalization for acute decompensation of liver cirrhosis with ascites as required by Inclusion Criterion #3v4 (Section 5.1). Within each stratum (ie, each unique combination of region and history of hospitalization for acute decompensation of liver cirrhosis), eligible subjects will be randomized in a 1:1 ratio into 1 of 2 treatment groups:

- SMT + Albutein 20%
- SMT (control group)

SMT + Albutein 20% Treatment Group

Approximately 205 subjects will be randomized to the SMT + Albutein 20% treatment group. Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administered on the same day as randomization at the dose of 1.5 g/kg body weight (maximum 100 grams per subject).Thereafter, subjects will receive Albutein 20% infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for the rest of the study (up to a maximum of 12 months). Subjects in this treatment group will also receive SMT (see Section 2.1).

SMT Group

Approximately 205 subjects will be randomized to the control group and receive SMT (see Section 2.1). Screening should occur while the subject is hospitalized. Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. Following randomization, subjects randomized to this group will receive SMT, which will be administered per institution standards. Several guidelines are published for the management of decompensated cirrhosis (2-5).

The treatment period in the study will be up to 12 months. See Appendix 1 for detailed study schedules. Subjects in both treatment groups (SMT + Albutein 20% and SMT alone) will be followed for up to 12 months.

The overall study schema is shown in Figure 4-1.

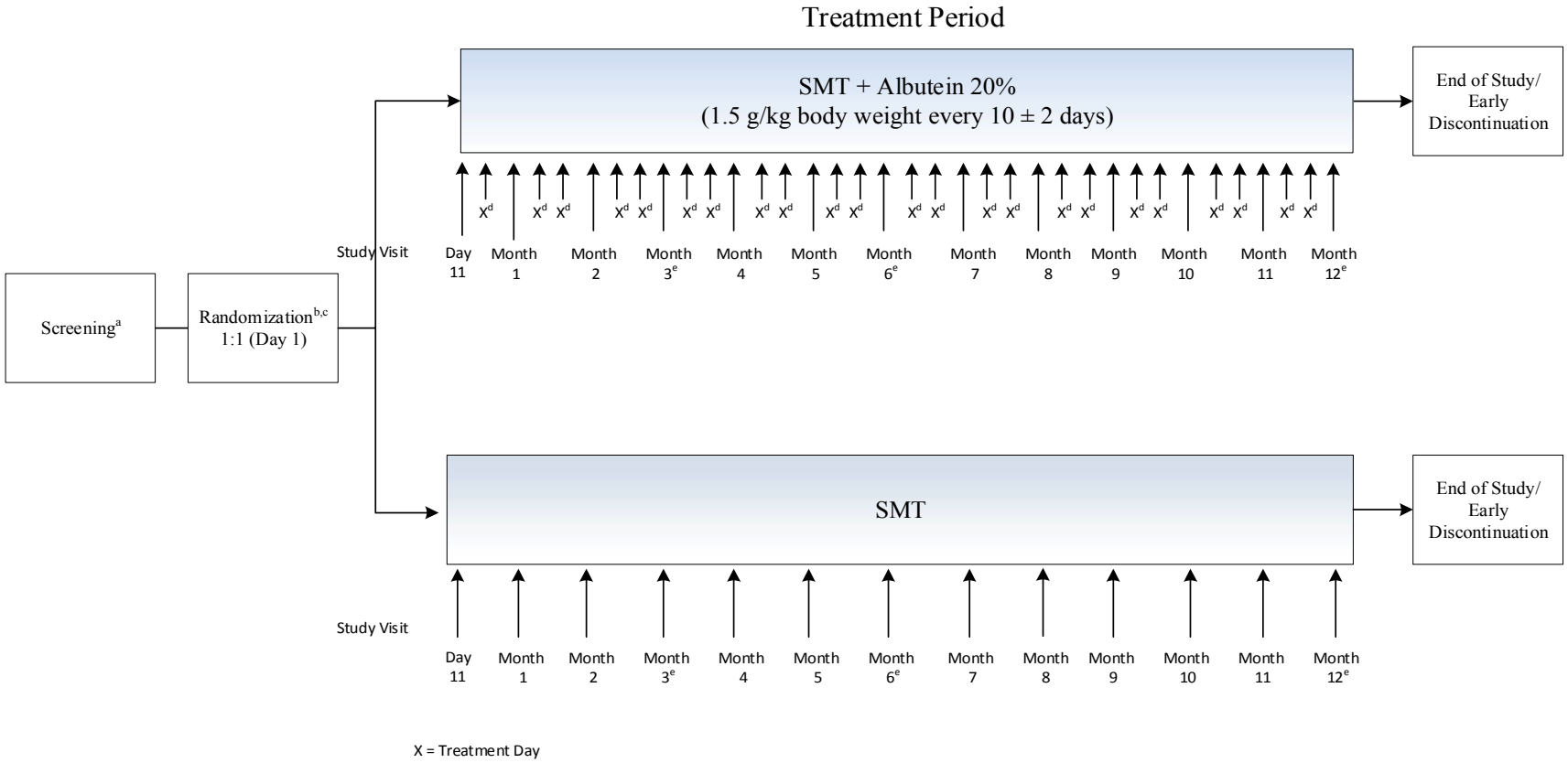


Figure 4-1 Overall Study Schema

[a] Screening must occur during hospitalization.

[b] Randomization (Day 1) must occur within 72 hours of screening (all efforts should be made to randomize subject as soon as they are determined eligible)

[c] Subjects in the SMT + Albutein 20% treatment group will receive infusion following randomization. Randomization and first Albutein 20% infusion should occur on the same day.

[d] Subjects in the SMT + Albutein 20% treatment group will receive infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for duration of the study (up to a maximum of 12 months)

[e] Overall and transplant-free survival will be assessed at 3, 6, and 12 months

4.3 Measures Taken to Minimize/Avoid Bias

4.3.1 Subject Numbering

Within each study center, subjects in the study will receive a consecutive subject number. Subject numbers are generated beginning with the study center number (3 digits, assigned by the sponsor) followed consecutively with a unique number for each subject (4 digits, beginning with the number "2"). For example, if the investigator's center number is 301, subject number will be 3012001, 3012002, 3012003, etc., in consecutive order. Subject numbers, once assigned, will not be reused at any center.

4.3.2 Randomization

Subjects who satisfy the inclusion criteria and who do not present any of the exclusion criteria will be enrolled in the study. Randomization must occur within 72 hours (3 days) of Screening. Randomization will be stratified by region (Europe or NA) and by whether or not subjects have been previously hospitalized for acute decompensation of liver cirrhosis prior to the most recent hospitalization for acute decompensation of liver cirrhosis with ascites as required by Inclusion Criterion #3v4 (Section 5.1). Within each stratum (ie, each unique combination of region and history of hospitalization for acute decompensation of liver cirrhosis), subjects will be randomly assigned to 1 of 2 treatment groups (SMT + Albutein 20% versus SMT alone) in a 1:1 ratio. Randomization will be centralized and will be performed via an Interactive Web Response System after subject inclusion. A computer-generated randomization list will be used for treatment allocation.

4.3.3 Blinding

Not applicable. This is an open-label study.

4.4 Study Treatments

4.4.1 Treatments to Be Administered

4.4.1.1 Standard Medical Treatment + Albutein 20%

Albutein 20% is the IP in this study and is purified from human plasma. The solution is normally clear to slightly opalescent. Do not use the IP if the solution is cloudy or has precipitated. The product must be warmed to room temperature before use.

Do not dilute with water for injection. Once the container is open, the content must be administered immediately. Unused content must be discarded.

For additional information, see the IB.

Subjects allocated to this treatment group will receive SMT + Albutein 20%.

4.4.1.2 Standard Medical Treatment

Subjects allocated to the control group will receive SMT, which will be administered per institution standards. Several guidelines are published for the management of decompensated cirrhosis (2-5). Diuretic therapy and dose modifications will be permitted on the basis of the evolution of ascites decompensation. Total paracentesis can be performed in the presence of tense ascites or symptoms due to the accumulation of abdominal fluid and will be followed by the administration of albumin. A TIPS will be considered when there are no other contraindications for this procedure for subjects with GI bleeding, refractory ascites, or hydrothorax. Complications such as bacterial infections, including SBP, or acute kidney injury, including type 1 HRS, will be treated according to current indications, which may require albumin infusion.

The usual treatment for comorbidities (ie, cardiac and respiratory diseases and diabetes) will be allowed according to the investigator's medical judgement. Any medication administered during the study (including any blood products) will be recorded in the electronic case report form (eCRF) as well as in the subject's medical record.

4.4.2 Labeling and Packaging of Investigational Product

Investigational products will be labeled according to the requirements of local law and legislation. Label text will be approved according to agreed Grifols procedures, and a copy of the labels will be made available to the study center. Each vial and corresponding box will have a label stating the protocol number and will specify that the product is only to be used in the clinical study.

4.4.3 Storage of Investigational Product

Albutein is stable for 3 years providing storage temperature does not exceed 25ºC. Protect from freezing. Do not use if the solution is turbid or if there is sediment in the bottle.

The product should be warmed to room temperature before use. Do not dilute with sterile water for injection. Once the container is open, the contents must be administered immediately. Do not begin administration more than 4 hours after the container has been entered. No remaining product in an opened container can be stored in the refrigerator and used at a later time. This product must not be used beyond the expiry date stated on the labeling.

Access to the IP must be strictly limited. The pharmacist or designee is responsible for maintain storage temperature records and for immediately reporting deviations in temperature to the study monitor.

4.5 Expected Duration of Subject Participation in the Study

The expected study duration of each subject in the study (after enrollment) will be up to 12 months.

4.6 Discontinuation Criteria for Individual Subjects and Study

4.6.1 Discontinuation Criteria for Individual Subjects

See Section 5.3 Subject Withdrawal Criteria.

4.6.2 Premature Termination of Study/Closure of Center

The sponsor, IRB/EC, and/or regulatory authorities have the right to close this study or a study center, and the investigator/sponsor has the right to close a center, at any time, although this should occur only after consultation between involved parties. The IRB/EC must be informed. Should the study/center be closed prematurely, all study materials (except documentation that has to remain stored at study center) must be returned to the sponsor. The investigator will retain all other documents until notification is given by the sponsor for destruction.

A study center can be closed for the following reasons:

- Lack of enrollment
- Non-compliance with the requirements of the study protocol
- Non-compliance with ICH GCP

4.7 Accountability Procedures for Investigational Product

Albutein 20% is to be used only for the study in accordance with the directions given in this protocol. The study pharmacist or designee is responsible for the distribution of the IP in accordance with directions given in the protocol and pharmacy manual.

The study pharmacist or designee is responsible for maintaining accurate records of the IP for his/her study center. Investigational product inventory/dispensing documentation verifying the receipt, dispensing, destruction, and return must be maintained and kept current by the pharmacist or designee. The unused inventory must be made available for inspection during the study by the monitor. Any unused material may be destroyed at the center according to their standard operating procedures (SOPs) or may be returned to the sponsor if the study center's SOPs do not allow local destruction.

Unused IP supplies must be accounted for by the monitor and inventory/dispensing logs must be verified by the monitor prior to IP destruction. Written documentation from Grifols or designee of any unused inventory is required. At the end of the study, a copy of the inventory/dispensing log(s) and Interactive Web Response System dispensing documents will be retrieved by the monitor and returned to Grifols.

4.8 Maintenance of Treatment Randomization Codes

Access to the actual randomization schedules or codes must be strictly controlled during the course of the study.

4.9 Data to Be Recorded Directly on the Case Report Forms

Not applicable.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

Eligible participants for this study include male and female adult subjects who are hospitalized with decompensated cirrhosis and ascites without ACLF at Screening. Subjects who fail to meet the eligibility criteria may not be re-screened.

Note that any criterion number containing v*5* was modified in the indicated protocol waws modified in the protocol (eg, 2v5 indicates that the original criterion #2 was last modified in Protocol Version 5).

5.1 Inclusion Criteria

Inclusion Criteria:

A subject must meet all the following inclusion criteria to be eligible for participation in this study:

1. Male or female subject ≥18 years of age.
2v5. Subjects with diagnosis of liver cirrhosis (based on clinical, laboratory, endoscopic, and ultrasonographic features or on histology).
3v5. Subjects who have been hospitalized for acute decompensation of liver cirrhosis with ascites (or with prior history of ascites requiring diuretic therapy) with or without ACLF at admission or during hospitalization but, without ACLF at Screening.
4v5. This criterion has been removed from Version 5 of the protocol.
5. In subjects with cirrhosis due to HBV, decompensation must occur in the setting of continuous (no less than 3 months) appropriate antiviral therapy.
6. In subjects with cirrhosis due to HCV, only decompensated patients who will not receive antiviral therapy during the study period will be included (subjects receiving antiviral therapy within 14 days prior to enrollment cannot be included in the study).
7. In subjects with cirrhosis due to autoimmune hepatitis, decompensation must occur in the setting of continuous immunosuppressive therapy.
8. Subjects must be willing and able to provide written informed consent or have an authorized representative able to provide written informed consent on behalf of the subject in accordance with local law and institutional policy.
9v5. CLIF-C score > 50 points at screening.

5.2 Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1v5. Subjects with ACLF at Screening.

2v5. Subjects with type 1 HRS currently on treatment with vasoconstrictors or hemodialysis.

3. Subjects with TIPS or other surgical porto-caval shunts.

4v5. Subject with refractory ascites as defined by ICA criteria without any other event of acute decompensation (Appendix 2).

5v5. This criterion has been removed from protocol Version 5.

6v5. Subjects receiving dual anti-platelet therapy or anti-coagulant therapy (exception: DVT prophylaxis).

7v5. Subjects with ongoing endoscopic eradication of esophageal varices with ≤2 endoscopic sessions completed before screening.

8v5. This criterion has been removed from protocol Version 5.

9. Subjects with evidence of current locally advanced or metastatic malignancy. Subjects with hepatocellular carcinoma within the Milan criteria [1 nodule ≤5 cm or 3 nodules ≤3 cm] [Appendix 6], non-melanocytic skin cancer, or controlled breast or prostate cancer can be included.

10v4. Subjects with acute or chronic heart failure (New York Heart Association [NYHA] Appendix 7).

11. Subjects with severe (grade III or IV) pulmonary disease (Global Obstructive Lung Disease [GOLD] Appendix 8).

12v5. Subjects with nephropathy with renal failure with a serum creatinine ≥2 mg/dL or systemic hypertension.

13v5. This criterion has been removed from protocol Version 5.

14. Subjects with severe psychiatric disorders.

15. Subjects with a known infection with human immunodeficiency virus (HIV) or have clinical signs and symptoms consistent with current HIV infection.

16. Females who are pregnant, breastfeeding, or, if of childbearing potential, unwilling to practice effective methods of contraception (oral, injectable, or implanted hormonal methods of contraception, placement of an intrauterine device (IUD) or intrauterine system, condom, or occlusive cap with spermicidal foam/gel/cream/suppository, male sterilization, or true abstinence*) throughout the study.

    * True abstinence: When this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of the clinical study, and withdrawal are not acceptable methods of contraception).

17. Subjects with previous liver transplantation.

18. Subjects with known or suspected hypersensitivity to albumin.

19. Subjects participating in another clinical study within 3 months prior to screening.

20v4. Subjects with active drug addiction (exceptions: active alcoholism or marijuana)

21. In the opinion of the investigator, the subject may have compliance problems with the protocol and the procedures of the protocol.

22. Subjects with ongoing or recent variceal bleeding. (subjects can be included 2 weeks after hemorrhagic episode).
23. Subjects with septic shock at screening.
24. Subjects with ongoing SBP infection (subjects can be included upon resolution).
25. Subjects with current infection of COVID19, those who are less than 14 days post recovery, or those who have clinical signs and symptoms consistent with COVID19 infection.

5.3 Subject Withdrawal Criteria

5.3.1 Screen Failures

Screening evaluations will be used to determine the eligibility of each subject for enrollment into this study. Subjects who fail to meet eligibility criteria during screening evaluations will be considered screen failures and will not participate in the study.

5.3.2 Discontinuation of Subjects from Treatment

If a subject receives a liver transplant, study treatment and all treatment-related assessments will be discontinued upon transplant. The criteria to determine when a subject becomes a candidate for liver transplantation is described in Section 5.3.2.1. The subject will continue to have subsequent planned visit assessment procedures performed.

Discontinuation of the treatment in subjects who receive a TIPS insertion will be at the discretion of the investigators. In case it is decided to discontinue the subject's treatment early, study treatment will be stopped and the subject will continue to have subsequent planned visit assessment procedures performed.

Adverse events that will determine temporary subject's discontinuation from study treatment include the following:

- Subjects developing severe bleeding, Albutein 20% infusions will be temporarily stopped until the bleeding episode is resolved. For subjects with variceal bleeding, Albutein 20% infusions will be temporarily stopped until the subject has received at least 2 banding sessions. Albutein 20% infusions will be resumed after the bleeding episode is resolved or after the subject has received at least 2 banding sessions, respectively.

Adverse events that will determine permanent subject's discontinuation from study treatment include the following:

- Anaphylactic shock or transfusion-related acute lung injury
- Severe AEs (ie, acute myocardial infarction, cerebral hemorrhage, cardiac failure, pulmonary edema, or intestinal necrosis) that precludes the continuation of the subject in the clinical study

Subjects with any one of these severe AEs will be discontinued from study treatment and all treatment-related assessments, and should continue to have subsequent planned visit assessment procedures performed.

5.3.2.1 Liver Transplantation Criteria

To confirm that a subject meets the criteria for a liver transplant, the MELD score and the Child Pugh score (Appendix 3) will be assessed. Priority status will be defined by the MELD score and should be considered once a subject with cirrhosis experiences an index complication such as ascites, HE, variceal hemorrhage, and/or hepatocellular dysfunction. Subjects with an index complication and a MELD score ≥15, a Child-Pugh score of ≥7, worsening renal dysfunction, or other evidence of rapid hepatic decompensation should be promptly evaluated for a liver transplant.

Additional criteria for liver transplantation in subjects with hepatocellular carcinoma or worsening within the Milan criteria (Appendix 6) with no CONTRAINDICATIONS as determined by the Organ Procurement and Transplantation Network (OPTN) Dynamic Imaging criteria (31) are as follows:

- Subject is not a candidate for total resection
- The hepatocellular carcinoma meets the definition of a Stage T2 lesion(s) that includes any of the following:
  - One lesion ≥2 cm and ≤5 cm
  - Two or 3 lesions ≥1 cm and ≤3 cm
  - No macrovascular involvement
  - No identifiable extrahepatic spread of tumor to surrounding lymph nodes, lungs, abdominal organs, or bone

5.3.3 Removal of Subjects

Subjects may withdraw or be withdrawn from the study for the following reasons:

1. At their own request or at the request of their legally acceptable representative.
2. If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being.
3. At the specific request of the sponsor*

* Subjects will only be removed at the request of the sponsor if a safety concern is identified that may potentially cause harm to a subject or if the risk of the specified treatment outweighs the benefit.

4. Post-consent decision due to inclusion error (determination of ineligibility based on safety or eligibility criteria).
5. Physician’s judgment following an AE after treatment.
6. Termination of the study by a regulatory authority.

In all cases, the reason for withdrawal must be recorded in the eCRF and in the subject's records.

5.3.4 Subject Replacement

Subjects who are randomized and withdrawn from the study will not be replaced.

5.3.5 Follow-up of Subjects Withdrawn from Study

If possible, blood samples will be obtained to complete all pertinent tests at the time of subject withdrawal. In all cases, the reason for withdrawal must be recorded in the eCRF and in the subject's records. No subject can be re-enrolled into the study after having been withdrawn from the study.

Subjects who receive any amount of IP and discontinue early from the study should have all Month 12/Early Discontinuation visit procedures performed at the time of withdrawal (preferably within 1 week of withdrawal).

All efforts should be made to collect the vital status of the subject (including data on liver transplantation, subject survival, and cause of death) at Months 3, 6, and 12.

6 TREATMENT OF SUBJECTS

See Section 4.4 for the treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration.

6.1 Administration and Timing of Investigational Products for Each Subject

6.1.1 Albutein 20% Dosage, Procedures, and Treatment Regimen

Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized or after discharge from the hospital. The first dose of Albutein 20% is administered on the same day as randomization at a dose of 1.5 g/kg body weight (maximum 100 grams per subject). Thereafter, subjects will receive Albutein 20% infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for the rest of the study (up to a maximum of 12 months). At each administration, the infusion rate should be 4 mL/min.

6.1.2 Standard Medical Treatment

Standard medical treatment will be administered within 3 days (72 hours) of screening, and following randomization, per institution standards. Several guidelines are published for the management of decompensated cirrhosis (2-5). Subjects will receive SMT (see Section 4.4.1.2) for the length of the study or as long as needed.

6.2 Prior and Concomitant Therapy

Prior (within 30 days prior to screening) and concomitant medications must be recorded in the eCRF and subject's source documents, including the trade or generic names of the medication, the dose, the route of administration, and the duration of the medication (frequency).

Any medication administered during the study (including any blood products) will be recorded on the eCRF and subject's source documents with the exception of anesthesia related products, fluid replacement therapies, and electrolyte supplementation. In addition, products administered at different doses on the same day to manage and maintain vital functions (eg, insulin, furosemide) will only be recorded once on the eCRF and subject's source documents with the dosage reported as pro re nata.

In the event that an AE or suspected adverse drug reaction (ADR) should occur, complete details of all concomitant medications administered will be reported to the sponsor.

Usual treatments in decompensated cirrhosis will be allowed.

Treatment with terlipressin and albumin will be allowed in both treatment groups only in subjects developing type-1 HRS according to the ICA criteria (Appendix 2).

6.2.1 Prohibited Medications Prior to Study Participation

Subjects receiving dual anti-platelet therapy or anti-coagulant therapy prior to enrollment (within 7 days prior to enrollment [exception: DVT prophylaxis]) cannot be included. In addition, subjects with HCV receiving antiviral therapy within 14 days prior to enrollment cannot be included.

6.2.2 Prohibited Concomitant Medications during the Study

Use of the following medications is prohibited during the study participation:

Albumin dialysis will not be allowed during the treatment period in the SMT + Albutein 20% treatment group.

6.2.3 Restricted Concomitant Medications during the Study

This section describes medications that are restricted but not prohibited during the study participation:

Platelet transfusion will be made in cases of severe thrombocytopenia (<20,000/mm^3), or in the case of bleeding complications when the platelet count is below 50,000/mm^3.

6.2.4 Cardiac Overload/Pulmonary Edema Treatment

In subjects with the diagnosis of cardiac overload/pulmonary edema, standard protocols will be applied as per institution standards and may include fluid restriction, IV administration of furosemide, and Renal Replacement Therapy if clinically indicated (diuresis lower than

0.5 mL/kg under high doses of furosemide). See Appendix 9 for an example of Algorithm for Cardiogenic Pulmonary Overload and Appendix 10 for guidelines on the management of pulmonary edema.

6.3 Treatment Compliance

Reasons for any deviation from the administration of less than 100% of the IP dose as prepared by the pharmacist or designee must be recorded in the eCRF and in the subject's medical records.

7 ASSESSMENT OF EFFICACY

7.1 Efficacy Variables

7.1.1 Primary Efficacy Variable

- Time to liver transplantation or death through 1 year after randomization.

7.1.2 Secondary Efficacy Variables

- Time to liver transplantation or death through 3 and 6 months after randomization, and time to death through 3 and 6 months after randomization
- Time to death through 1 year after randomization
- Total number of paracenteses and incidence of refractory ascites according the ICA criteria (Appendix 2)

7.1.3 Exploratory Variables

- Incidence of ACLF (3, 6, and 12 months)
- Organ function subscores (liver, renal, cerebral, coagulation, circulation, and respiration), according to the CLIF-C OF score and the total CLIF-C OF score (Appendix 3)
- Number and length of hospital readmission for an acute complication of cirrhosis
- Number and length of ICU admission
- Incidence of disease-related complications (see Section 8.3.10)
- Serum albumin concentration and albumin functional capacity in serum (albumin binding capacity [Table 7-1])
- Incidence of insertion of TIPS for refractory ascites
- CLIF-C ACLF score ,CLIF-C AD score, MELD score, and Child Pugh score
- Quality of life: EQ-5D-5L health questionnaire (Appendix 5)

7.2 Methods and Timing for Assessing, Recording, and Analyzing Efficacy Parameters

7.2.1 Observations and Measurements

The following is a description of the procedures/assessments to take place at each study visit. See the Schedules of Study Procedures and Events in Appendix 1.

7.2.1.1 Screening Visit

Following signature of the informed consent form (ICF), subjects will be screened while hospitalized. Local laboratory testing will be used for screening to determine inclusion and exclusion criteria have been met, prior to initiating treatment. Local laboratory tests performed for screening purpose only will be kept in subjects' source documents but will not be entered into the eCRF. In addition, Central Laboratory testing will be collected for the purpose of safety assessment and data analyses.

If a subject is ineligible for participation, their demographic data and specific reason for ineligibility will be captured on the subject's source documents and eCRF.

The screening assessments and activities will include:

- Assign subject number
- Add subject's data into the Screening Log
- Review of the inclusion and exclusion criteria which include local labs (see Sections 5.1 and 5.2, respectively) to determine subject's eligibility
- Documentation of demographics: date of birth, gender, race, and ethnic origin
- Documentation of medical history, including etiology of cirrhosis, history, and significant concomitant disease conditions
- Urine pregnancy test (human chorionic gonadotrophin [HCG]-based urine assay) for women of childbearing potential (to be performed locally at the investigative study center)
  - Women of child-bearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal (postmenopausal is defined as amenorrhea for more than 12 consecutive months or women on hormone replacement therapy with documented serum follicle stimulating hormone level <35 mIU/mL). Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an IUD or barrier methods to prevent pregnancy or practicing abstinence or where their sexual partner is sterile (eg, vasectomy) should be considered to be of child-bearing potential.
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 2)
- Hepatic encephalopathy evaluation (Appendix 4)
- Hemodynamics assessment (mean arterial pressure [MAP])

- Peripheral capillary oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score (Appendix 3).
- Record endoscopic findings (if performed in the last 6 months)
- Record abdominal ultrasound (if performed in the last 6 months)
- Record ACLF grade (see Table 2-1)
- Record prior (within the last 30 days) concomitant medications
- Record AEs

7.2.1.2 Treatment Period (Day 1 through Month 12)

7.2.1.2.1 TREATMENT DAY 1

Randomization must occur within 72 hours of screening, and it can occur while the subject is hospitalized of after discharge from the hospital. The first dose of Albutein 20% is administered on the same day as randomization. All assessments and activities will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Randomization (after confirmation of subject eligibility, hospital discharge (if applicable), and prior to treatment)
- Record vital signs (systolic and diastolic blood pressure [BP], heart rate [HR], respiratory rate [RR], temperature [T]) (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group)
- Ascites assessment according to the ICA criteria (Appendix 2)
- Record weight
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group)
  - Systemic inflammatory assessment
  - Albumin functional capacity assessment (albumin binding capacity)
  - Blood biomarker retains (all clinical blood samples will need to be drawn first)
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire [Appendix 5])
- Record AEs
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

7.2.1.2.2 TREATMENT DAY 11 AND MONTHLY VISITS (MONTH 1 THROUGH MONTH 11)

Subjects will have assessments on Day 11 (±2 days), Month 1 (±2 days), and then monthly (±2 days) for up to 11 months: Days 31, 61, 91, 121, 151, 181, 211, 241, 271, 301, 331.

All assessments and activities will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Ascites assessment according to the ICA criteria (Appendix 2)
- Hepatic encephalopathy evaluation (Appendix 4)
- Hemodynamics assessment (MAP)
- Peripheral capillary oxygen saturation (SpO_2) assessment
- Assessment of TIPS insertion
- Record weight
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group
  - Systemic inflammatory assessment
  - Albumin functional capacity assessment (albumin binding capacity [Day 11, Months 1, 3, 6, and 9 only])
  - Blood biomarker retains (optional, all clinical blood samples will need to be drawn first) (Months 1, 3, 6, and 9 only)
- Record ACLF grade (see Table 2-1)
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score (Appendix 3).
- Physical examination (Month 6 only)
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire [Appendix 5]) (Months 1, 3, 6, and 9 only)
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death (Months 3 and 6 only)
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

7.2.1.2.3 SMT + ALBUTEIN 20% TREATMENT DAYS 21, 41, 51, 71, 81, 101, 111, 131, 141, 161, 171, 191, 201, 221, 231, 251, 261, 281, 291, 311, 321, 341, 351

Subjects in the SMT + Albutein 20% treatment group will receive infusions every 10 (± 2) days for up to 12 months in the study. Study assessments and activities will include:

All treatment day assessments and activities will be performed prior to treatment in the SMT + Albutein 20% treatment group only. Vital signs will also be recorded in the SMT + Albutein 20% treatment group only after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Record weight
- Record concomitant medications
- Record AEs
- Albutein 20% infusion (SMT + Albutein 20% treatment group only)

7.2.1.2.4 MONTH 12 VISIT/ EARLY DISCONTINUATION VISIT

All assessments and activities at the Month 12 (Day 361 ±2 days)/Early Discontinuation Visit will be performed prior to treatment. In the SMT + Albutein 20% treatment group, vital signs and serum albumin concentration will also be recorded after treatment. Study assessments and activities will include:

- Record vital signs (systolic and diastolic BP, HR, RR, T) (measured immediately prior to and immediately after the end of the Albutein 20% infusion in the SMT + Albutein 20% treatment group)
- Ascites assessment according to the ICA criteria (Appendix 2)
- Hepatic encephalopathy evaluation (Appendix 4)
- Hemodynamics assessment (MAP)
- Peripheral capillary oxygen saturation (SpO_2) assessment
- Assessment of TIPS insertion
- Record weight
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group [Month 12 Visit only])
  - Systemic inflammatory assessment
  - Albumin functional capacity assessment in serum (albumin binding capacity)

- Blood biomarker retains (optional, all clinical blood samples will need to be drawn first)

- Record ACLF grade (see Table 2-1)
- Illness severity scores evaluation: CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score (Appendix 3).
- Physical examination
- Record concomitant medications
- Quality of life assessment (EQ-5D-5L health questionnaire [Appendix 5])
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death
- Albutein 20% infusion (SMT + Albutein 20% treatment group [Month 12 Visit only])

7.2.1.3 End of Study Visit for Subjects who Complete Study Treatment

Subjects will return to the study center within 1 week of study completion. End of study assessments in subjects who complete study treatment (up to 12 months) will include:

- Record concomitant medications
- Record AEs

7.2.1.4 Follow-Up for Early Discontinued Subjects (Months 3, 6, 12)

In subjects who discontinue from the study early, the following assessments will be performed at Months 3, 6, and 12:

- Record data on liver transplantation, subject survival, and cause of death

7.2.2 Description of Laboratory Tests and Procedures

Detailed descriptions of laboratory test procedures are located in the study Laboratory Manual. Table 7-1 provides a summary of the laboratory tests conducted for this study.

Table 7-1 Name, Description, and Location of Laboratory Tests and Procedures

Test Panel	Description	Location
Hematology[a]	Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count with differential	Central
Chemistry[a]	Sodium, potassium, creatinine, blood urea nitrogen, calcium, magnesium, lactate dehydrogenase, AST, ALT, gamma-glutamyl transpeptidase, ALP, glucose, total bilirubin, direct and indirect bilirubin, C-reactive protein (CRP), lactate, serum albumin	Central
Coagulation[a]	International normalized ratio (INR)[a], fibrinogen, platelet count, prothrombin time	Central
Urine pregnancy test	Qualitative urine β-HCG will be performed at Screening	Local
Albumin functional capacity assessment	Albumin binding capacity	Central
Systemic inflammation assessment	Procalcitonin, WBC (hematology panel), CRP (chemistry panel)	Central
Biomarkers retains[b,c]	Blood	Central

Abbreviations: ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase; BUN – blood urea nitrogen; CRP – C-reactive protein; GGT – gamma-glutamyl transpeptidase; HCG - Human chorionic gonadotropin; INR – international normalized ratio; LDH – lactate dehydrogenase; WBC – white blood cells.

[a] Laboratory tests required for determination of illness severity scores are presented in Appendix 3. A central laboratory will be used for all laboratory testing, including results required for illness severity scoring (eg, creatinine, bilirubin, INR). At the Screening visit only, local laboratory tests will be performed in order to determine subject eligibility. Screening local laboratory test results will be kept in subjects' source documents but will not be entered into the eCRF.

[b] Biomarker retains will be drawn for potential analysis of cytokines (interleukin [IL] 6 (IL-6), IL-8, IL-10, and tumor necrosis factor alpha [TNFα]) found in the blood which are significantly correlated with disease activity. Blood samples will be stored for possible future analysis of other biomarkers that may be identified throughout the length of the clinical study and beyond. These samples will be frozen and retained for 15 years at which time if no analysis is performed, the samples will be destroyed. All clinical blood samples will need to be drawn first.

[c] For subjects in the SMT + Albutein 20% treatment group, biomarker retains should be collected prior to infusion.

7.2.3 Drug Concentration Measurements

As indicated, serum albumin concentration (clinical chemistry laboratory assessment) will be measured on Day 1 and throughout the treatment period (see Appendix 1). In the SMT + Albutein 20% treatment group, measurements will be collected prior to and after treatment administration.

8 ASSESSMENT OF SAFETY

8.1 Safety Variables

Safety of Albutein 20% will be evaluated in this study.

- The number of suspected ADRs, including ARs, and incidence rate of subjects with suspected ADRs during the treatment period and within 72 hours after Albutein 20% infusion completion (or after Albutein 20% infusion stops) will be considered as the main safety variables. The number and incidence rate of overall AEs and SAEs, as well as deaths and discontinuations due to AEs will be also collected and analyzed.
- Vital signs will be recorded at each scheduled visit and in particular before and after each Albutein 20% administration.
- Clinical laboratory test parameters (blood cell counts, platelet count, INR, fibrinogen, etc.) will be assessed according to the study schedule.
- Physical examinations will be assessed according to the study schedule.
- According to the investigator's assessment, all clinically relevant changes in vital function and laboratory testing parameters findings will be considered AEs.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

For timing of safety assessments, please see Section 7.2.1.

8.2.1 Adverse Events

Adverse events (includes suspected ADRs) occurring at any time between signing of the subject's ICF and the last day of the subject's participation (End of Study or Early Discontinuation Visit) in the clinical study will be reported and recorded on the appropriate subject's eCRF entry and subject's source documents.

It is the investigator's responsibility to ensure that all AEs including suspected ADRs are appropriately recorded.

8.2.2 Vital Signs

Vital signs will be measured by a medically certified individual or a nurse according to his/her standard clinical practice.

The following vital signs will be assessed:

- Temperature
- Blood pressure (systolic and diastolic BP)
- Heart rate
- Respiratory rate

Vital signs will be routinely monitored by the study staff as detailed in Appendix 1. The investigator will be required to classify vital signs abnormalities as clinically significant or not according to his/her criteria. Results will be recorded in source documents and on the subject eCRF. Vital signs abnormalities judged by the investigator as clinically significant will be considered AEs.

8.2.3 Clinical Laboratory Evaluations

Considering the medical history inherent in subjects with decompensated cirrhosis, certain laboratory abnormalities, while clinically significant, will be expected in this medical context, and would not indicate treatment-emergent worsening or a new AE (being instead an extension of medical history/underlying pre-existing conditions). Examples include: low platelet count, decreased albumin, prolonged prothrombin time/INR, elevated bilirubin, and abnormal transaminases. These conditions would be expected laboratory consequences of cirrhosis/decompensated liver diseases. These results would not be considered individual AEs as they most likely reflect manifestations of existing cirrhosis and hepatic decompensation, unless abnormalities worsen after initiation of study treatment and become accentuated to a medically significant degree in a treatment-emergent fashion.

Central clinical laboratory data for renal, hepatic, and hematological parameters will be listed for each clinical study subjects.

The investigator will be required to classify laboratory results out of the normal range reported by the central laboratory as clinically significant or not according to his/her criteria.

Laboratory results out of the normal range judged by the investigator as clinically significant will be considered AEs.

8.2.4 Physical Examinations

A medically certified individual will conduct a physical examination.

8.3 Procedures for Eliciting Reports of and for Recording and Reporting Adverse Event and Intercurrent Illnesses

The investigator is responsible for ensuring that all staff involved in the clinical study are familiar with the content of this section.

8.3.1 Warnings/Precautions

For complete information on Albutein 20% refer to the IB.

8.3.2 Adverse Event Monitoring

Subjects must be carefully monitored for AEs. This monitoring includes clinical and laboratory tests and physical signs. Adverse events should be assessed in terms of their seriousness, severity, and causal relationship to the IP.

Adverse events will be elicited by spontaneous reporting by the study individual or by a non-leading inquiry or direct observation by the study staff.

8.3.3 Adverse Event Definitions

8.3.3.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product or study treatment and which does not necessarily have a causal relationship with this administration. An AE can therefore be any unfavorable and unintended sign (including any abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This includes worsening of a pre-existing condition or increase in frequency of a pre-existing condition.

An AE is also defined as any occurrence that is new in onset, expected, or aggravated in severity or frequency from a baseline condition, or abnormal results or diagnostic procedures including laboratory test abnormalities.

In this clinical study, deterioration of the targeted disease and symptoms associated with the targeted disease will not be considered an AE as long as the deterioriation is anticipated and there is no evidence suggesting a causal relationship between the study drug and the AE. Anticipated disease progression includes, but not limited to: HE, GI bleeding, portal hypertension, ascites, SBP, hyponatremia and HRS (see Section 8.3.10).

8.3.3.2 Suspected Adverse Drug Reactions/Adverse Reactions

All noxious and unintended responses to a medicinal product or study treatment related to any dose should be considered suspected ADRs. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product or study treatment and an AE is at least a reasonable possibility. An AR is a subset of the suspected ADRs that are deemed as definitely related to the treatment. See below for additional details.

8.3.4 Assessment of Causality of Adverse Event

The investigator is required to provide a causality assessment for each AE reported to the sponsor. The sponsor will consider the investigator’s causality assessment and also provide its own assessment. Causal relationship to the study drug will be established according to medical judgment on whether there is a **reasonable possibility of a causal relationship between the AE and the study drug.**

The investigator must determine and classify the AE causality according to the following categories:

Unrelated/Not related: there is not a reasonable possibility of causal relationship between the AE and the study drug.

Possibly related: there is evidence to suggest a causal relationship between the study drug and the AE.

Definitively related: there is a reason to conclude that the study drug caused the AE.

Criteria to assess the causal relationship should take into account of the following conditions:

- A plausible temporal sequence from the study drug administration to the AE onset
- Whether the event follows a known response pattern to the suspected treatment
- Whether the AE could be reasonably explained by the subject's clinical state, comorbidities, or concomitant medications
- The occurrence of improvement on stopping/reducing the treatment (positive dechallenge) and/or reappearance of the event on repeated exposure (positive rechallenge)

For expedited safety reporting purposes, AEs assessed as either "definitively related" or "possibly related" will be considered POTENTIALLY RELATED or just RELATED.

8.3.5 Severity of Adverse Event or Suspected Adverse Drug Reaction

AEs including suspected ADRs will be classified according to severity, depending on the intensity of the event, as follows:

Mild: an AE which is well tolerated by the subject, causing minimum degree of malaise and without affecting activities of daily living (ADL).

Moderate: an AE that interferes with ADL.

Severe: an AE that prevents ADL.

Severity gradation of AEs including suspected ADRs must be distinguished from their seriousness gradation, which is defined according to event consequence. For example, headache can be mild, moderate or severe but not necessarily serious in all these cases. See Section 8.3.7 for Seriousness of AEs.

The investigator will be responsible for assessing the severity of AEs including suspected ADRs during the clinical study.

8.3.6 Expectedness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR is considered "unexpected" if the nature, seriousness, severity or outcome of the reaction(s) is not consistent with the reference information. The expectedness shall be determined by the sponsor according to the reference document (ie, IB or Summary of Product Characteristics) for any serious ADRs (potentially related serious adverse events [SAEs]) for expedited safety reporting purposes.

8.3.7 Seriousness of Adverse Event or Suspected Adverse Drug Reaction

Adverse Events including suspected ADRs are considered "serious" if, in the view of either the investigator or sponsor, they result in any of the following outcomes:

- Death

- Life-threatening AE (life-threatening in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- In-patient hospitalization or prolongation of existing hospitalization*
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- An important medical event (important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening, or result in death, or hospitalization, but from medical and scientific judgment may jeopardize the subject or/and may require medical or surgical intervention to prevent one of the other outcomes listed above)

* After the initial hospital discharge, any hospitalization is to be considered only hospital stay for equal or more than 24 hours. The following hospitalizations should not be reported as SAEs:

- Hospitalization or prolongation of hospitalization as part of a routine procedure followed by the center or clinical practice (according to the investigator's criteria) for the treatment of decompensated cirrhosis
- Hospitalization for a survey visit, annual physicals, or social reasons
- Elective or pre-planned hospitalizations for a pre-existing condition that had not worsened from Baseline (eg, elective or scheduled surgery arranged prior to start of the study)
- Admissions not associated with an AE (e.g. social hospitalization for purposes of respite care)

This definition permits either the sponsor or the investigator to decide whether an event is "serious." If either the sponsor or the investigator believes that the event is serious, the event must be considered "serious" and evaluated by the sponsor for expedited reporting.

8.3.8 Adverse Event Documentation

All AEs and SAEs occurring after the subject has **signed the ICF through the Final Visit (ie, End of Study or Early Discontinuation Visit)** must be fully recorded in the subject's eCRF and SAE form (if serious) as well as in the medical record. If no AE has occurred during the study period, this should also be indicated in the eCRF.

It is the responsibility of the investigator to ensure that AEs are appropriately recorded.

At each visit, AEs will be elicited by asking the individual a non-leading question such as "Do you feel different in any way since the last visit?" Moreover, AEs will also be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms, and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible.

The following variables must be recorded in the AE eCRF:

- The verbatim term (a diagnosis is preferred)
- Date/time of onset
- Date/time of resolution
- Severity (mild, moderate, severe)
- Causality (unrelated/not related, possibly related, definitely related)*
- Seriousness (yes, no)
- Action taken (with regard to IP)
- Other action (to treat the event)
- Outcome and sequel (follow-up on AE)

* An AE occurring before subject's exposure to treatment will be labeled as "unrelated/not related".

If it is a preexisting AE that gets worse regarding intensity or frequency, it should be indicated.

For AEs that occur during infusions, the infusion rate in effect at the time of onset of the AE (if applicable), the time of onset of the AE and the time of AE change materially in intensity and/or resolve will be captured in the eCRF.

In addition to the investigator's own description of the AEs, each AE will be encoded according to the Medical Dictionary for Regulatory Activities (MedDRA).

For example, a laboratory test abnormality considered clinically significant, eg, causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged clinically significant in the context of the subject's medical history by the investigator, should be reported as an AE. Each event must be described in detail along with start and stop dates, severity, relationship to treatment, action taken, and outcome. Each event must be adequately supported by documentation as it appears in the subject's medical or case file.

8.3.9 Event of Special Interest

There are no events of special interest related to Albutein 20%.

8.3.10 Disease Progression

In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the IP is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated disease progression includes, but not limited to: HE, GI bleeding, portal hypertension, ascites, SBP, hyponatremia and HRS.

The development of sequelae of decompensated cirrhosis during the clinical study should be considered as disease progression and not an AE, unless there is evidence suggesting a causal relationship between the study drug and the AE. Manifestations of decompensated cirrhosis will be treated as disease progression and will be documented in the eCRF and subject's medical records, but not immediately reported to the sponsor.

Hospitalization due to disease progression and/or the signs and symptoms of disease progression should not be reported as an SAE during the study.

8.3.11 Reporting of Serious Adverse Events, Deaths, and Pregnancy

8.3.11.1 Reporting of Serious Adverse Events

Any SAE (see Section 8.3.7) that occurs after **signing the study ICF through the Final Visit (ie, End of Study or Early Discontinuation Visit)** must be expeditiously reported whether or not considered attributable to the study drug. Each SAE must be fully recorded in the subject's eCRF and SAE Report Form. In addition, any SAE that occurs after the end of study/early discontinuation should be reported if the investigator feels that the event is related to the use of IP.

SAEs will be reported using the designated SAE Report Form. When the investigator becomes aware of an SAE, she/he must submit a completed, signed, and dated SAE Report Form (in English) **within 24 hours** to the sponsor by email/fax. The date of this SAE discovery by the study center staff should be documented in the source documents (ie, medical records).

Each SAE must be followed up until resolution or stabilization. After the initial report, all relevant information for SAE follow-up, and for the outcome, must also be supplied to the sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form. In addition, the sponsor or contract research organization may request additional information and/or reports.

All SAE Report Forms must be reported to:

Grifols Global Pharmacovigilance

Email: [redacted]
FAX (back-up only): [redacted] (USA)
[redacted] (International)

When required, and according to local law and regulations, SAEs must be reported to the IRB/EC and regulatory authorities. Copies of the investigator's reports must be sent to the sponsor.

8.3.11.2 Reporting of Deaths

The following considerations should be made for all deaths occurring during the clinical study:

- Any death that is unequivocally due to disease progression should not be reported as an SAE, but should be documented in the eCRF as well as in the subject's medical record.
- Any death that is not unequivocally due to disease progression should be immediately reported as an SAE (see Section 8.3.11.1) as well as documented in the subject's medical record. Every effort should be made to establish a cause of death. Reporting of the SAE should include a comment regarding the co-involvement of progression of disease, if appropriate, and should assign a single primary cause of death (AE) together with any contributing causes.

8.3.11.3 Reporting Pregnancy

While pregnancy itself is not a true "AE," pregnancy occurring in a clinical study must be followed to collect information regarding the experiences of gestation and pregnancy. The investigator must report any pregnancy that occurs in a study subject or partner of a male subject from the first treatment exposure until 28 days after the last dose of treatment. A pregnancy will not be considered an AE unless a relation to the treatment is suspected. A Pregnancy Report Form must be completed and sent as soon as possible to the sponsor. A copy of the form should be filed at the study center for pregnancy follow-up until delivery or to the end of pregnancy. Any anomalies, complications, abnormal outcomes, or birth defect observed in the child must be reported as an SAE within 24 hours of the investigator or study personnel's first knowledge.

Please use the email address or fax numbers (back up only) in Section 8.3.11.1 for reporting pregnancy.

8.4 Type and Duration of the Follow-Up of Subjects after Adverse Events

In so far as is possible, all individuals will be followed up until the AE or suspected ADR has been resolved. If an AE/suspected ADR/SAE is present when the subject has completed the study, the course of the event must be followed until the final outcome is known, or the event has been stabilized and no further change is expected and the investigator decides that no further follow-up is necessary.

9 STATISTICS

9.1 Statistical Methods

Unless otherwise specified, descriptive statistics will include the number of non-missing observations, mean, standard deviation (SD), median, minimum and maximum values for the continuous/quantitative data or absolute and relative frequency counts and percentages for categorical/qualitative data.

Data handling and evaluation procedures will be described in the Statistical Analysis Plan (SAP).

9.1.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized by treatment group. For quantitative variables, mean, SD, median, minimum, and maximum will be provided. For qualitative variables, the frequency and percentage will be provided.

9.1.2 Efficacy Analysis

9.1.2.1 Primary Endpoint

This is a study to demonstrate superiority of SMT + Albutein 20% to SMT alone on 1-year transplant-free survival. The null hypothesis is that the hazard functions for liver transplantation or death are the same for the 2 treatment groups, or equivalently, the hazard ratio is equal to 1. The alternative hypothesis is that the hazard functions for liver transplantation or death are not the same for the 2 treatment groups, or equivalently, the hazard ratio is not equal to 1. The effect of SMT + Albutein 20% administration on the transplant-free survival at 1 year after randomization versus SMT alone will be summarized by means of Kaplan-Meier survival estimates and curves and compared between treatment groups by means of the Log-rank test stratified by region (Europe or NA) and history of hospitalization for acute decompensation of liver cirrhosis (yes or no). An un-stratified analysis will be performed as a sensitivity analysis.

In the event that clinically and statistically significant imbalances are observed between treatment groups in any of subjects' baseline characteristics, sensitivity analyses will be performed in which treatment effects will be adjusted by those characteristics by fitting an appropriate Cox Proportional-Hazards (PH) model. In addition, subgroup analyses by age group, sex, race, region (Europe vs NA), and history of hospitalization for acute decompensation of liver cirrhosis (yes vs no) will be carried out for the primary endpoint.

The primary efficacy analysis will be carried out on the ITT population and repeated on the Per-Protocol (PP) population (see Section 9.7 for description of subject populations for analysis).

9.1.2.2 Secondary Endpoints

The analysis of the secondary endpoints will be performed using the ITT population.

The effects of SMT + Albutein 20% administration on the overall survival at 3, 6, and 12 months and transplant-free survival at 3 and 6 months after randomization versus SMT alone will be assessed by carrying out the same analyses for the primary efficacy variable described above.

Total number of paracenteses and incidence of refractory ascites will be listed for each subject, summarized by treatment group, and compared between treatment groups by means of analysis of covariance (ANCOVA) or Student's t test (for normally-distributed variables),

Poisson regression model (for count variables), and Fisher's exact test or Chi-square test (for binomial variables).

The fixed-sequence testing method will be used to adjust for multiplicity in the analyses of the secondary efficacy endpoints. Specifically, the superiority of SMT + Albutein 20% versus SMT alone for the secondary efficacy endpoints will only be tested if the superiority for the primary efficacy endpoint is demonstrated at the 2-sided significance level of 5%. For the secondary efficacy endpoints, each subsequent hypothesis is tested only if the superiority for the previous comparison(s) has been demonstrated at a 2-sided significance level of 5%. The order in which the null hypotheses will be tested is predetermined for the secondary efficacy endpoints as follows:

1. Time to death through 1 year after randomization
2. Time to liver transplantation or death through 6 months after randomization
3. Time to death through 6 months after randomization
4. Time to liver transplantation or death through 3 months after randomization
5. Time to death through 3 months after randomization
6. Total number of paracenteses
7. Incidence of refractory ascites according the ICA criteria

9.1.2.3 Exploratory Endpoints

As appropriate to the data, the original values or changes from baseline (last measurement taken prior to the start of study treatment on Day 1) of the following exploratory endpoints will be listed for each subject, summarized by treatment group, and compared between treatment groups by means of ANCOVA or Student's t tests (for normally-distributed variables), non-parametric Mann-Whitney U test (for non-parametric variables), Kaplan-Meier estimates and Log-rank test (for time-to-event variables), Poisson regression model (for count variables), and Fisher's exact test or Chi-square test (for binomial variables):

Incidence of ACLF, CLIF-C OF score, CLIF-C ACLF score, CLIF-C AD score, MELD score, Child Pugh score, number and length of hospital readmission for an acute complication of cirrhosis, number and length of ICU admission, incidence of disease-related complications, serum albumin concentration, albumin functional capacity (albumin binding capacity), incidence of insertion of TIPS for refractory ascites, and the EQ-5D-5L health questionnaire.

For the normally-distributed variables, the ANCOVA model will include change from baseline as the dependent variable, treatment as a fixed factor, and baseline value as a covariate. If any potential confounders are identified among subjects' baseline characteristics, they will be included as additional covariates in the ANCOVA models to adjust treatment effects. In addition, for the longitudinal measurements taken repeatedly over time, the treatment effects may be explored by using the mixed-effect model repeated measures analysis.

9.1.3 Safety Analysis

The safety analyses will be addressed by listing and tabulation of AEs (including suspected ADRs), vital signs, physical assessments, and clinical laboratory tests. Data will be described using descriptive analyses and treatment comparisons will be based on review of descriptive statistics. The safety analyses will be based on the Safety population.

9.1.3.1 Adverse Events

Adverse events will be coded and classified using MedDRA® terms (system organ class and preferred terms).

When a causal relationship of an AE is classified by the investigator as definitively or possibly related, the event will be defined as a suspected ADR. A suspected ADR with a causal relationship of "definitively related" will be defined as an AR. The sponsor will consider the investigator's causality assessment and also provide its own assessment. If there is any disagreement in the causality assessment between the investigator and the sponsor, a separate summary of suspected ADRs/ARs will be provided.

For summary purpose, AEs will be classified as treatment-emergent AEs (TEAEs) or non-TEAEs depending on the comparison of AE onset date/time with the start date/time of study treatment. A TEAE will be defined as an AE which occurs between the start of study treatment and the final visit of the clinical study. A non-TEAE will be defined as an AE which occurs prior to the start of study treatment. Non-TEAEs and TEAEs will be summarized separately.

In addition, TEAEs, including suspected ADRs, will be summarized by each treatment group, system organ class, preferred term, causal relationship, severity, and seriousness (serious versus non-serious) using descriptive statistics. At each level of summarization, a subject will only be counted once per system organ class or preferred term using the most severe AE or the AE with the strongest causal relationship to the treatment.

AEs temporally associated with the infusion of the IP (ie, infusional AEs, including infusional suspected ADRs) will be summarized by presenting infusion/subject incidences and percentages and listed.

Subjects with SAEs, AEs leading to death, or who withdraw from the study because of an AE will also be individually listed and summarized.

9.1.3.2 Clinical Laboratory Values

All clinical laboratory data (Table 7-1) will be listed for each subject and summarized by treatment group.

The investigator will be required to classify out of the normal range laboratory results reported by the laboratory as clinically significant or not according to his/her criteria.

Out of the normal range laboratory results judged by the investigator as clinically significant in the context of the subject's medical history will be considered AEs.

9.1.3.3 Vital Signs

Vital signs (T, RR, HR, systolic and diastolic BP) will be listed for each subject and summarized by treatment group. In case a subject presents a clinically significant abnormality of vital signs during an infusion, the event will be flagged and reported as an AE temporally associated with the infusion.

Clinically significant vital signs abnormalities will be presented as AEs. Clinical relevance will be based on the investigator's criteria.

9.1.3.4 Physical Assessment

Physical assessment findings (normal and abnormal) will be listed for each subject and summarized by treatment group. Any clinically significant abnormality, as determined by the investigator, experienced by a subject during the clinical study and not already present at baseline will be reported as AE.

9.2 Determination of Sample Size

Based on the CANONIC study, the overall 1-year transplant-free mortality rate in the current study population is assumed to be about 46% (24,26). Assuming a potential drop-out rate of 20% and 5% type-1 error for a 2-sided Chi-square test, a global sample size of 410 patients (205 per treatment group) will allow an 80% statistical power to detect an absolute reduction of 15% (15) for the 1-year mortality rate in subjects treated with SMT + Albutein 20%.

Alternatively, the sample size was calculated based on the log-rank test as follows. Assuming the 1-year transplant or mortality rate is 46% for the control group, or equivalently, the transplant-free survival rate is 54%, which translates to a hazard rate of 0.0017 assuming an exponential distribution. Using a log-rank test at the 2-sided significant level of 5%, a total of 125 events would provide at least 80% power to detect an absolute reduction of 15% in transplant or mortality rate (a 31% 1-year transplant or mortality rate, or equivalently, a 69% transplant-free survival rate) in subjects treated with SMT + Albutein 20%. This absolute difference in transplant or mortality rates translates to a hazard ratio of 0.6022, or an approximate 40% risk reduction. With all subjects followed for the entire 1-year duration of the study, a total of 322 subjects would be required to obtain 125 events. Assuming a dropout rate of 20%, a global sample size of 404 subjects (202 per treatment group) is required. This sample size calculation was performed using Proc Power with the Twosamplesurvival statement in SAS version 9.4.

Taking a conservative approach, the larger of the 2 sample sizes (ie, the one based on the Chi-square test) will be used to ensure the study has sufficient power.

9.3 Level of Significance to Be Used

All statistical tests will be 2-sided and the significance level will be fixed at 0.05.

More details including approaches for multiplicity adjustments (if needed) will be provided in the SAP.

9.4 Criteria for Termination of the Study

The criteria for termination of the study are listed in Section 4.6.2. Stopping rules for the study will also be provided separately in the DSMB charter.

Study stopping rules for individual subjects is presented in Section 5.3.

9.5 Procedure for Accounting for Missing, Unused, and Spurious Data

The procedure for accounting for missing, unused, and spurious data will be detailed in the SAP.

9.6 Reporting Deviations from the Statistical Analysis Plan

The detailed statistical analysis methodologies will be documented in the SAP. If there are any deviations from the originally planned analyses in the SAP, they will be fully described and justified in the protocol amendment(s) and/or final clinical study report.

9.7 Subject Population for Analysis

There will be 3 analysis populations in this study; 2 populations for efficacy assessment and 1 population for safety evaluation.

The ITT population is defined as all subjects who are randomized. The ITT population will be used for all efficacy analyses. Subjects will be grouped according to the treatment to which they are randomized in all efficacy analyses.

The PP population is defined as the subset of subjects included in the ITT population who do not present major protocol violations which might have an impact on the primary efficacy analysis, complete at least 80% of the scheduled treatments and are not excluded from the study for interrupting the Albutein 20% treatment for more than 4 consecutive weeks (SMT + Albutein 20% treatment group subjects). The primary efficacy analyses will be carried out using the PP population in order to confirm the results based on the ITT population.

The Safety population is defined as the subset of patients who receive at least one SMT plus Albutein 20% treatment or SMT. Safety analyses will be based on this population. Subjects will be grouped according to the treatment they actually received in all safety analyses.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The data will be recorded and kept current in eCRFs by the study center personnel directly responsible for the information and reviewed for completeness by the monitor. Grifols personnel or designee can review the records.

In accordance with ICH GCP guidelines, the monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the eCRFs for consistency and to verify adherence to the protocol, and the completeness, consistency, and accuracy of data entered. "Source documentation" includes individual subject files, separate from the eCRFs, which should be maintained and include visit dates, laboratory results, concomitant treatment, vital signs, medical history, examinations, AEs, IP dispensing logs, and other notes as appropriate. The investigator agrees to cooperate with the monitor to ensure that any problems noted during the course of these monitoring visits are resolved.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Monitoring and auditing procedures defined/agreed by the sponsor will be followed, in order to comply with ICH GCP guidelines. Each center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, ICH GCP and legal aspects. The on-site verification of the eCRF for completeness and clarity will include cross checking with source documents, and clarification of administrative matters. Query verification of data will be described in the Data Management Plan.

Representatives of regulatory authorities or of Grifols may conduct audits or inspections or audits of the investigator study center. If the investigator is notified of an audit or inspection by a regulatory authority, the investigator agrees to notify the Grifols representative (eg, Clinical Assessment Monitor, Program Leader, or Program Manager) immediately. The investigator agrees to provide to representatives of a regulatory agency or Grifols access to records, facilities, and personnel for the effective conduct of an audit or inspection.

12 ETHICS

12.1 Institutional Review Board/Ethics Committee

Documented approval from appropriate IRBs/ECs will be obtained for all participating centers/countries prior to study start, according to ICH GCP guidelines, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IRB's/EC's approval must be obtained and also forwarded to the sponsor. Copies of the investigator's report to the IRBs/ECs must be sent to the sponsor. The IRBs/ECs must supply to the sponsor, upon request, a list of the IRB's/EC's members involved in the vote and a statement to confirm that the IRB's/EC's is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

12.2 Ethical Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by ICH GCP guidelines. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the sponsor representatives and/or an inspection by regulatory authority representatives at any time. The investigator must agree to the audit or inspection of study-related records by the sponsor representatives and/or

regulatory authority representatives, and must allow direct access to source documents to the sponsor and/or regulatory authority representatives.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/EC/sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IRB/EC/sponsor. Any deviations from the protocol must be fully explained and documented by the investigator. Protocol deviations will be submitted to the IRB/EC according to the requirements of each institution.

No medical waivers for protocol inclusion/exclusion criteria will be allowed by the sponsor, and in case the need for a change to the protocol is identified, it will be submitted as a protocol amendment to the competent regulatory authority and/or ethics committee as applicable per regulations.

12.3 Regulatory Authority Approvals/Authorizations

Regulatory authority approvals/authorizations/ notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for investigator study centers responsible for conducting the study will be posted on a publicly accessible clinical registry(ies) as required by local law.

12.4 Subject Information and Consent

Subject information and ICF will be provided to investigator study centers. Prior to the beginning of the study, the investigator must have the IRB/EC written approval/favorable opinion of the written ICF and any other written information to be provided to subjects. The written approval of the IRB/EC together with the approved subject information/ICF must be filed in the study files and a copy of the documents must also be provided to the sponsor by the investigator's study center.

Written informed consent must be obtained before any study-specific procedure takes place. Participation in the study and the date the informed consent was given by the subject should be documented appropriately in the subject's files. A signed copy of the subject ICF will be provided to the subject or subject's authorized representative.

12.5 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the sponsor. Only the subject code number will be recorded in the eCRF, and if the subject's name appears on any other document (eg, pathologist report), it must be redacted before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the sponsor,

IRB/EC, or regulatory authorities may inspect their medical records and personal health information to verify the information collected, and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects' records to be identified.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Handling

The study data will be recorded and kept current in the eCRF by the study center study personnel directly responsible for the information. Entries made in the eCRF must be verifiable against source documents, or must have been directly entered into the eCRF, in which case the entry in the eCRF will be considered the source data. The data in the eCRF will be monitored at the study center by Grifols representatives at regular intervals and reviewed for completeness and compared with the source documents. Examples of acceptable source documents include individual subject medical records, prospective information gathered on source documentation worksheets, lab reports and other diagnostics pertinent to this study which are separate from the eCRFs. The listing of types of source documents which will be defined in the source data agreement will be filed in TMF.

All AEs and SAEs must be recorded. All SAEs must be recorded on the SAE form. The SAE form must be kept in study center records with a copy provided to the designated person as detailed in the study file.

13.2 Record Retention

At study completion, all study data will be transferred to Grifols according to ICH GCP guidelines, local laws, regulations, and Grifols requirements. The study file and all source data should be retained until notification is given by the sponsor for destruction.

An investigator is required by ICH GCP guidelines to retain the study files. If an investigator moves, withdraws from an investigation or retires, the responsibility for maintaining the records may be transferred to another person (eg, other investigator). Grifols must be notified in writing of the person responsible for record retention and the notification will be retained in the sponsor study file and the investigator study center file.

14 FINANCING AND INSURANCE

In the event of subject injury as a direct result of either administration of treatment or any non-standard-of-care study procedure, sponsor will pay for the costs of treatment, provided the subject has followed the instructions given by the study doctor and the illness or injury is not due to the natural progression of any conditions existing before the subject participated in

the study. Financial compensation for such things as lost wages, disability, or discomfort due to any research-related injury is not available.

Sponsor shall maintain comprehensive general liability insurance or self-insurance in amounts adequate to cover any damage, demand, claim, loss or liability caused or incurred by sponsor, or as otherwise required by applicable laws and/or regulations.

15 PUBLICATION POLICY

Institution and the investigator agree that the first publication shall be made in conjunction with the presentation of a joint, multicenter publication of the study results from all appropriates study centers. If such a multicenter publication is not submitted within 12 months after conclusion of the study at all study centers or after Grifols confirms there will be no joint, multicenter publication, then institution and/or investigator shall have the right, at their discretion, to publish, either in writing or orally, the results of the study performed under the protocol, subject to the conditions outlined below:

- The results of the study will be reported in the publicly accessible registry(ies).
- The institution and/or investigator shall furnish Grifols with a copy of any proposed publication at least 30 days in advance of the date of submission for publication.
- Within said 30-day period, Grifols shall:
  - Review such proposed publication for confidential information (other than study results) and for subject information subject to the Health Insurance Portability and Accountability Act of 1996 (“HIPAA”) and other applicable privacy laws;
  - Review such proposed publication for the unauthorized use of the name, symbols and/or trademarks of Grifols;
  - By written notice to the investigator, identify with specificity the text or graphics in such proposed publication that Grifols contends contains confidential information, protected subject information, or the unauthorized use of Grifols’s name, symbols and/or trademarks so that the proposed publication may be edited appropriately to remove such text or graphics before publication; and
  - By written request, Grifols may delay proposed publications up to 60 days to allow Grifols to protect its interests in Grifols inventions described in such publications.
- Institution and/or investigator shall give Grifols the option of receiving an acknowledgment for its sponsorship of the study in all such publications or presentation.

16 REFERENCES

1. Albutein® 5%, 20%, and 25% Investigator's Brochure. Instituto Grifols, S.A. May 2017.
2. Angeli P, Gines P, Wong F, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. Gut 2015;64:531-537.
3. Gluud LL, Christensen K, Christensen E, et al. Terlipressin for hepatorenal syndrome. Cochrane Database Syst Rev 2012. doi:10.1002/14651858.CD005162.pub3.
4. Runyon BA. Introduction to the revised American Association for the Study of Liver Diseases. Practice Guideline management of adult patients with ascites due to cirrhosis 2012. Hepatology 2013; 57: 1651-3
5. European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 2010; 53: 397-417
6. Arroyo V, García-Martinez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. J Hepatol 2014; 61: 396-407
7. García-Martinez R, Caraceni P, Bernardi M, et al. Albumin: Pathophysiological basis and its role in the treatment of cirrhosis and its complications. Hepatology 2013; 58: 1836-46
8. Fanali G, di Masi A, Trezza V, et al. Human serum albumin: from bench to bedside. Mol Aspects Med 2012; 33: 209-90
9. Sort P, Navasa M, Arroyo V, et al. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. N Engl J Med 1999; 341: 403-9.
10. Moore KP, Wong F, Gines P, et al. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. Hepatology 2003;38(1):258-66.
11. Blei AT, Córdoba J; Practice Parameters Committee of the American College of Gastroenterology. Hepatic Encephalopathy. Am J Gastroenterol 2001;96(7):1968-76.
12. Moreau R, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. Hepatology 2003;37(2):233-43.
13. Gentilini P, Casini-Raggi V, Di Fiore G, et al. Albumin improves the response to diuretics in patients with cirrhosis and ascites: results of a randomized, controlled trial. J Hepatol 1999; 30: 639-45
14. Romanelli RG, La Villa G, Barletta G, et al. Long-term albumin infusion improves survival in patients with cirrhosis and ascites: an unblinded randomized trial. World J Gastroenterol 2006; 12: 1403-1407
15. Bernardi M, Riggio O, Angeli P, et al. Long-Term Use of Human Albumin for the Treatment of Ascites in Patients With Hepatic Cirrhosis: The Interim Analysis of the ANSWER Study.
16. Salerno F, Gerbes A, Gines P, et al. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. Gut 2007;56:1310-1308.

17. Luca A, García-Pagán JC, Bosch J, et al. Beneficial effects of intravenous albumin infusion on the hemodynamic and humoral changes after total paracentesis. Hepatology 1995;22:753-758.
18. Uriz J, Gines P, Cardenas A, et al. Terlipressin plus albumin infusion: an effective and safe therapy of hepatorenal syndrome. J Hepatol 2000;33:43-48.
19. Duvoux C, Zanditenas D, Hezode C, et al. Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome: A pilot study. Hepatology 2002;36:374-380.
20. Angeli P, Albino G, Carraro P, , et al. Cirrhosis and muscle cramps: evidence of a causal relationship. Hepatology 1996;23:264-273.
21. Moeller S, Henriksen JH. Cardiovascular complications of cirrhosis. Postgrad Med J 2009; 85: 44-54.
22. Peters T Jr. All about albumin. Biochemistry, genetics and medical applications. Academic Press, San Diego, 1996: 188-249.
23. Clinical Study Protocol IG0802. Long-term effects of prolonged administration of albumin 20% on cardiocirculatory and renal function and hepatic hemodynamics in patients with advanced cirrhosis and ascites. Instituto Grifols, SA. 14 Jun 2012.
24. Murray J, Ezzati M, Flaxman A, et al. The global burden of disease study 2010: a multi-investigator collaboration for global comparative descriptive epidemiology. Lancet 2012;380: 2055-2058.
25. Moreau R, Ginès P, Jalan R, et al. Acute-on-chronic liver failure is a distinct syndrome developing in subjects with acute decompensation of cirrhosis. Gastroenterology 2013;144:1426-37.
26. Bernal W, Jalan R, Quaglia A, et al. Acute-on-chronic liver failure. Lancet 2015; 386:1576–87.
27. Dirchwolf M and Ruf AE. Role of systemic inflammation in cirrhosis: From pathogenesis to prognosis. World J Hepatol 2015 August 8; 7(16): 1974-1981.
28. Garcia-Tsao G, Friedman S, Iredale J, et al. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. Hepatology 2010; 51(4): 1445–1449.
29. Era S, Kuwata K, Imai H, et al. Age-related change in redox state of human serum albumin. Biochim Biophys Acta. 1995;1247(1):12-16.
30. Oettl K and Marsche G. Redox state of human serum albumin in terms of cysteine-34 in heath and disease. Methods Enzymol 2010;474:181-195.
31. Clària J, Stauber R, Coenraad M, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. Hepatology. 2016;64(4):1249-1264.
32. Wald C, Russo M, Heimbach J, Hussain H, Pomfret E, Bruix J. New OPTN/UNOS policy for liver transplant allocation: standardization of liver imaging, diagnosis, classification, and reporting of hepatocellular carcinoma. Radiology. 2013 Feb; 266(2):376-382.
33. The EuroQol Group. EuroQol – A new facility for the measurement of health-related quality of life. Health Policy 1990;16:199-208.

34. Longo D, Fauci A, Kasper D, et al. Harrison's Principles of Internal Medicine, 18th Edition: www.accessmedicine.com.
35. Purvey M, Allen G. Managing acute pulmonary oedema. Aust Prescr 2017;40:59-63. https://doi.org/10.18773/austprescr.2017.013

17 APPENDICES

Appendix 1 Schedule of Study Procedures and Events

Procedures and Evaluation / Study Period / Study Day	Screening	Treatment Period[a]				End of Study: Completed Subjects	Follow-up: Discontinued Subjects[d]
		Study Visits[b]		Treatment Days[c]	Month 12/Early Discontinuation		
		Day 1	Day 11 Months 1-11				
Inclusion/exclusion criteria	X	X					
Medical history & demographics	X						
Abdominal ultrasound	X[k]						
Endoscopic procedures	X[l]						
Urine pregnancy test	X						
Physical exam	X[m]		X[n]		X		
Randomization		X					
Vital signs[e]		X	X	X	X		
Ascites assessment	X	X	X		X		
Hepatic encephalopathy evaluation	X		X		X		
Hemodynamics assessment (MAP)	X		X		X		
Peripheral capillary oxygen saturation (SpO_2) assessment	X		X		X		
Weight		X	X	X	X		
Clinical laboratory assessments[f]	X	X	X		X		
Serum albumin concentration[g]		X	X		X[r]		
Record ACLF grade	X		X		X		
Systemic inflammation assessment[h]		X	X		X		
Illness severity scores evaluation[i]	X		X		X		
Quality of life assessment (EQ-5D-5L health questionnaire)		X	X[o]		X		
Prior and concomitant medications	X	X	X	X	X	X	
Albumin infusion		X[c]	X[c]	X[c]	X[r]		
Assess TIPS insertion			X		X		
Albumin functional capacity assessment (albumin binding capacity)		X	X[p]		X		
Adverse events	X	X	X	X	X	X	
Biomarker retains[j]		X	X[o]		X		
Record liver transplantation, survival, cause of death data			X[q]		X		X

Footnotes continued on next page

[a] The treatment period will be up to 12 months.
[b] Subjects will have assessments on Day 11 (±2 days), Month 1 (±2 days), and then monthly (±2 days) for up to 12 months.
[c] Within 72 hours (3 days) of Screening, subjects will be randomized on Day 1. Following randomization, subjects in the SMT + Albutein 20% treatment group will receive the first infusion on the same day at the dose of 1.5 g/kg body weight (maximum 100 grams per subject). Thereafter, subjects will receive Albutein 20% infusions at the dose of 1.5 g/kg body weight (maximum 100 grams per subject) every 10 ± 2 days for the rest of the study.
[d] Subjects who discontinue from the study early will have follow-up vital status assessments performed at Months 3, 6, and 12.
[e] Vital signs include systolic BP, diastolic BP, HR, T, and RR. Vital signs will be measured immediately prior to and immediately after the end of the Albutein 20% administration in the SMT + Albutein 20% treatment group.
[f] Clinical laboratory assessments will include hematology, chemistry, and coagulation performed by the central lab. At the Screening visit only, local laboratory tests will be performed in order to determine subject eligibility. Screening local laboratory test results will be kept in subjects' source documents but will not be entered into the eCRF (see Table 7-1).
[g] Serum albumin concentration will be measured immediately prior to treatment. In the SMT + Albutein 20% treatment group, serum albumin concentration will also be measured immediately after the end of the Albutein 20% administration.
[h] Systemic inflammation assessment includes testing for procalcitonin, WBC, and CRP (see Table 7-1).
[i] Illness severity scores include CLIF-C OF score, CLIF-C AD score, CLIF-C ACLF score, MELD score, and Child Pugh score (see Appendix 3 for illness severity scores).
[j] Biomarker retains will be drawn for potential analysis of cytokines (interleukin [IL] 6 (IL-6), IL-8, IL-10, and tumor necrosis factor alpha [TNFα]) found in the blood which are significantly correlated with disease activity (see Table 7-1). All clinical blood samples will need to be drawn first before biomarker retains are drawn.
[k] Findings from abdominal ultrasounds performed in the last 6 months will be recorded.
[l] Findings from endoscopic procedures performed in the last 6 months will be recorded.
[m] Full physical examination (excluding breast and genitourinary examination).
[n] Physical examination performed on Month 6 only.
[o] EQ-5D-5L health questionnaire and optional collection for blood biomarker retains on Months 1, 3, 6, and 9. All clinical blood samples will need to be drawn first.
[p] Assessed on Day 11 and Months 1, 3, 6, and 9.
[q] Assessed on Months 3 and 6.
[r] Month 12 Visit only.

Appendix 2 European Association for the Study of the Liver Clinical Practice Guidelines on the Management of Ascites/International Club of Ascites

Uncomplicated ascites: Ascites that is not infected and which is not associated with the development of the hepatorenal syndrome. Ascites can be graded as follows:

Grading of Ascites and Suggested Treatment

Grade of Ascites	Definition	Treatment
Grade 1 ascites	Mild ascites only detectable by ultrasound	No treatment
Grade 2 ascites	Moderate ascites evident by moderate symmetrical distention of abdomen	Restriction of sodium intake and diuretics
Grade 3 ascites	Large or gross ascites with marked abdominal distension	Large-volume paracentesis followed by restriction of sodium intake and diuretics (unless patients have refractory ascites)

Definition and Diagnostic Criteria for Refractory Ascites in Cirrhosis:

Diuretic-resistant ascites	Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of a lack of response to sodium restriction and diuretic treatment
Diuretic-intractable ascites	Ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that preclude the use of an effective diuretic dosage
Requisites	
1. Treatment duration	Patients must be on intensive diuretic therapy (spironolactone 400 mg/day and furosemide 160 mg/day) for at least 1 week and on a salt-restricted diet of less than 90 mmol/day
2. Lack of response	Mean weight loss of <0.8 kg over 4 days and urinary sodium output less than the sodium intake
3. Early ascites recurrence	Reappearance of grade 2 or 3 ascites within 4 weeks of initial mobilization
4. Diuretic-induced complications	Diuretic-induced HE is the development of encephalopathy in the absence of any other precipitating factor.
	Diuretic-induced renal impairment is an increase of serum creatinine by >100% to a value >2 mg/dL (177 µmol/L) in patients with ascites responding to treatment. Diuretic-induced hyponatremia is defined as a decrease of serum sodium by >10 mmol/L to a serum sodium of <125 mmol/L. Diuretic-induced hypo- or hyperkalemia are defined as a change in serum potassium to <3 mmol/L or >6 mmol/L, respectively, despite appropriate measures

Source: (5)

Diagnostic criteria of hepatorenal syndrome (HRS) type of acute kidney injury (AKI) in patients with cirrhosis

Box 1. Diagnostic criteria of hepatorenal syndrome (HRS) type of acute kidney injury (AKI) in patients with cirrhosis

HRS-AKI

- Diagnosis of cirrhosis and ascites
- Diagnosis of AKI according to ICA-AKI criteria
- No response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin 1 g per kg of body weight
- Absence of shock
- No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast media, etc.)
- No macroscopic signs of structural kidney injury*, defined as:
  - absence of proteinuria (>500 mg/day)
  - absence of microhaematuria (>50 RBCs per high power field),
  - normal findings on renal ultrasonography

*Patients who fulfil these criteria may still have structural damage such as tubular damage. Urine biomarkers will become an important element in making a more accurate differential diagnosis between HRS and acute tubular necrosis.
ICA, International Club of Ascites; NSAIDs, non-steroidal anti-inflammatory drugs; RBCs, red blood cells.

Source: (2)

Appendix 3 Illness Severity Scores

Criteria to Define Organ Failure (CLIF-C OF Score)

CLIF-Organ Failure score system.

Organ / System	Sub-score = 1	Sub-score = 2	Sub-score = 3
Liver	Bilirubin <6 mg/dL	6 ≤ Bilirubin <12 mg/dL	Bilirubin ≥12 mg/dL
Kidney	Creatinine <2 mg/dL	2 ≤ Creatinine <3.5 mg/dL	Creatinine ≥3.5 mg/dL or renal replacement
Brain (West-Haven grade for HE*)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR <2.0	2.0 ≤ INR <2.5	INR ≥2.5
Circulatory	MAP ≥70 mm/Hg	MAP <70 mm/Hg	Use of vasopressors
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - >200 >214- ≤357	≤200 (#) ≤214 (#)

*The shaded area describes criteria for diagnosing organ failures; *HE: Hepatic Encephalopathy; # patients enrolled in the study with MV were considered as presenting respiratory failure (respiratory sub-score=3) except for patients intubated due to severe HE or to other reasons and not to a respiratory failure, in whom respiratory failure is defined according to the PaO2/FiO2 ratio.*

CLIF-C ACLF Score

CLIF-C ACLFs = 10*[0.33*CLIF-C OFs + 0.04*Age {years} + 0.63*Ln(white cell count {10^9 cells/L}) - 2]

CLIF-C AD Score

CLIF-C ADs= 10[0.03*Age {years} +0.66*Ln(Creatinine {mg/dL}) +1.71*Ln(INR)+0.88*Ln(white cell count {10^9 cells/L}) - 0.05*Sodium {mmol/L}+8]

MELD Score

MELD Score = 9.57 Ln(Creatinine {mg/dL}) + 3.78 Ln(Bilirubin {mg/dL}) + 11.2 Ln(INR) + 6.43

Child Pugh Score

Child-Turcotte-Pugh Classification for Severity of Cirrhosis

	Points*		
	1	**2**	**3**
Encephalopathy	None	Grade 1-2 (or precipitant induced)	Grade 3-4 (or chronic)
Ascites	None	Mild to moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	<2	2-3	>3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
INR	<1.7	1.7-2.3	>2.3

*Child-Turcotte-Pugh Class obtained by adding score for each parameter (total points)

Class A = 5 to 6 points (least severe liver disease)

Class B = 7 to 9 points (moderately severe liver disease)

Class C = 10 to 15 points (most severe liver disease)

Appendix 4 West Haven Criteria for Hepatic Encephalopathy

WEST HAVEN CRITERIA OF ALTERED MENTAL STATUS IN HEPATIC ENCEPHALOPATHY

Stage	Consciousness	Intellect and Behavior	Neurologic Findings
0	Normal	Normal	Normal examination; impaired psychomotor testing
1	Mild lack of awareness	Shortened attention span; impaired addition or subtraction	Mild asterixis or tremor
2	Lethargic	Disoriented; inappropriate behavior	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

Appendix 5 EuroQoL 5 Dimensions 5 Levels (EQ-5D-5L) Health Questionnaire

Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY

I have no problems walking ❑
I have slight problems walking ❑
I have moderate problems walking ❑
I have severe problems walking ❑
I am unable to walk ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

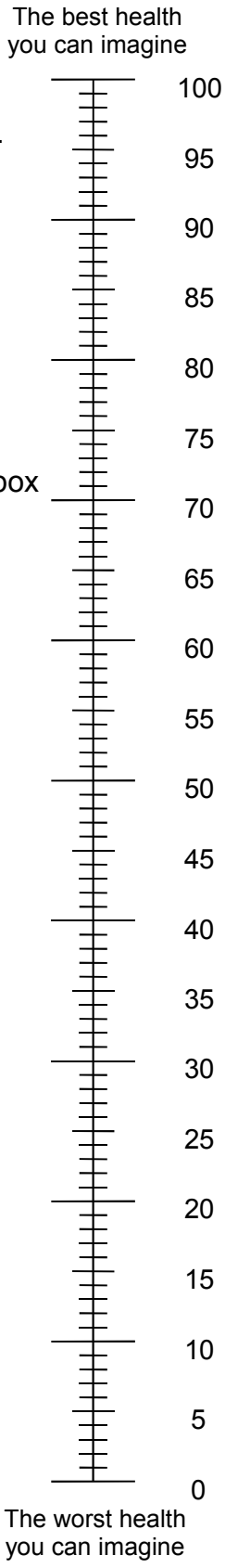
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Source: (33)

Appendix 6 Milan Criteria

The Milan criteria are a generally accepted set of criteria used to assess suitability in patients for liver transplantation with cirrhosis and hepatocellular carcinoma.

In order to be suitable for a liver transplantation, one needs to have the following:

- Single tumor with diameter ≤5 cm or up to 3 tumors each with diameter ≤3 cm
- No extra-hepatic involvement
- No major vessel involvement

Appendix 7 NYHA Classification – The Stages of Heart Failure

In order to determine the best course of therapy, physicians often assess the stage of heart failure according to the NYHA functional classification system. This system relates symptoms to everyday activities and the patient's quality of life.

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

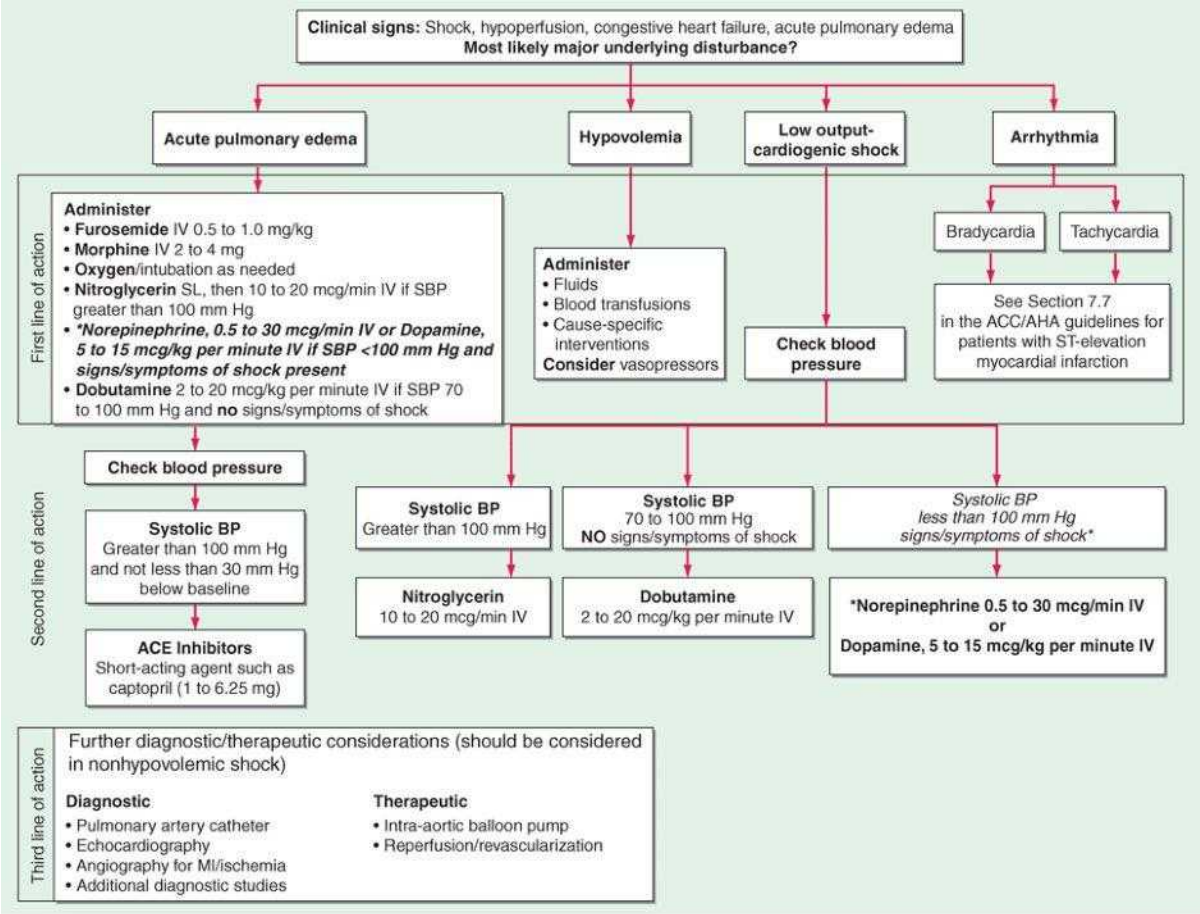
Appendix 8 GOLD Staging System for COPD Severity

The European Respiratory Society (ERS) diagnostic criteria for COPD include the following symptoms: coughing, sputum production and/or dyspnea, as well as a history of exposure to risk factors for COPD. The diagnosis is confirmed by a post-bronchodilator $FEV_1/FVC < 0.7$ by spirometry, as sign of the airflow limitation that is not fully reversible.

Stage	Description	Findings (based on postbronchodilator FEV_1)
0	At risk	Risk factors and chronic symptoms but normal spirometry
I	Mild	FEV_1/FVC ratio less than 70 percent FEV_1 at least 80 percent of predicted value May have symptoms
II	Moderate	FEV_1/FVC ratio less than 70 percent FEV_1 50 percent to less than 80 percent of predicted value May have chronic symptoms
III	Severe	FEV_1/FVC ratio less than 70 percent FEV_1 30 percent to less than 50 percent of predicted value May have chronic symptoms
IV	Very severe	FEV_1/FVC ratio less than 70 percent FEV_1 less than 30 percent of predicted value Or FEV_1 less than 50 percent of predicted value plus severe chronic symptoms

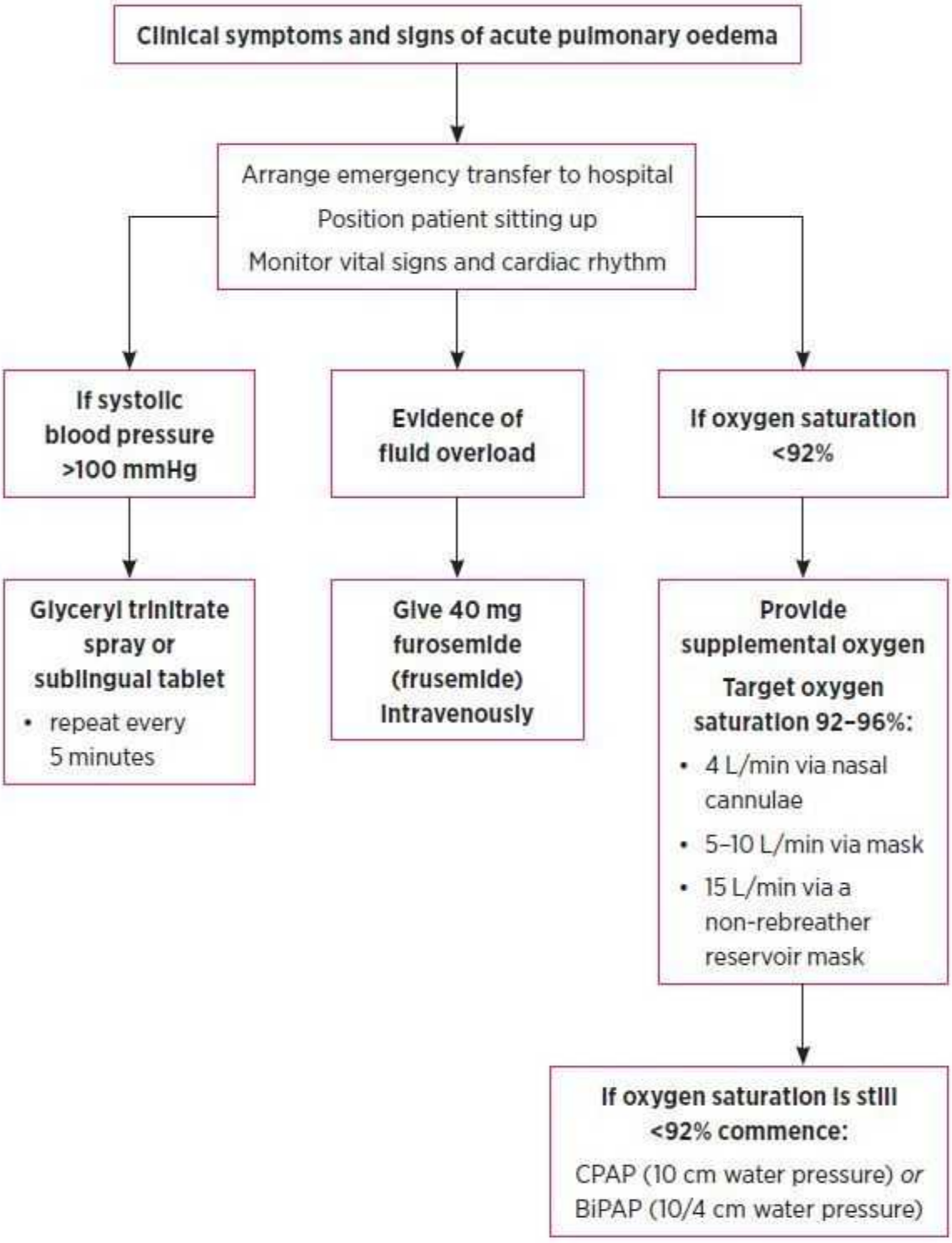
GOLD = Global Initiative for Chronic Obstructive Lung Disease; COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity

Appendix 9 Algorithm For Cardiogenic Pulmonary Overload



Source: (34)

Appendix 10 Management of Acute Pulmonary Edema



Source: (35)

Appendix 11 Summary of Changes for Amendment 5

(Note: Administrative changes including minor administrative corrections are not included in Protocol Summary of Changes.)

Sections	Change From: (Version 5) (Strikethrough is added to highlight deleted text.)	Change To: (Version 6) (Underline is added to highlight new text.)	Rationale:
Synopsis, 5.2	*Note: no deleted text.*	25. Subjects with current infection of COVID19, those who are less than 14 days post recovery, or those who have clinical signs and symptoms consistent with COVID19 infection.	New exclusion criteria added regarding infection of COVID19.