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Clinical Study Protocol

Protocol Title:	Effects of Plasma Exchange with Human Serum Albumin 5% (PE-A 5%) on Short-term Survival in Subjects with "Acute-On-Chronic Liver Failure" (ACLF) at High Risk of Hospital Mortality
Investigational Product:	Albutein® 5%
Sponsor's Name and Address:	Instituto Grifols, S.A. Can Guasch, 2 08150, Parets del Vallès, Barcelona Spain
Study Identification:	IG1407
Additional Identifier:	APACHE (ACLF Plasma Exchange)
EUDRACT Number:	2016-001787-10
IND/CTA Number:	17377
Development Phase:	3
Sponsor Signatories:	[redacted], MD, FASN [redacted] Grifols Therapeutics LLC Email address: [redacted] Telephone number: [redacted] [redacted] [redacted] Scientific & Innovation Office Grifols Email address: [redacted] Telephone number: [redacted] See Sponsor Signatures on the cover page of the protocol

PROTOCOL VERSION HISTORY

Protocol Version	Approval Date/Effective Date
6 Amendment 5 + Integrated Protocol	See Left Margin
5 Amendment 4 + Integrated Protocol	08 Jul 2020
4 Amendment 3 + Integrated Protocol	19 Jun 2020
3 Amendment 2 + Integrated Protocol	31 Mar 2020
2.0 Amendment 1 + Integrated Protocol	27 Apr 2018
1.0 Original	12 Dec 2017

Amendment 5

The protocol for IG1407 (Version 5, dated 08 July 2020) has been amended and reissued as Protocol Amendment 5, Version 6. See Appendix 11 for a summary of changes from Protocol Version 5.0 to 6.0.

PROTOCOL SYNOPSIS

Title of Study:

Effects of Plasma Exchange with Human Serum Albumin 5% (PE-A 5%) on Short-term Survival in Subjects with "Acute-On-Chronic Liver Failure" (ACLF) at High Risk of Hospital Mortality

Study Number: IG1407

Phase: 3

Study Objectives:

Primary Efficacy Objective:

- To evaluate the effect of standard medical treatment (SMT) plus PE-A 5% (SMT+PE-A 5%) on 90-day overall survival.

Secondary Efficacy Objectives:

- To evaluate the effect of SMT+PE-A 5% on 90-day transplant-free survival.
- To evaluate the effect of SMT+PE-A 5% on 28-day overall survival.

Exploratory Objectives:

The effects of SMT+PE-A 5% on the following parameters will be evaluated:

- 90-day overall, 90-day transplant-free, and 28-day overall survival after randomization in subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- In-patient hospital stay (including intensive care unit [ICU] stay) overall and transplant-free survival in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- 28-day transplant-free survival in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- Incidence of individual organ failures: liver, renal, brain, coagulation, circulation, and respiration as defined by the Chronic Liver Failure-Consortium Organ Failure score (CLIF-C OF score) and the total CLIF-C OF score, during the treatment period and at the end of treatment and of hospitalization (or liver transplantation or death)
- ACLF course (resolution, improvement, steady course, or worsening), CLIF-C ACLF score, Child-Pugh score, and Model for End-stage Liver Disease (MELD) score
- Systemic inflammation as measured by C-reactive protein (CRP), white blood cell (WBC), and procalcitonin
- Rate of all new infections
- Time-to-hospital discharge

- Pre and post plasma exchange (PE) albumin levels in serum

Safety Objective:

- To determine the safety and tolerability profile of PE-A 5% in subjects with ACLF.

Overall Study Design and Description:

Approximately 380 subjects with cirrhosis, ACLF, and high risk of hospital mortality (ACLF-1b, ACLF-2, or ACLF-3a) (Table 2-1) will be included in this study after obtaining written informed consent. In case of hepatic encephalopathy (HE), written informed consent will be obtained from a relative or a legally authorized representative if the subject is considered incompetent to consent.

There will be a screening period of no more than 10 days for each subject during which subjects will be screened for enrollment in the study. Subjects with ACLF-1b, ACLF-2, or ACLF-3a will be included at diagnosis of the syndrome. Subjects with ACLF-1a will be followed up and included if they deteriorate to ACLF-1b, ACLF-2, or ACLF-3a within the 10-day Screening Period. Subjects with ACLF-3b will be followed up and included if they improve to ACLF-3a, ACLF-2, or ACLF-1b within the 10-day Screening Period.

Randomization of subjects will be stratified by region (European Union [EU] or North America [NA]) and the 3 ACLF grades (ACLF-1b, ACLF-2, or ACLF-3a). Within each stratum (ie, each unique combination of region and ACLF grade), subjects will be randomized in a 1:1 ratio into 2 treatment groups below:

- SMT+PE-A 5% (treatment group)
- SMT (control group)

SMT+PE-A 5% Treatment Group:

Approximately 190 subjects will be randomized to the SMT+PE-A 5% treatment group. PE-A 5% will be performed using 5% albumin (Albutein® 5%) as the main replacement fluid administered intravenously. Fresh frozen plasma (FFP) will be administered after the 5% albumin administration to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration.

The treatment schedule consists of 2 initial PE-A 5% sessions on consecutive days followed by every other day PE-A 5% sessions. A minimum of 4 and a maximum of 9 PE-A 5% sessions will be performed in subjects randomized to receive SMT+PE-A 5% (maximum treatment period: 17 days).

The exact number of sessions will be determined by the pattern of response (achieving complete response or no improvement/deterioration of ACLF) to PE-A 5% therapy. See Section 6.1.1.3, Table 6-1, and Table 6-2 for further details. IVIGs will be administered at a dose of 200 mg/kg 1 day after every 2 PE sessions in order to prevent the development of

hypogammaglobulinemia and infection. For subjects ending the treatment at an odd-number PE session (eg, #5, #7, or #9), IVIGs will be administered at a dose of 100 mg/kg 1 day after this last PE session.

SMT Group:

Approximately 190 subjects will be randomized to the control group and receive SMT for the length of the study or as long as needed. Subject's treatment period as specified in this protocol will be 7 days for all subjects and will then be prolonged depending on subject's ACLF evolution to up to 17 days:

- Subjects in the SMT group with ACLF-1b, -2, or -3a who achieve complete response (ACLF resolution or improve to ACLF-1a) within the treatment period (from Day 1 to Day 7), will be assessed 4 more days after the ACLF resolution or improvement day and then will be followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.
- Subjects in the SMT group with ACLF-1b, -2, or -3a who maintain or worsen their ACLF grades throughout the treatment period (from Day 1 to Day 7), will be further assessed for up to Day 17 and will then be followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.

The Treatment Period will be up to 17 days. See Appendix 1 and Appendix 2 for detailed study schedules.

Subjects in both the SMT+PE-A 5% treatment group and the SMT control group will be followed for 90 days after randomization. During the entire study, the safety of both groups will be monitored by a Data Safety Monitoring Board.

Primary Endpoint and Secondary Endpoints

The primary efficacy endpoint is to compare the 90-day overall survival in the intent-to-treat (ITT) population (see Section 9.7 for definition) between the SMT+PE-A 5% treatment group and the SMT control group.

The secondary efficacy endpoints are to assess the effects of PE-A 5% on: 1) 90-day transplant-free survival and 2) 28-day overall survival versus SMT alone.

Number of Subjects Planned:

A total of approximately 380 subjects will be enrolled in this study. These subjects will be randomized in a 1:1 ratio into the SMT+PE-A 5% treatment group or the SMT control group.

Study Centers Planned

Approximately 40 study centers in the United States (US), Canada, and Europe will participate in this study.

Diagnosis and Main Criteria for Inclusion:

Note that any criterion number containing v3 was modified in the protocol (eg, 2v3 indicates that the original criterion #2 was last modified in Protocol Version 3).

Inclusion Criteria:

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

1. Male or female cirrhotic subjects between 18 and 79 years of age.
2. Subjects with ACLF-1b, ACLF-2, or ACLF-3a detected either at admission or during hospitalization (must be ACLF-1b, -2, or -3a within the Screening Period [a maximum of 10 days]) (see Table 2-1 for ACLF grades).
3. 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.
4. In case of HE, informed consent will be provided by a relative or a legally authorized representative if the subject is considered incompetent to consent.

Exclusion Criteria:

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

1. Subjects without ACLF.
2. Subjects with ACLF-1a or ACLF-3b (see Table 2-1 for ACLF grades) after the Screening Period.
3. Subjects fulfilling inclusion criteria that improve to no ACLF or to ACLF-1a or worsen to ACLF-3b during the Screening Period (between initial evaluation and time of randomization).
4. Subjects with ACLF for more than 10 days prior to randomization.
5. Subjects with acute or subacute liver failure without underlying cirrhosis.

6v3 Subjects with septic shock requiring use of norepinephrine (> 0.3 mcg/kg/min) or need for a second vasopressor (including terlipressin).

7v3 Subjects with active bacterial or fungal infection: who have received less than 24h of appropriate antibiotic treatment.

8v3 Subjects with severe respiratory failure with PaO2/FiO2 ≤200.

9. Subjects with active or recent bleeding (unless controlled for >48 hours).
10. Subjects with severe thrombocytopenia ($\leq 20 \times 10^9$/L) (based on local laboratory assessment).

11. Subjects with chronic renal failure and currently receiving hemodialysis.
12. 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 5]), non-melanocytic skin cancer, and controlled breast or prostate cancer can be included).
13. Subjects with severe chronic heart failure (New York Heart Association [NYHA] class III or IV) (Appendix 6).
14. Subjects with severe pulmonary disease (Global Obstructive Lung Disease [GOLD] stage III or IV) (Appendix 7).
15. Subjects with severe myopathy as defined clinically.
16. Subjects with a known infection with human immunodeficiency virus (HIV) or have clinical signs and symptoms consistent with current HIV infection.
17. Females who are pregnant, breastfeeding, or if of childbearing potential, unwilling to practice a highly effective method 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 a trial, and withdrawal are not acceptable methods of contraception).
18. Subjects with previous liver transplantation.

19v3 Subjects receiving anti-platelet or anti-coagulant therapy (low-molecular-weight heparin [LMWH] for deep vein thrombosis [DVT] prophylaxis is allowed).

20. Participation in another clinical study within at least 30 days prior to screening.

21v3 Subjects with active drug addiction (exceptions: active alcoholism or marijuana)

22. Subjects with a do-not-resuscitate order.
23. In the opinion of the investigator, the subject may have compliance problems with the protocol and the procedures of the protocol.
24. 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® 5% is the investigational product.

PE-A 5% will be performed using 5% albumin (Albutein® 5%) as the main replacement fluid administered intravenously. One-point-two (1.2) plasma volumes will be exchanged per PE-A 5% session. Total blood volume (TBV) will be calculated according to Nadler's formula (Appendix 3). Plasma volume will be estimated from the calculated TBV and the

hematocrit (HCT). The volume to be exchanged will be considered as the volume to be infused (not the fluid to be removed).

The formula for calculating plasma volume to obtain the total volume to be exchanged is as follows:

$$\text{Plasma volume} = \text{TBV} \times (1 - \text{HCT})$$

$$\text{Total volume to be exchanged} = \text{Plasma volume} \times 1.2$$

Fresh frozen plasma (FFP) will be administered after the 5% albumin administration to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration. A minimum of 4 and a maximum of 9 PE-A 5% sessions will be performed in subjects randomized to receive SMT+PE-A 5% (maximum treatment period: 17 days). The aim of the study is to achieve complete response (resolution of ACLF or improvement to ACLF-1a). The number of PE-A 5% sessions will be determined by the pattern of response to PE-A 5% treatment.

Intravenous immunoglobulins (IVIGs) will be administered at a dose of 200 mg/kg 1 day after every 2 PE-A 5% sessions in order to prevent the development of hypogammaglobulinemia and infection. In those subjects receiving an odd number of PE-A 5% sessions (eg, #5, #7, or #9 PE-A 5% sessions when they are the last PE session for the subject), IVIGs will be administered 1 day after this last PE-A 5% session at a dose of 100 mg/kg. Initial infusion rate of IVIGs will be 0.5 mL/kg/hour, increasing to a maximum rate of 2 mL/kg/hour if tolerance is adequate.

Duration of Treatment:

Subject participation (from screening to the final visit): Up to 100 days

Reference Therapy, Dose, and Mode of Administration:

Control Group: SMT per institution standards

Key Study Variables:

Primary Efficacy:

- The primary efficacy variable will be time to death through Day 90 after randomization of SMT+PE-A 5% versus SMT alone.

Secondary Efficacy Variables:

- Time to transplant or death through Day 90 after randomization of SMT+PE-A 5% versus SMT alone.
- Time to death through Day 28 after randomization of SMT+PE-A 5% versus SMT alone.

Exploratory Efficacy Variables:

- Time to death through Day 90, time to transplant or death through Day 90, and time to death through Day 28 after randomization in subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Time to death and time to transplant or death through in-patient hospital stay (including ICU stay) in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Time to transplant or death through Day 28 after randomization in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Incidence of individual organ failures: liver, renal, brain, coagulation, circulation, and respiration, as defined by the CLIF-C OF score and the total CLIF-C OF score, during the treatment period and at the end of treatment, and of hospitalization (or liver transplantation or death).
- ACLF course (resolution, improvement, steady course, or worsening), CLIF-C ACLF score, Child-Pugh score, and MELD score.
- Systemic inflammation as measured by CRP, WBC and procalcitonin.
- Rate of all new infections.
- Time-to-hospital discharge.
- Albumin levels in serum will be measured pre and post PE-A 5% to monitor the status of infused albumin in subjects during the course of the study.

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 PE-A 5% completion (or after PE-A 5% stops) will be considered as the main safety variables. The number and incidence rate of overall adverse events (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 PE-A 5% session.
- Clinical laboratory test parameters (blood cell counts, platelet count, international normalized ratio, fibrinogen, etc.) will also 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 Period (Day -10 to Day -1):

Following signature of the informed consent form, screening procedures will be performed. 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 electronic case report form.

- Assign subject number
- Add subject's data to Screening Log
- Review of inclusion and exclusion criteria to determine subject's eligibility
- Documentation of demographics: year of birth, gender, race, and ethnic origin
- Documentation of medical history, including etiology of cirrhosis and significant concomitant disease conditions for the last 12 months
- Chest x-ray
- Electrocardiogram (ECG)
- Abdominal ultrasound
- 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)
- Record vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], heart rate [HR], respiratory rate [RR], temperature [T])
- Ascites assessment according to the International Club of Ascites (ICA) criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamics assessment (mean arterial pressure [MAP])
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for clinical laboratory assessments (eg, hematology, chemistry, coagulation)
- Infection surveillance using microbiology (cultures) including blood, urine, ascetic fluid, sputum, and pleural fluid. If a positive result occurs at screening, a Day 3 result will be collected. If a thoracentesis or paracentesis has been performed within the last 5 days prior to screening, results of the fluid cultures should be documented in the medical notes. Microbiology cultures will be performed at local labs and the results will be captured in the subject's source documents but will not be the entered into the eCRF. All

new infections will be recorded as AEs and recorded in subject's source documents and the eCRF. Additional samples will be obtained upon clinical suspicion.

- Microbiological tests (Cytomegalovirus polymerase chain reaction, and galactomannan antigen index)
- Record ACLF grade (Table 2-1)
- Illness severity scores: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups
- Record prior (30 days prior to screening) and concomitant medications
- Record AEs

Evaluation of cardiac function at screening will be optional in subjects without vascular failure but mandatory in those with shock. This evaluation will be performed according to local guidelines.

Treatment Period (Days 1 to 17):

All screening laboratory results and assessments must be available and all inclusion and exclusion criteria must have been satisfied prior to initiating treatment. The Treatment Period is from Day 1 until Day 17. For subjects in the SMT+PE-A 5% treatment group, PE-A 5% treatment may stop early depending on clinical response. Subjects who remain in the hospital will continue to have Treatment Period assessments performed up through Hospital Discharge.

Baseline/Treatment Day 1:

- Randomization (after confirmation of subject eligibility and prior to treatment)
- Record vital signs (SBP, DBP, HR, RR, T) measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group
- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation) (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)

- Serum albumin concentration (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
- Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
- Plasma biomarker retains for subjects in the SMT+PE-A 5% treatment group (plasma removed will be collected from the plasma bag after the first 15 minutes and the last 15 minutes of the PE-A 5% session)

- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- PE-A 5% (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

Treatment Days 2, 4, 6, 8, 10, 12, 14, 16:

- Record vital signs (SBP, DBP, HR, RR, and T) measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group
- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation) (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Systemic inflammation assessment (CRP [in chemistry panel], , WBC [in hematology panel], and procalcitonin)
  - Plasma biomarker retains for subjects in the SMT+PE-A 5% treatment group (plasma removed will be collected from the plasma bag after the first 15 minutes and the last 15 minutes of each PE-A 5% session)
- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)

- PE-A 5% (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

Treatment Days 3, 5, 7, 9, 11, 13, 15, 17:

Record vital signs (SBP, DBP, HR, RR, T) on days when IVIG is administered, measured immediately prior to the IVIG administration

- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Infection surveillance if positive result obtained at Screening Visit using microbiology (cultures) including blood, urine, ascetic fluid, sputum, and pleural fluid. Day 3 sample will be collected if positive result at screening. Additional samples will be obtained upon clinical suspicion. All new infections will be recorded as AEs.
- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, MELD score(Appendix 4)
- IVIG 200 mg/kg infusion administered after every 2 PE-A 5% sessions (Days 3, 7, 11, or 15) (SMT+PE-A 5% treatment group only). See Table 6-1 and Table 6-2.
- IVIG 100 mg/kg infusion administered following the last, odd-numbered PE session on either Days 9, 13, or 17 (SMT+PE-A 5% treatment group only). See Table 6-1 and Table 6-2.
- Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups (Days 3, 7, and 17 only)
  - Blood sample will be collected before IVIG administration (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

Follow-Up Period:

Hospital Discharge:

- Record vital signs (SBP, DBP, HR, RR, T)

- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 10)
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel] and procalcitonin)
  - Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Record ACLF grade (Table 2-1)
- Record prior and concomitant medications
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death

Subjects who remain in the hospital will continue to have treatment period assessments performed up through Hospital Discharge. Follow-up assessments for Days 21, 28, 60, and 90 will be performed for all subjects.

Follow-Up Visits (Day 21 [+1 Day], and Days 28 [+2 Days], 60 [+2 Days], 90 [+2 Days]):

- Record vital signs (SBP, DBP, HR, RR, T)
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 10)
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for:
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel] and procalcitonin)

- Blood biomarker retains (Days 28, 60, and 90) for subjects in the SMT and SMT+PE-A 5% groups

- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Record ACLF grade (Table 2-1)
- Record prior and concomitant medications
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death on Days 28 and 90

Subjects who discontinue from the study early should have all Day 90 procedures performed at the time of withdrawal (preferably within 1 week of withdrawal).

Statistical Methods:

Study Populations

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 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 and receive at least the first 2 PE-A 5% sessions (SMT+PE-A 5% treatment group) or survive more than 2 days following randomization (SMT control group). 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 subjects who receive at least one PE-A 5% 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.

Primary Efficacy Analyses:

The effects of PE-A 5% treatment on the 90-day overall survival after study 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 (EU or NA) and the 3 ACLF grades (ACLF-1b, ACLF-2, or ACLF-3a). 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 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.

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

Secondary Efficacy Analyses:

The effect of SMT+PE-A 5% treatment on 90-day transplant-free survival and on 28-day overall survival will be assessed by carrying out the same analyses specified for the primary efficacy variable described above.

The analysis of the 2 secondary endpoints will be performed using the ITT population. The fixed-sequence testing method will be used to adjust for multiplicity in the analyses of the secondary efficacy endpoints.

Exploratory Endpoints:

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

The analyses of the primary and secondary endpoints will be repeated for each sub-group of subjects with ACLF-1b, ACLF-2, or ACLF-3a for exploratory purposes. Additional sub-group analyses by age group, sex, race, and region (EU versus NA) may be performed for the primary endpoint.

All exploratory efficacy analyses related to in-patient hospital stay (including ICU stay) overall and transplant-free survival, and 28-day transplant-free survival will be assessed by carrying out the same analyses specified for the primary efficacy variable described above. These analyses will also be repeated for each sub-group of subjects with ACLF-1b, ACLF-2, and ACLF-3a for exploratory purposes.

Changes from baseline (last measurement taken prior to the start of study treatment on Day 1), during and after the treatment period and at the end of hospitalization (or before liver transplantation or death) in organ function parameters and sub-scores and in CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score and systemic inflammation parameters will be compared between treatment groups by means of analysis of covariance (ANCOVA) with 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 baseline subjects' characteristics, they will be accounted for to adjust treatment effects in the ANCOVA models for each of these exploratory variables. 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. CLIF-SOFA score and ESR assessments were removed in protocol Version 3.0, and any data collected prior to this protocol version may be listed but will not be subject to any summaries or formal statistical analyses.

The number and percentage of subjects showing a specific ACLF course (resolution, improvement, steady course, or worsening) or a specific organ failure as well as the number and percentage of new infections at the end of hospitalization will be compared between treatment groups by means of Fisher's exact or Chi-square test. As sensitivity analyses, treatment effects will be adjusted for clinically and statistically significant imbalances in baseline characteristics by fitting appropriate Logistic Regression model or by means of the Cochran-Mantel-Haenszel test.

Time-to-hospital discharge will be estimated by the Kaplan-Meier method and compared between treatment groups by the Log-rank test stratified by region and the 3 ACLF grades.

Albumin level in serum will listed for each subject and summarized by treatment group.

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

The overall 90-day mortality rate is assumed to be about 50% in the whole study population. Assuming a potential drop-out rate of 10% and 5% type-1 error for a 2-sided Chi-square test, a global sample size of 380 subjects (190 per treatment group) will allow 80% statistical power to detect an absolute reduction of 15% (a 35% 90-day mortality rate) in subjects treated with SMT+PE-A 5%.

Alternatively, the sample size was calculated based on the log-rank test as follows. Assuming the 90-day mortality rate is 50% for the control group, or equivalently, the survival rate is 50%, which translates to a hazard rate of 0.0077 assuming an exponential distribution. Using a log-rank test at the two-sided significance level of 5%, a total of 141 events would provide at least 80% power to detect an absolute reduction of 15% in mortality rate (a 35% 90-day mortality rate, or equivalently, a 65% survival rate) in subjects treated with SMT + PE-A 5%. This absolute difference in mortality rates translates to a hazard ratio of 0.6215, or an approximate 38% risk reduction. With all subjects followed for the entire 90-day duration of the study, a total of 332 subjects would be required to obtain 141 events. Assuming a dropout rate of 10%, a global sample size of 370 subjects (185 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 two sample sizes (ie, the one based on the Chi-square test) will be used to ensure the study has sufficient power.

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GLOSSARY AND ABBREVIATIONS

ACLF	Acute-on-Chronic Liver Failure
AD	Acute decompensation
ADL	Activities of daily living
ADR	Adverse drug reactions
AE(s)	Adverse event(s)
ALF	Acute liver failure
ALI	Acute lung injury
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
AR	Adverse reaction
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
BUN	Blood urea nitrogen
CLIF	Chronic Liver Failure
CLIF-C ACLF	CLIF-Consortium ACLF score
CLIF-C OF	CLIF-Consortium Organ Failure
CLIF-SOFA	Chronic Liver Failure Sequential Organ Failure
CMH	Cochran-Mantel-Haenszel
CMV	Cytomegalovirus
CRP	C-reactive protein
DAMPS	Damage associated molecular patterns
DBP	Diastolic blood pressure
DSMB	Data Safety Monitoring Board
DVT	Deep vein thrombosis
EASL	European Association for the Study of the Liver
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EU	European Union
FIO_2	Fraction of inspired oxygen
FFP	Fresh frozen plasma
GCP	Good clinical practice
GGT	Gamma glutamil transpeptidase
GI	Gastrointestinal
GOLD	Global Obstructive Lung Disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCG	Human chorionic gonadotrophin
HCT	Hematocrit
HE	Hepatic encephalopathy
HIV	Human Immunodeficiency Virus
HNA2	Human non-mercaptalbumin-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
IL	Interleukin
INR	International Normalized Ratio
IP	Investigational product
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
IVIG	Intravenous immunoglobulin
IWRS	Interactive Web Response System
LMWH	Low-molecular-weight heparin
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
PAMPS	Pathogen associated molecular patterns
PCR	Polymerase Chain Reaction
PE	Plasma Exchange
PH	Proportional-Hazards
PP	Per Protocol
RCT	Randomized Controlled Trial
ROS	Reactive oxygen species
RR	Respiratory rate
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SD	Standard deviation
SMT	Standard medical treatment
SOP	Standard operating proceure
SpO_2	Peripheral oxygen saturation
T	Temperature
TBV	Total blood volume
TEAE	Treatment-emergent adverse event
TRALI	Transfusion-related acute lung injury
US	United States
USA	United States of America
WBC	White blood cell

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

2.1 Name and Description of the Investigational Product

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

The investigational product (IP) in this study is Human Albumin Grifols 5% (Albutein® 5%), which will be administered via plasma exchange (PE) as a treatment for Acute-on-Chronic Liver Failure (ACLF). Albutein 5% is a solution containing 50 g/L of total protein of which at least 95% is human albumin. See Section 4.5 Study Treatments for detail.

Standard medical treatment (SMT) will be administered per institution standards. There are several published guidelines for management of complications and comorbidities of ACLF (1,2,3,4,5).

2.2 Relevant Findings from Nonclinical and Clinical Trials

Preliminary Results of Effects of Plasma Exchange on Fulminant Hepatic Failure and ACLF

Several studies suggest that PE may be effective and safe in the treatment of subjects with fulminant hepatic failure (6,7). A recent multicenter randomized controlled trial (RCT) (8) utilizing high volume PE was compared to SMT in 182 subjects with acute liver failure (ALF). The procedure consisted of the exchange of 10 L of plasma/day during 3 consecutive days, starting within 24 hours after the onset of hepatic encephalopathy (HE). Plasma exchange significantly improved hospital survival (58.7% vs. 47.8%; p=0.008). The incidence of adverse events (AEs) was similar in the 2 groups. There was evidence that PE may be effective in improving liver function recovery in cirrhotic subjects with severe hepatic failure and that PE offers beneficial effects as a liver support system for removing

circulating toxins that cause HE and hemodynamic instability in subjects with decompensated cirrhosis (9,10,11).

There is no study examining PE with human serum albumin 5% (PE-A 5%) in ACLF with the exception of a recent pilot investigation performed in the Hospital Clinic in Barcelona sponsored by Instituto Grifols S.A. (12) Ten subjects with acute decompensation (AD) of cirrhosis, serum bilirubin ≥5 mg/dL and HE (grade 2 or more), and/or renal failure (serum creatinine ≥2 mg/dL) were treated with 6 sessions of PE-A 5% within 10 days (exchange of 1.1 plasma volumes in each session). The main objective of the study was to assess safety of PE-A 5% in ACLF. All subjects received 5% albumin as the main replacement fluid (approximately 70% of the plasma exchanged). Fresh frozen plasma (FFP) will be administered after the 5% albumin administration to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration. Fresh frozen plasma was administered at the end of each PE-A 5% session (approximately 30% of total plasma removed). Based on the data obtained from this trial, severe thrombocytopenia (platelet count $\leq 20\times10^9$/L) was observed in 3 subjects with low platelet count at baseline. No hemorrhagic events were observed. PE-A 5% treatment improved liver, renal, cardiovascular, and cerebral function and attenuated inflammatory response and endothelial dysfunction. Serum albumin concentration increased from 24 to 28 g/L, indicating that PE using 5% albumin solutions has a modest impact on serum albumin. Short-term mortality rate was 30% at 28 days and 40% at 90 days. These mortality rates were lower than those observed in 40 matched subjects with ACLF included in the CANONIC Study (57.5% and 65% respectively) (13). This pilot study suggests that PE-A 5% is safe in subjects with ACLF, and may improve organ function.

2.3 Known and Potential Risks and Benefits to Human Subjects

The potential beneficial effects of PE-A 5% in ACLF are related to 2 features. Plasma exchange removes endogenous and exogenous substances accumulated in subjects with ACLF as a consequence of organ failure(s) and systemic inflammation. Many of these substances are normally transported in blood bound to serum albumin. However, since most binding sites of human serum albumin in subjects with cirrhosis and ACLF are presumably occupied, many of these substances circulate free in plasma and interact with specific cell sites producing adverse reactions, predisposing subjects with ACLF to bacterial infections (14). The net effect of these features is severe hypoalbuminemia and impairment in albumin function, which further increase the plasma concentration of free molecules with toxic effects.

The second potential beneficial effect of PE-A 5% is related to the substitution of the saturated endogenous albumin by exogenous albumin with preserved biological functions (15), thus improving the process of transportation and reducing the levels of free toxic molecules that accumulate in plasma as consequence of organ failure(s) and systemic inflammation.

Fluid removal and replacement during PE-A 5% will be performed simultaneously. Routine practice is to exchange 1.1 to 1.5 plasma volumes during a PE-A 5% session. Removing larger volumes will prolong the procedure, expose the patient to more replacement fluid and

more anticoagulant, and thus increases the risk of complications without a relevant benefit (8).

Administering FFP at the beginning of a PE-A 5% session would result in the removal of most coagulation factors administered. Therefore, the administration of FFP will be performed at the end of the PE-A 5% session to prevent coagulopathy.

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

2.4.1 Administration of Investigational Products

Plasma exchange treatment will be performed using Albutein 5% (5% sodium chloride albumin solutions). Fresh frozen plasma will be given after albumin to prevent coagulopathy. In subjects without severe coagulopathy (International Normalized Ratio [INR] ≤3), two-thirds of the volume exchanged will be replaced with albumin 5% and one-third with FFP. In subjects with severe coagulopathy (INR >3), half of the volume exchanged will be replaced with albumin and half with FFP. In total, a minimum of 4 and a maximum of 9 PE-A 5% sessions will be performed in each subject. Intravenous immunoglobulins (IVIGs) will be administered to prevent the development of hypogammaglobulinemia and infection. See Section 6.1.1 for detailed treatment administration.

2.4.2 Justification for Selection of Doses/Timing of Investigational Products

One-point-two (1.2) plasma volumes will be exchanged per PE-A 5% session. Total blood volume (TBV) will be calculated according to Nadler's formula (Appendix 3). Plasma volume will be estimated from the calculated TBV and the hematocrit (HCT). The volume to be exchanged will be considered as the volume to be infused (not the fluid to be removed). PE-A 5% sessions will be scheduled as follows: 2 consecutive sessions initially to rapidly reduce systemic inflammation followed by every other day sessions to enable the liver to produce the coagulation factors removed during PE-A 5%.

Evidence suggests that ACLF is caused predominantly by intense and sustained stimulation of the innate immune system causing systemic inflammation. Plasma levels of cytokines increase across ACLF grades and the clinical course of the syndrome varies in parallel with changes in the plasma levels of cytokines and systemic oxidative stress. Removal of plasma inducers of inflammation and pro-inflammatory mediators (cytokines and reactive oxygen species [ROS]) by PE-A 5% is, therefore, a rational therapy for ACLF.

In this study, a minimum of 4 PE-A 5% sessions are scheduled to adequately remove all these inflammatory mediators and inducers. There is a lack of evidence to support additional PE sessions in patients showing no response. Therefore, the maximum number of PE-A 5% sessions in patients with no response will be 4. In contrast, in patients showing progressive improvement, treatment will be maintained until complete response with a maximum number of 9 sessions.

It can be considered that with 6 PE sessions, the subject plasma has been completely exchanged. The number of PE sessions required depends on the condition or disease treated, but normally ranges between 3 and 6 sessions. Subjects with acute conditions such as Guillain-Barré syndrome, and hemolytic uremic syndrome often require therapeutic cycles of 5 or more PE sessions (16). Considering rebound and re-equilibration between the extra vascular system and the intravascular system of large molecular weight substances is relatively slow (1 to 3%/hour), several consecutive PE treatments are essential to remove a substantial percentage of the total body burden. In many disease states, such as cirrhosis of the liver which causes hypoalbuminemia, it is suggested to begin with a therapeutic course of 5 to 7 exchanges and increase/decrease accordingly depending on the patient's condition (17).

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 diagnosed with ACLF-1b, ACLF-2, or ACLF--3a after obtaining the informed consent (See Table 2-1 for ACLF grades). This detailed classification of the ACLF is based on the review of the CANONIC data (13). The syndrome of ACLF has not been well characterized until recently when the CANONIC study was completed (a multicenter, prospective, observational study conducted in 1343 subjects hospitalized with AD of cirrhosis in 29 European hospitals) (13). To help ensure the inclusion of a homogeneous population of subjects in terms of mortality and potential reversibility of ACLF, the review of the CANONIC data corresponding to subjects with ACLF was to assess the following points:

- Whether ACLF grading at day 3 to 4 following diagnosis improves the prediction of short-term mortality with respect to ACLF grading at diagnosis. In this case, waiting 3 to 4 days prior randomization might increase the homogeneity of the series.
- Whether prognosis in subjects with ACLF-1 can be refined by subdividing the group in 2 subgroups: ACLF-1a including only subjects with single renal failure without cerebral dysfunction; ACLF-1b including subjects with any type of single organ failure (either renal or non-renal) associated with renal and/or cerebral dysfunction. Similarly, ACLF-3 can be further divided into 2 subgroups: ACLF-3a and ACLF-3b. See Table 2-1 below.

Table 2-1 Grades of ACLF

Grades of ACLF	
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: (13)

Table 2-2 shows the main results of this analysis:

- Re-evaluating ACLF grade 3 to 4 days following diagnosis does not substantially change the prediction of mortality from ACLF assessment at diagnosis. Therefore, delaying the study randomization by 3 to 4 days would not contribute to reduce the heterogeneity of the study population.
- Subjects with ACLF grade 1 can be divided into 2 subgroups with different prognosis.
- Subjects with ACLF grade 3 can be divided into 2 subgroups with different prognosis.
- The inclusion in the study of subjects with ACLF-1b, ACLF-2 or ACLF-3a at the time of diagnosis, excluding subjects with ACLF-1a and ACLF-3b, would reduce heterogeneity from a 90-day mortality rate ranging between 25 to 90% and 42 to 66% without a major reduction in the study population (only 25%).
- ACLF-1a resolves with SMT in a high proportion of subjects. Moreover, these subjects show relatively low 90-day mortality (25%) and may reach liver transplantation on a standard wait list. Treating ACLF-1a with PE-A 5% seems, therefore, unnecessary.
- In contrast, 90-day mortality rate in subjects with ACLF-3b is extremely high (90%) making them poor candidates for an invasive procedure such as proposed in the current trial.

Table 2-2 28-Day and 90-day Mortality Rates in Patients with Different Grades of ACLF at Diagnosis and 3-4 Days Later (CANONIC Study)

	28-day mortality		90-day mortality	
	At diagnosis	3-4 days after diagnosis	At diagnosis	3-4 days after diagnosis
NO ACLF				
Prevalence	-	23%	-	-
Mortality	-	10%	-	18%
ACLF-1a				
Prevalence	20%	10%	-	-
Mortality	15%	13%	25%	27%
ACLF-1b				
Prevalence	30%	18%	-	-
Mortality	31%	16%	42%	31%
ACLF-2				
Prevalence	36%	23%	-	-
Mortality	30%	37%	44%	52%
ACLF-3a				
Prevalence	9%	18%	-	-
Mortality	60%	58%	66%	65%
ACLF-3b				
Prevalence	5%	8%	-	-
Mortality	90%	91%	90%	97%
ACLF-1b/2/3a				
Prevalence	74.5%	58%	-	-
Mortality	34%	37%	46%	50%

Source: (13)

2.7 Relevant Data and Literature Review

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 (18). 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 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 has not been well characterized until recently when the CANONIC study was completed (13). 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 (EASL) Chronic Liver Failure (EASL-CLIF) Consortium. The EASL-CLIF Consortium defined ACLF as a syndrome that develops in patients with cirrhosis characterized by AD, organ failure(s), and high short-term mortality.

The CANONIC study (13) and other investigations (3,19,20,21) strongly suggest that ACLF occurs in the setting of systemic inflammation. Patients admitted to hospital with decompensated cirrhosis and ACLF show significantly higher levels of leukocytes and serum C-reactive protein (CRP) levels than those without ACLF. Moreover, blood leukocytes and serum CRP levels increase in parallel with the grade of ACLF. In 30% of patients, systemic inflammation occurs in association with a bacterial infection; in 25% of patients, with acute alcoholic liver injury; and in the rest of the patients, with no clear precipitating event.

Diagnostic Criteria of Organ Failure and of ACLF in Cirrhosis

The diagnostic criteria of ACLF by the EASL-CLIF Consortium were based on the assessment of: a) clinical features, b) course, and c) prognosis (28-day and 90-day survival) of the patients included in the CANONIC study (13).

First, the CLIF Sequential Organ Failure (CLIF-SOFA) Score (Appendix 4) was the original scale used to define organ failure in the CANONIC study. Liver, renal, cerebral, coagulation, circulation, and respiration are the organs/systems assessed by the score, each one classified into 6 categories. A simplified version of the CLIF-SOFA score, the CLIF-Consortium Organ Failure (CLIF-C OF) Score (Appendix 4) with identical criteria to diagnose organ failure and similar prognostic accuracy, has been developed post analysis of the CANONIC data (3).

The second assumption/criterion is that according to clinical experience, ACLF occurs in the setting of AD.

The third predefined criterion for the diagnosis of ACLF was an expected 28-day mortality ≥15%. In the CANONIC study, this criterion was present in patients with 2 or more organ failures, but not in patients with one organ failure (a 28-day mortality of 14.6%). Additional risk factors were used to further categorize patients in this low risk subgroup (Figure 2-1) (13).

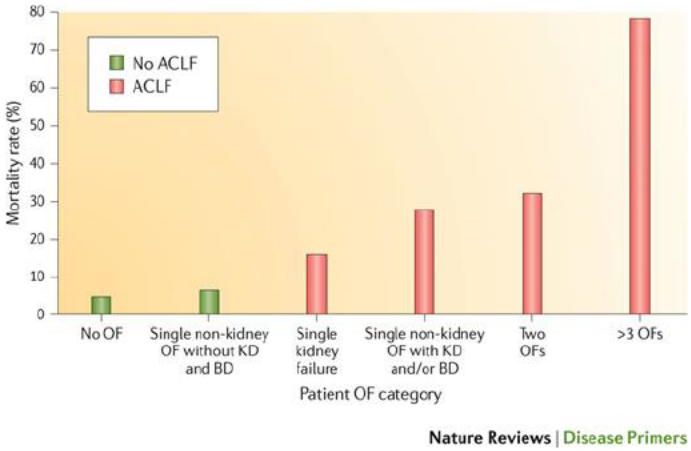

Figure 2-1 28-Day Mortality Rates of Patients with Decompensated Cirrhosis with (Red Bars) and without (Green Bars) ACLF according to the Diagnostic Criteria Proposed in the CANONIC Study

Patients are divided into the following categories: patients with no organ failure (OF); patients with a single non-kidney organ failure without kidney dysfunction (KD; a serum creatinine level of 1.5-1.9mg/dl) or brain dysfunction (BD; grade 1-2 HE); patients with a single kidney failure; patients with a single non-kidney organ failure with kidney dysfunction and/or brain dysfunction; patients with 2 organ failures; and patients with 3 or more organ failures. The groups included under the diagnosis of ACLF are represented in red.

Prevalence of ACLF in Cirrhosis and Associated Mortality

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 (13,22). Mortality rates at Day 28 and Day 90 are shown in Table 2-3:

Table 2-3 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: (13,22)

Two new prognostic scores, the CLIF-Consortium ACLF score (CLIF-C ACLF score) for patients with ACLF and the CLIF-Consortium AD score for patients with AD without ACLF were derived from the CANONIC study cohort (Appendix 4) (3,20). These 2 scores were designed because a single score was insufficient to satisfactorily delineate the prognosis of these 2 populations of patients. The CLIF-C ACLF score comprises the CLIF-C OF score, age, and white blood cell (WBC) count. The CLIF-Consortium AD score comprises age, serum sodium level, serum creatinine level, WBC count, and INR. Both scores range

between 0 and 100, have been validated by other studies and are more accurate than the Model of End-Stage Liver Disease (MELD) score (Appendix 4), the MELD sodium score (Appendix 4), and the Child-Pugh score (Appendix 4). The performance of both scores also improved over the period of follow-up, indicating that they should be updated daily.

Clinical Course of ACLF

ACLF is a very dynamic syndrome. It may improve, follow a steady course, or worsen during hospitalization (Table 2-1). Although there is considerable variability between subjects, some broad principles regarding the course of the condition can be put forward. First, improvement to no ACLF (resolution of ACLF) during hospitalization is frequent in subjects with ACLF-1 (55%), relatively frequent in subjects with ACLF grade 2 (35%), and infrequent (16%) in subjects with ACLF-3 (19). Few subjects with ACLF-1 (21%) progress to ACLF-2 or -3. Most subjects with ACLF-2 (51%) follow either a steady course (26%) or progress to ACLF-3 (26%). In addition, most subjects with ACLF-3 (68%) do not improve with the current medical treatment. Finally, 28-day and 90-day mortalities correlate with clinical course.

Table 2-4 28-day Clinical Course of ACLF (CANONIC Study)

Initial grade at diagnosis	Final ACLF grade			
	No ACLF	ACLF-1	ACLF-2	ACLF-3
ACLF-1 (n=202)	55% (improvement)	24% (no change)	9% (worsening)	12% (worsening)
ACLF-2 (n=136)	35% (improvement)	14% (improvement)	26% (no change)	26% (worsening)
ACLF-3 (n=50)	16% (improvement)	4% (improvement)	12% (improvement)	68% (no change)

Source: (13)

Association of ACLF development with precipitating events and prior history of acute decompensation.

ACLF in cirrhosis frequently develops in the setting of an acute event that acts as a precipitating factor (13). The most frequent precipitating events of ACLF in Europe and North America are bacterial infections and acute alcoholic hepatitis. ACLF usually occurs in young cirrhotic patients who are also alcoholics due to a systemic inflammatory reaction caused by bacterial infections or acute alcoholic liver injury. In 40% of these patients, however, precipitating events could not be identified (13). In Asia, ACLF often occurs due to acute viral hepatitis type A, B, and E superimposed to cirrhosis (23,24).

ACLF can develop at any stage from compensated to decompensated cirrhosis (13). In approximately 25% there is no prior history of AD. In these patients, therefore, AD associated to ACLF is the initial complication of cirrhosis. In another 25% of patients ACLF develops within a very short period (3 months) after the first episode of AD. Therefore, ACLF is infrequently a complication of an end-stage long-term chronic liver failure.

The clinical course and prognosis of ACLF depends partially on the presence and type of precipitating event. ACLF caused or complicated by infection shows a worse prognosis than that observed in ACLF subjects without infection. ACLF in subjects with no prior history of AD is also more severe (higher grade of ACLF and mortality) than in those with prior history of AD (13).

Mechanism of ACLF and Organ Failure in Cirrhosis and Pathophysiological Basis of Use of Plasma Exchange in Treatment of ACLF.

Evidence suggests that ACLF is caused predominantly by an intense and sustained stimulation of the innate immune system causing systemic inflammation (13,21,25,26,27).

There are 2 categories of ACLF: those in which the inducer (or inducers) of inflammation (for example, bacterial infections or excessive alcohol intake) are identified and those in which there is no clinical trigger (or triggers). Inducers of inflammation vary according to etiology.

Removal of plasma exogenous and endogenous inducers of inflammation (pathogen associated molecular patterns [PAMPs] and damage associated molecular patterns [DAMPs]), pro-inflammatory mediators (cytokines and ROS) and other biologically active substances such as endogenous vasodilators (nitric oxide, prostaglandins and bradykinin) by PE-A 5% is, therefore, a rational therapy for ACLF.

Systemic inflammation may induce organ failure by 2 different mechanisms. The first is related to severe impairment of cardio-circulatory function and organ hypo-perfusion due to intense release of vasodilators by endothelial and smooth muscle cells promoted by the inflammatory mediators. The second is related to the extension of systemic inflammation into the organs leading to direct deleterious effects of inflammatory mediators on microcirculatory homeostasis, mitochondrial function and cell survival. There is evidence that both mechanisms are important in the pathogenesis of organ failure in patients with ACLF (21).

Impairment of endogenous albumin function in cirrhosis. Dysfunctional albumin as a second target in the treatment of ACLF with PE-A 5%

Albumin is a critical determinant of the plasma oncotic pressure and therefore of circulatory homeostasis (28). 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) (29). Finally, the third and 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 (30).

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 (31,32).Oxidation of albumin to human non-mercaptalbumin-2 (HNA2) is irreversible and causes intense modifications of the protein structure and function and rapid clearance from the extracellular compartment (33,34).

Approximately 50% of circulating albumin in decompensated cirrhosis is oxidized and 10% is irreversible oxidized to HNA2 (21). 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. This data indicates a significant reduction in the scavenger capacity of serum albumin for inflammatory inducers and mediators. This feature may participate in the exaggerated response to the pro-inflammatory precipitating events observed in these patients.

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 PE-A 5% in patients with ACLF.

Current treatment of ACLF

Liver transplantation represents the only effective therapeutic option for patients with ACLF currently available. However, this treatment is far from ideal due to the shortage of organs for transplantation. The long-term probability of survival of patients included in the CANONIC study with ACLF grade 2 and grade 3 transplanted within the first 28 days after diagnosis was 81% (19). The corresponding figures of other studies rage between 74% and 90%, a number similar to that observed in patients transplanted for other indications (35,36,37,38,39). A major problem however, is that the feasibility of the procedure in severe ACLF is very low since in contrast to patients with acute liver failure, patients with ACLF cannot currently be included in the high-urgency transplantation list. In fact, current data indicate that less than half of the patients with ACLF are listed and of these, the procedure is feasible in only 10 to 25%, as >50 to 70% of the listed patients die on the waiting list (40).

Current medical management of ACLF consists of early recognition and treatment of the precipitating event and supportive care. Approximately 50% of patients with ACLF are admitted to intensive care units (ICUs) for organ support (13). There is no specific treatment for these patients. The Molecular Adsorbent Recirculating System (an extracorporeal liver support system based on albumin dialysis) and other artificial liver support devices improve cerebral and renal function but not the function of other organs or prognosis (41,42). On the other hand, circulatory support with terlipressin and intravenous (IV) albumin infusion, which improves renal function and may increase survival in some patients with cirrhosis and renal failure, is rarely effective in patients with ACLF and severe systemic inflammation (unpublished data from the CANONIC study). Therefore, there is urgent need for novel treatments to improve the high mortality associated to this syndrome.

PE-A 5% as a potential treatment of ACLF

Plasma exchange is a well standardized and safe therapeutic procedure widely used in many diseases requiring rapid and/or long-term removal of exogenous or endogenous substances with deleterious effects on organ function (16). At present, the most commonly used replacement fluid in PE is 4 to 5% human albumin. Fresh frozen plasma is used in a limited number of disorders (coagulation factor deficiencies). Plasma exchange has been safely tested in subjects with acute liver failure, a condition that reproduces many features of ACLF including extrahepatic organ failure(s), systemic inflammation and high short-term mortality.

A recent RCT in subjects with ALF, a condition also characterized by hepatic insufficiency associated to systemic inflammation and extra-hepatic organ/system failure, have shown that PE improves systemic inflammation, reduces organ failure(s) and renal replacement requirements and significantly increase survival in these subjects (43).

Moreover, a recent study on ACLF suggests that the potential beneficial effect of PE-A 5% in treating ACLF relies on 2 principles. The first is that it would remove endogenous and exogenous inducers of systemic inflammation (PAMPs and DAMPs), pro-inflammatory mediators (cytokines and ROS) and other biologically active substances (nitric oxide, prostaglandins and bradykinin) that participate in the pathogenesis of organ failure(s). The second is that PE-A 5% will exchange highly oxidized endogenous albumin with saturated binding sites by exogenous albumin with higher functionality and its process may modulates the severity of systemic inflammation (21).

3 STUDY OBJECTIVES AND PURPOSE

The current study aims to assess PE-A 5% as a treatment for ACLF. Subjects with ACLF-1b to -3a in severity will be included in this study to assess the impact on survival of SMT plus PE-A 5% (SMT+PE-A 5%) relative to SMT alone.

3.1 Efficacy Objectives

3.1.1 Primary Efficacy Objective

- To evaluate the effect of SMT+PE-A 5% on 90-day overall survival.

3.1.2 Secondary Efficacy Objectives

- To evaluate the effect of SMT+PE-A 5% on 90-day transplant-free survival.
- To evaluate the effect of SMT+PE-A 5% on 28-day overall survival.

3.1.3 Exploratory Efficacy Objectives

The effects of SMT+PE-A 5% on the following parameters will be evaluated:

- 90-day overall, 90-day transplant-free, and 28-day overall survival after randomization in subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- In-patient hospital stay (including ICU stay) overall and transplant-free survival in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- 28-day transplant-free survival in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a
- Incidence of individual organ failures: liver, renal, brain, coagulation, circulation, and respiration as defined by the CLIF-C OF score and the total CLIF-C OF score, during the

treatment period and at the end of treatment and of hospitalization (or liver transplantation or death)

- ACLF course (resolution, improvement, steady course, or worsening), CLIF-C ACLF score, Child-Pugh score, and MELD score
- Systemic inflammation as measured by CRP, WBC, and procalcitonin
- Rate of all new infections
- Time-to-hospital discharge
- Pre and post PE-A 5% albumin levels in serum

3.2 Safety Objectives

- To determine the safety and tolerability profile of PE-A 5% in subjects with ACLF.

4 STUDY DESIGN

4.1 Primary Endpoint and Secondary Endpoints

The primary efficacy endpoint is to compare the 90-day overall survival in the intent-to-treat (ITT) population (see Section 9.7 for definition) between the SMT+PE-A 5% treatment group and the SMT control group.

The secondary efficacy endpoints are to assess the effects of PE-A 5% on: 1) 90-day transplant-free survival and 2) 28-day overall survival versus SMT alone.

4.2 Study Design and Plan

This is a phase 3, multicenter, randomized, controlled, parallel-group, open-label study to evaluate the effects of PE-A 5% in ACLF subjects. The study will involve hospitals with expertise in the management of subjects with ACLF. This clinical study is planned to be performed in approximately 40 study centers in the United States, Canada, and Europe. The protocol must be approved by the Institutional Review Board (IRB) or Ethics Committee (EC) of each of the participant centers.

Approximately 380 subjects with cirrhosis, ACLF, and high risk of hospital mortality (ACLF-1b, ACLF-2, or ACLF-3a) (Table 2-1) will be included in this study after obtaining written informed consent. In case of HE, written informed consent will be obtained from a relative or a legally authorized representative if the subject is considered incompetent to consent.

There will be a screening period of no more than 10 days for each subject during which subjects will be screened for enrollment in the study. Subjects with ACLF-1b, ACLF-2, or ACLF-3a will be included at diagnosis of the syndrome. Subjects with ACLF-1a will be followed up and included if they deteriorate to ACLF-1b, ACLF-2, or ACLF-3a within the

10-day Screening Period. Subjects with ACLF-3b will be followed up and included if they improve to ACLF-3a, ACLF-2, or ACLF-1b within the 10–day Screening Period.

Randomization of subjects will be stratified by region (European Union [EU] or North America [NA]) and the 3 ACLF grades (ACLF-1b, ACLF-2, or ACLF-3a). Within each stratum (ie, each unique combination of region and ACLF grade), subjects will be randomized in a 1:1 ratio into 2 treatment groups below:

- SMT+PE-A 5% (treatment group)
- SMT (control group)

SMT+PE-A 5% Treatment Group

Approximately 190 subjects will be randomized to the SMT+PE-A 5% treatment group. PE-A 5% will be performed using 5% albumin (Albutein 5%) as the main replacement fluid administered intravenously. Fresh frozen plasma will be given after each PE-A 5% session to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration. The treatment schedule consists of 2 initial PE-A 5% sessions on consecutive days followed by every other day PE-A 5% sessions. A minimum of 4 and a maximum of 9 PE-A 5% sessions will be performed in subjects randomized to receive SMT+PE-A 5% (maximum treatment period: 17 days).

The exact number of PE sessions will be determined by the pattern of response (achieving complete response or no improvement/deterioration of ACLF) to PE-A 5% therapy. See Section 6.1.1.3, Table 6-1, and Table 6-2 for further details. IVIGs will be administered at a dose of 200 mg/kg 1 day after every 2 PE sessions in order to prevent the development of hypogammaglobulinemia and infection. For subjects ending the treatment at an odd-number PE session (eg, #5, #7, or #9), IVIGs will be administered at a dose of 100 mg/kg 1 day after this last PE session. Initial infusion rate of IVIGs will be of 0.5 mL/kg/hour, increasing to a maximum rate of 2 mL/kg/hour if well tolerated. See Table 6-1 and Table 6-2 for details.

SMT Group

Approximately 190 subjects will be randomized to the control group and receive SMT (see Section 2.1) for the length of the study or as long as needed. Subject's treatment period as specified in this protocol will be 7 days for all subjects and will then be prolonged depending on subject's ACLF evolution for up to 17 days:

- Subjects in the SMT group with ACLF-1b, -2, or -3a who achieve complete response (ACLF resolution or improve to ACLF-1a) within the treatment period (from Day 1 to Day 7), will be assessed for 4 more days after the ACLF resolution or improvement day and then will be followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.
- Subjects in the SMT group with ACLF-1b, -2, or -3a who maintain or worsen their ACLF grades throughout the treatment period (from Day 1 to Day 7), will be further assessed for up to Day 17 and will be then followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.

The Treatment Period will be up to 17 days. See Appendix 1 and Appendix 2 for detailed study schedules.

Subjects in both the SMT+PE-A 5% treatment group and the SMT control group will be followed for 90 days after randomization. During the entire study, the safety of both groups will be monitored by a DSMB (Section 1).

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

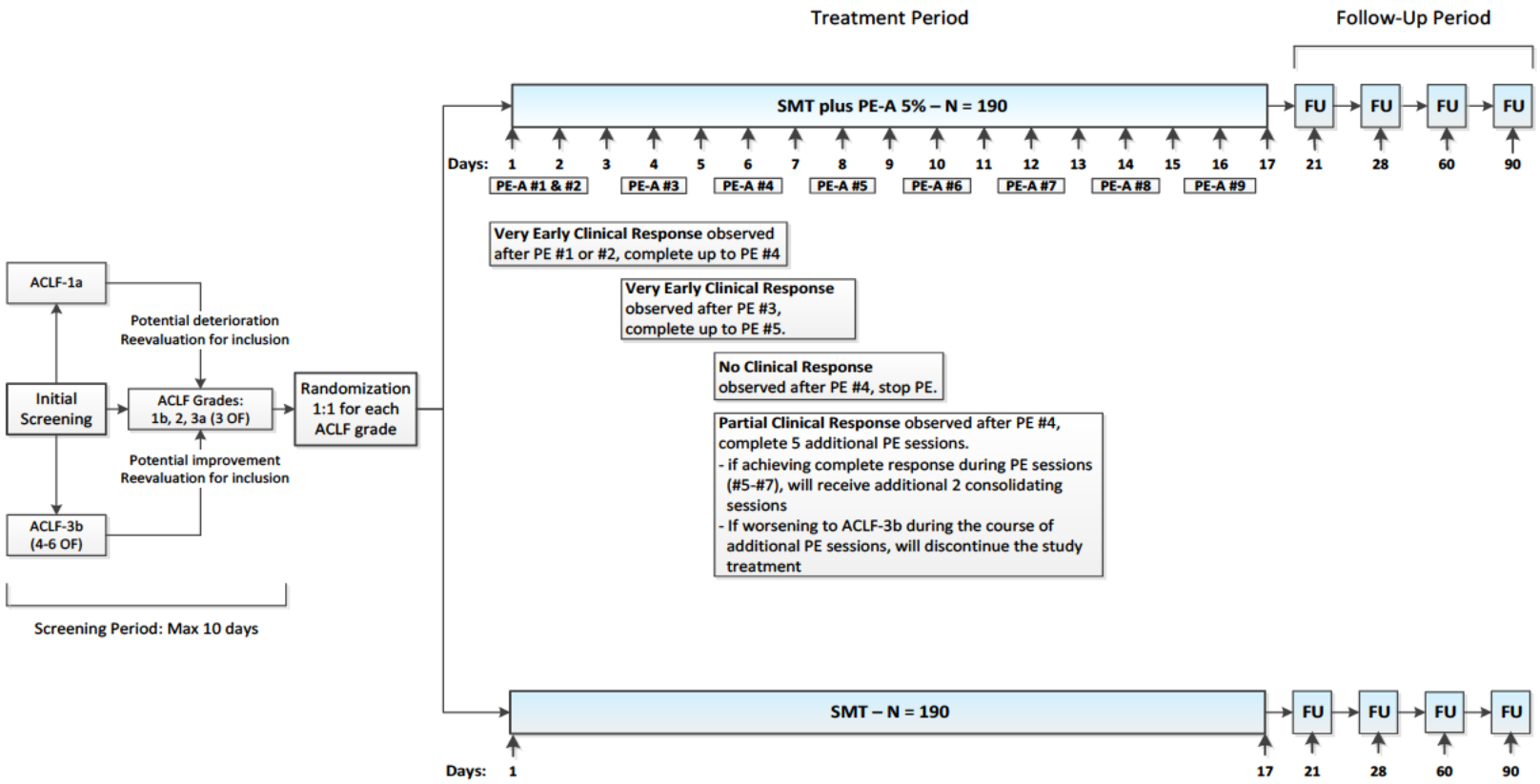

Figure 4-1 Overall Study Schema

FU = follow up visit

IVIGs will be administered intravenously at a dose of 200 mg/kg 1 day after every 2 PE-A 5% sessions in order to prevent the development of hypogammaglobulinemia and infection. For subjects ending the treatment at an odd-number PE session (eg, #5, #7, or #9), IVIGs will be administered at a dose of 100 mg/kg 1 day after this last PE session. Initial infusion rate of IVIGs will be of 0.5 mL/kg/hour, increasing to a maximum rate of 2 mL/kg/hour if well tolerated.

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 will be 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 "1"). For example, if the investigator's center number is 301, subject number will be 3011001, 3011002, 3011003, 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 will be stratified by region (EU or NA) and the 3 ACLF grades (ACLF-1b, ACLF-2, or ACLF-3a). Within each stratum (ie, each unique combination of region and ACLF grade), subjects will be randomly assigned to 1 of the 2 treatment groups (SMT vs. SMT+PE-A 5%) in a 1:1 ratio. Randomization will be centralized and will be performed via an Interactive Web Response System (IWRS) 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 SMT+PE-A 5%

Subjects allocated to this treatment group will receive SMT+PE-A 5%. PE-A 5% will be performed using 5% albumin and FFP as replacement fluids Fresh frozen plasma will be administered after the 5% albumin administration to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration. The amount of Albutein 5% and FFP to be infused will be determined by the INR (See Section 6.1.1.1). IVIG will be administered intravenously after every 2 PE-A 5% sessions in order to prevent the development of hypogammaglobulinemia and infection (See Section 6.1.1.3).

4.4.1.2 Albutein® 5%

Albutein 5% 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 body 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.

4.4.1.3 Standard Medical Treatment

Subjects allocated to the control group will receive SMT. Standard medical treatment will be administered per institution standards. Several guidelines are published for management of complications and comorbidities of ACLF (1,2,3,4,5).

4.4.2 Labeling of Investigational Product

Investigational products will be labeled according to the requirements of local law and regulations. 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 25°C. 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 maintaining 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 beginning with the screening will be up to 100 days.

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 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 5% 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 and IWRS 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 eCRFs

Not applicable.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

Eligible participants for this study include male or female subjects who are between 18 to 79 years of age and have a diagnosis of ACLF-1b, -2, or -3a. Subjects who initially fail to meet the ACLF eligibility criteria may be re-screened within a maximum of 10 days.

Subjects who fail to meet eligibility criteria upon re-screen are Screen Failures and will not be eligible to participate in the study.

Note that any criterion number containing v3 was modified in the protocol (eg, 2v3 indicates that the original criterion #2 was last modified in Protocol Version 3).

5.1 Inclusion Criteria

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

1. Male or female cirrhotic subjects between 18 and 79 years of age.
2. Subjects with ACLF-1b, ACLF-2, or ACLF-3a detected either at admission or during hospitalization (must be ACLF-1b, -2, or -3a within the Screening Period [a maximum of 10 days]) (see Table 2-1 for ACLF grades).
3. 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.
4. In case of HE, informed consent will be provided by a relative or a legally authorized representative if the subject is considered incompetent to consent.

5.2 Exclusion Criteria

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

1. Subjects without ACLF.
2. Subjects with ACLF-1a or ACLF-3b (See Table 2-1 for ACLF grades) after the Screening Period.
3. Subjects fulfilling inclusion criteria that improve to no ACLF or to ACLF-1a or worsen to ACLF-3b during the Screening Period (between initial evaluation and time of randomization).
4. Subjects with ACLF for more than 10 days prior to randomization.
5. Subjects with acute or subacute liver failure without underlying cirrhosis.

6v3 Subjects with septic shock requiring use of norepinephrine (> 0.3 mcg/kg/min) or need for a second vasopressor (including terlipressin).

7v3 Subjects with active bacterial or fungal infection: who have received less than 24h of appropriate antibiotic treatment.

8v3 Subjects with severe respiratory failure with PaO2/FiO2 ≤200.

9. Subjects with active or recent bleeding (unless controlled for >48 hours).
10. Subjects with severe thrombocytopenia ($\leq 20 \times 10^9$/L) (based on local laboratory assessment).
11. Subjects with chronic renal failure and currently receiving hemodialysis.

12. 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 5]), non-melanocytic skin cancer, and controlled breast or prostate cancer, can be included).
13. Subjects with severe chronic heart failure (New York Heart Association [NYHA] class III or IV) (Appendix 6).
14. Subjects with severe pulmonary disease (Global Obstructive Lung Disease [GOLD] stage III or IV) (Appendix 7).
15. Subjects with severe myopathy as defined clinically.
16. Subjects with a known infection with human immunodeficiency virus (HIV) or have clinical signs and symptoms consistent with current HIV infection.
17. Females who are pregnant, breastfeeding, or if of childbearing potential, unwilling to practice a highly effective method 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 a trial, and withdrawal are not acceptable methods of contraception).

18. Subjects with previous liver transplantation.

19v3 Subjects receiving anti-platelet or anti-coagulant therapy (LMWH for DVT prophylaxis is allowed).

20. Participation in another clinical study within at least 30 days prior to screening.

21v3 Subjects with active drug addiction (exceptions: active alcoholism or marijuana)

22. Subjects with a do-not-resuscitate order.
23. In the opinion of the investigator, the subject may have compliance problems with the protocol and the procedures of the protocol.
24. 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.

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 Days 21, 28, 60, and 90 follow-up procedures performed.

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

- New septic shock with norepinephrine requirements >0.5 mcg/kg/min
- Severe bleeding (gastrointestinal or other)
    - Any intracranial bleeding (excluding microhemorrhages <10 mm evident only on gradient-echo MRI)
    - Clinically overt signs of hemorrhage associated with a drop in hemoglobin of ≥5 g/dL, or a ≥15% absolute decrease in hematocrit, or requirement of ≥4 U of blood
    - Fatal bleeding (bleeding that directly results in death within 7 days)
- Anaphylactic shock or severe transfusion-related acute lung injury (TRALI) defined as new acute lung injury (ALI)/ARDS characterized by sudden development of dyspnea, bilateral infiltrates in chest X-ray, moderate or severe hypoxemia ($PaO_2/FiO_2 < 200$) with or without hypotension, and fever that develop during or within six hours after blood product administration
- Any other severe AEs (ie, acute myocardial infarction, cerebral hemorrhage, or intestinal necrosis) that preclude the continuation of the subject in the trial

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, subjects should be evaluated according to current clinical practice guidelines which include, but are not limited to, the use of the MELD score and the Child Pugh score (Appendix 4). Priority status will be defined by the MELD score and should be considered once a subject 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. Considering, frequent and unpredictable complications may be associated with ACLF limiting the utility of the above tools; a decision to proceed with LT should be individualized.

Additional criteria for liver transplantation in subjects with hepatocellular carcinoma or worsening within the Milan criteria (Appendix 5) with no CONTRAINDICATIONS as

determined by the Organ Procurement and Transplantation Network (OPTN) Dynamic Imaging criteria (45) 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 from the Study

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 adverse event (AE) post-treatment.
6. Termination of the trial by a regulatory authority.

In all cases, the reason for withdrawal must be recorded in the electronic case report form (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 the 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 Day 90 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 Hospital Discharge, Day 28, and Day 90.

6 TREATMENT OF SUBJECTS

6.1 Administration and Timing of Investigational Product for Each Subject

6.1.1 PE-A 5% Dosage, Procedures, and Treatment Regimen

See Section 4.4 for the treatments to be administered, including the name of the product, the dose, the dosing schedule, and the route/mode of administration.

6.1.1.1 Plasma Exchange Procedures

Plasma exchange treatment (PE-A 5%) will be performed using 5% albumin solution (Albutein 5%). Fresh frozen plasma will be administered after the 5% albumin administration to prevent coagulopathy, and the PE-A 5% session is considered completed at the end of the FFP administration. In subjects without severe coagulopathy (INR ≤3), two-thirds of the volume exchanged will be replaced with albumin 5% and one-third with FFP. In subjects with severe coagulopathy (INR >3), half of the volume exchanged will be replaced with albumin and half with FFP.

One-point-two (1.2) plasma volumes will be exchanged per PE-A 5% session. Total blood volume will be calculated according to Nadler's formula (Appendix 3). Plasma volume will be estimated from the calculated TBV and the HCT. The volume to be exchanged will be considered as the volume to be infused (not the fluid to be removed).

The formula for calculating plasma volume to obtain the total volume to be exchanged is as follows:

$$\text{Plasma volume} = \text{TBV} \times (1 - \text{HCT})$$

$$\text{Total volume to be exchanged} = \text{Plasma volume} \times 1.2$$

Note: plasma volume is in mL; HCT = hematocrit, TBV = total blood volume in mL

Plasma exchange will be performed using centrifugation or plasma filtration techniques (44,46). Citrate or heparin will be used as anticoagulant therapy. Vital signs of each subject and coagulation parameters will be controlled during each PE-A 5% session.

6.1.1.2 Treatment of Potential Complications Related to Plasma Exchange

Cardiovascular monitoring, Cardiac Overload Treatment, Coagulation Disturbances, and IVIGs Side Effects

Evaluation of cardiovascular function will be performed by continuous monitoring of vital signs during hospital admission (mean, systolic and diastolic arterial pressure, HR, and SpO_2). These vital parameters will be strictly monitored before, during, and after each PE-A 5% session. Arterial pressure will be measured non-invasively in subjects with hemodynamic stability and invasively, through an arterial line, in any subject experiencing shock during the procedure. Central venous pressure will be periodically monitored in all subjects through a central line catheter. Estimation of cardiac function will be mandatory in subjects with shock and can be performed by different techniques according to local guidelines (echocardiography, pulse wave analysis, Pulse index Contour Continuous Cardiac Output, Lithium Dilution Cardiac Output, Swan-Ganz).

Plasma Exchange is an euvolemic procedure. Therefore, cardiac overload/pulmonary edema is an unexpected side effect in subjects receiving PE-A 5%. In subjects with the diagnosis of cardiac overload (unrelated side effect), standard protocols will be applied as per institution standards and may include fluid restriction, IV administration of furosemide, and Renal Replacement Therapy (RRT) if clinically indicated (diuresis lower that 0.5 mL/kg under high doses of furosemide). See Appendix 8 for an example of Algorithm for Cardiogenic Pulmonary Overload.

In the case of clinically relevant alterations in the coagulation parameters after PE (fibrinogen <1 g/L, platelet <20,000/µL), transfusion (fibrinogen or platelets) will be carried out in order to prevent bleeding complications related to the procedure.

If an AE occurs during the infusion of IVIGs, the rate of the infusion should be reduced or the infusion should be stopped depending on the nature and severity of the event until all symptoms disappear. After re-evaluation of the AE, the infusion may be restarted and increased to a tolerated rate. If a subject experiences a second AE, the infusion should be stopped immediately.

6.1.1.3 PE-A 5% Treatment Regimen

A minimum of 4 and a maximum of 9 PE-A 5% sessions will be performed in each subject. The number of PE-A 5% sessions will be determined by the pattern of response (complete response, no response, or partial response) to PE-A 5% treatment (Table 6-1 and Table 6-2) and is described briefly below:

Treatment schedule consists of 2 initial PE-A 5% sessions on consecutive days followed by every other day PE-A 5% sessions. The aim of the study is to achieve complete response (resolution of ACLF or improvement to ACLF-1a):

- Subjects with very early complete response after 1 or 2 PE-A 5% sessions (resolution of ACLF or improvement to ACLF-1a) will receive 3 or 2 additional PE-A 5% sessions, respectively, to consolidate the response because the total number of PE-A 5% sessions should not be lower than 4. See Table 6-1 for details.
- Subjects with very early complete response after 3 PE-A 5% sessions (resolution of ACLF or improvement to ACLF-1a) will receive 2 additional PE-A 5% sessions to consolidate the response.

- Subjects with complete response after 4 PE-A 5% sessions (resolution of ACLF or improvement to ACLF-1a) will receive 2 additional PE-A 5% sessions to consolidate the response. See Table 6-1 for details.
- Subjects with no response (subject with no change or worsening of ACLF grade) after 4 PE-A 5% sessions will discontinue treatment so the total number of PE-A 5% sessions will be 4.

Table 6-1 PE Schedule (Number of Sessions) according to the Clinical Response to Treatment (Complete and No Clinical Responses)

Very early complete clinical response after the first 1 or 2 PE sessions[a]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■					
Day	1	2	3	4	5	6	7					
Very early complete clinical response after the first 3 PE sessions[b]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■	▲ #5	●			
Day	1	2	3	4	5	6	7	8	9			
Complete clinical response after the first 4 PE sessions[c]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■	▲ #5		▲ #6	■	
Day	1	2	3	4	5	6	7	8	9	10	11	
No clinical response after the first 4 PE sessions[d]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■					
Day	1	2	3	4	5	6	7					

[a] These subjects will receive 3 or 2 additional PE-A 5% sessions following complete clinical response and therefore will receive a total of 4 PE-A 5% sessions.

[b] These subjects will receive 2 additional PE-A 5% sessions following complete clinical response and therefore will receive 5 PE-A 5% sessions.

[c] These subjects will receive 2 additional PE-A 5% sessions following complete clinical response and therefore will receive 6 PE-A 5% sessions.

[d] These subjects with no response after 4 PE-A 5% sessions will discontinue the treatment so the total number of PE-A 5% sessions will be 4.

▲ PE = PE-A 5% session, ■ IGIV = 200 mg/kg every 2 PE sessions, ● IGIV = 100 mg/kg 1 day after the last, odd-numbered PE session

- Subjects with partial response after the first 4 PE-A 5% sessions (reduction of 1 organ failure or more to ACLF-1b or ACLF-2) will be treated accordingly: as described below:
  - Subjects with partial response who in the course of the additional PE-A 5% sessions show complete response will receive 2 consolidating sessions after achieving complete response. See Table 6-2 for details.
  - Subjects with partial response who in the course of the additional PE-A 5% sessions worsen to ACLF-3b will be discontinued for the PE-A 5% sessions.
- In summary, regardless of the type of response, the total number of sessions should never be lower than 4 or higher than 9.

Table 6-2 PE Schedule (Number of Sessions) according to the Clinical Response to Treatment (Partial Clinical Responses after the First 4 PE Sessions)

Achieve complete response at 5th PE session[a]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■	▲ #5		▲ #6	■	▲ #7
Day	1	2	3	4	5	6	7	8	9	10	11	12
PE	●											
Day	13											

Achieve complete response at 6th PE session[b]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■	▲ #5		▲ #6	■	▲ #7
Day	1	2	3	4	5	6	7	8	9	10	11	12
PE		▲ #8	■									
Day	13	14	15									

Achieve or do not achieve complete response after 7th PE session[c]												
PE	▲ #1	▲ #2	■	▲ #3		▲ #4	■	▲ #5		▲ #6	■	▲ #7
Day	1	2	3	4	5	6	7	8	9	10	11	12
PE		▲ #8	■	▲ #9	●							
Day	13	14	15	16	17							

[a] These subjects will receive 2 additional PE-A 5% sessions following complete clinical response at the 5th PE-A 5% session and therefore will receive a total of 7 PE sessions.

[b] These subjects will receive 2 additional PE-A 5% sessions following complete clinical response at the 6th PE-A 5% session and therefore will receive a total of 8 PE-A 5% sessions.

[c] Regardless of the response after the 7th or 8th session, subjects will receive the remaining 2 or 1 PE-A 5% sessions so the total number of PE-A 5% sessions will be 9.

▲ PE = PE-A 5% session, ■ IGIV = 200 mg/kg every 2 PE sessions, ● IGIV = 100 mg/kg 1 day after the last, odd-numbered PE session

IVIGs will be administered intravenously at a dose of 200 mg/kg 1 day after every 2 PE-A 5% sessions in order to prevent the development of hypogammaglobulinemia and infection. In those subjects receiving an odd number of PE-A 5% sessions (eg, #5, #7, or #9 PE-A 5% sessions when they are the last PE session for the subject), IVIGs will be administered 1 day after this PE-A 5% session at a dose of 100 mg/kg. Initial infusion rate of IVIGs will be of 0.5 mL/kg/hour, increasing to a maximum rate of 2 mL/kg/hour if tolerance is adequate.

The entire follow-up period will be 90 days after randomization. Detailed procedures can be found in Figure 4-1, Section 7.2.1, Appendix 1, and Appendix 2.

6.1.2 Standard Medical Treatment

Standard medical treatment will be administered per institution standards. Several guidelines are published for the management of complications and comorbidities of ACLF (1,2,3,4,5). Subjects will receive SMT (see Section 2.1) for the length of the study or as long as needed. Subject's treatment period specified in this protocol will be 7 days for all subjects and will be prolonged, depending on subject's ACLF evolution, to up to 17 days:

- Subjects in the SMT group with ACLF-1b, -2, or -3a who achieve complete response (ACLF resolution or improve to ACLF-1a) within the treatment period (from Day 1 to Day 7), will be assessed for 4 more days after the ACLF resolution or improvement and then will be followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.
- Subjects in the SMT group with ACLF-1b, -2, or -3a who maintain or worsen their ACLF grades throughout the treatment period (from Day 1 to Day 7), will be further assessed for up to Day 17 and will be then followed up at Hospital Discharge (if applicable) and on Days 21, 28, 60, and 90.

The entire follow-up period for subjects who receive SMT will be 90 days after randomization.

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 name of the medication, the dose, the route of administration, and the duration and frequency of the medication.

Any medication administered during the study (including any blood products [including IVIG and SMT]) 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 medication 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 hepatorenal syndrome (HRS) according to the International Club of Ascites (ICA) criteria (47). These subjects should follow the current EASL guidelines (48) in which management of type-1 HRS and indications of albumin are described. Noradrenalin plus albumin or the combination of octreotide, albumin and midodrine are considered a second line therapy of HRS. These treatments will be allowed in type-1 HRS only in countries where terlipressin is not available (United States of America [USA] and Canada).

6.2.1 Prohibited Medications Prior to Study Participation

Subjects receiving anti-platelet therapy or anti-coagulant therapy prior to enrollment (within 7 days prior to enrollment) cannot be included. In addition, subjects with HCV receiving antiviral therapy prior to enrollment cannot be included. Subjects antiviral therapy which was completed 14 days prior to randomization can be included in the study.

6.2.2 Prohibited Concomitant Medications during the Study

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

During the treatment period (from Day 1 to Day 7, which could be prolonged to up to Day 17 depending on the clinical responses in the SMT+PE-A 5% treatment group or ACLF evolution in the SMT group), the administration of nephrotoxic (non-steroidal anti-inflammatory drugs, angiotensin II receptor antagonists, angiotensin converting enzyme inhibitors) or V2 receptor antagonists (Vaptans) will not be allowed. Oral β-blockers could be maintained in subjects receiving this treatment prior to enrolment. However, their replacement by IV somatostatin is recommended during the treatment period in subjects allocated to receive PE-A 5% in order to assure hemodynamic stability.

Albumin dialysis will not be allowed during the treatment period in the SMT+PE-A 5% group.

Bioartificial liver support systems are not considered SMT in ACLF. Therefore, this therapy will not be allowed in both treatment groups during the 90-day study period.

6.2.3 Restricted Concomitant Medications during the Study

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

Nephrotoxic drugs (Nonsteroidal anti-inflammatory drugs, aminoglycosides, colistin) may not be administered and should be restricted in subjects with infections by multiresistant bacteria unless there is no equivalent treatment available.

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.

6.3 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

Definitions

Diagnosis of cirrhosis will be established by histology or by clinical, analytical, and ultrasonographic findings. Organ failures will be defined according to the CANONIC Study criteria (Table 2-1). Liver failure: serum bilirubin ≥12.0 mg/dL; kidney failure: serum creatinine level ≥2.0 mg/dL or renal-replacement therapy; coagulation failure: International Normalized Ratio ≥2.5; cerebral failure: grade III or IV HE according to the West-Heaven criteria (Appendix 9) (49); circulatory failure: use of any vasopressor to maintain hemodynamic stability (the use of terlipressin for the treatment of HRS is not included in this definition); and respiratory failure: ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (FIO_2) ≤200 or pulse oximetric saturation to FIO_2 ratio ≤214. Kidney dysfunction will be defined by a serum creatinine level ranging from 1.5 to 1.9 mg/dL. Mild-to-moderate HE will be defined by grade I or II HE.

ACLF-1a will be diagnosed in subjects with single kidney failure. ACLF-1b will be diagnosed in subjects with single organ failure with serum creatinine between 1.5 and 1.9 mg/dL and/or HE grade 1-2; ACLF-2 in the presence of 2 organ failures; and ACLF-3a in the presence of 3 organ failures, and ACLF-3b in the presence of 4 or more organ failures (Table 2-1). Complete response will be defined as resolution of ACLF or improvement to ACLF-1a and partial response as a reduction of 1 organ failure or more to ACLF-1b or ACLF-2.

Septic shock will be diagnosed in the presence of circulatory failure associated with inadequate oxygen utilization by the cells/inadequate tissue perfusion requiring vasopressor drugs. The presence of hypotension, although commonly present (MAP less than 60 to 65 mmHg), will not be required to define shock (50). Refractory septic shock will be diagnosed in subjects with high vasopressor requirements (norepinephrine dose >0.5 mcg/kg/min) (51). Diagnosis of ARDS will be established on the basis of a ratio PaO_2/FiO_2 ≤300 or a pulse oximetric saturation (SpO_2) to FiO_2 ratio ≤357 (52) add in the presence of bilateral lung infiltrates and normal left ventricular function.

Illness Severity Scores

The CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4) will be used to assess the clinical condition severity of the subjects.

7.1.1 Primary Efficacy Variable

- The primary efficacy variable will be time to death through Day 90 after randomization of SMT+PE-A 5% versus SMT alone.

7.1.2 Secondary Efficacy Variables

- Time to transplant or death through Day 90 after randomization of SMT+PE-A 5% versus SMT alone.
- Time to death through Day 28 after randomization of SMT+PE-A 5% versus SMT alone.

7.1.3 Exploratory Efficacy Variables

- Time to death through Day 90, time to transplant or death through Day 90, and time to death through Day 28 after randomization in subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Time to death and time to transplant or death through in-patient hospital stay (including ICU stay) in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Time to transplant or death through Day 28 after randomization in the entire study population and in the subgroups of subjects with ACLF-1b, ACLF-2, or ACLF-3a.
- Incidence of individual organ failures: liver, renal, brain, coagulation, circulation and respiration, as defined by the CLIF-C OF score and the total CLIF-C OF score, during the treatment period and at the end of treatment, and of hospitalization (or liver transplantation or death).
- ACLF course (resolution, improvement, steady course, or worsening), score, CLIF-C ACLF score, Child-Pugh score, and MELD score
- Systemic inflammation as measured by CRP, WBC, and procalcitonin
- Rate of all new infections.
- Time-to-hospital discharge.
- Albumin levels in serum measured pre and post PE-A 5% to monitor the status of infused albumin in subjects during the course of the study.

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 and Appendix 2 for summaries of study visits and the procedures to be conducted at each visit.

7.2.1.1 Screening Period (Day -10 to Day -1)

Following signature of the informed consent form (ICF), screening procedures will be performed. 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 Period assessments and activities will include:

- Assign subject number
- Add subject's data into the Screening Log
- Review of inclusion and exclusion criteria (see Sections 5.1 and 5.2, respectively) to determine subject's eligibility
- Documentation of demographics: year of birth, gender, race, and ethnic origin
- Documentation of medical history, including etiology of cirrhosis and significant concomitant disease conditions for the last 12 months
- Chest x-ray
- Electrocardiogram (ECG)
- Abdominal ultrasound
- 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 intrauterine device 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)
- Record vital signs (systolic blood pressure [SBP], diastolic blood pressure [DBP], HR, respiratory rate [RR], temperature [T])

- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamics assessment (mean arterial pressure [MAP])
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for clinical laboratory assessments (eg, hematology, chemistry, coagulation)
- Infection surveillance using microbiology (cultures) including blood, urine, ascetic fluid, sputum, and pleural fluid. If a positive result occurs at screening, a Day 3 result will be collected. If a thoracentesis or paracentesis has been performed within the last 5 days prior to screening, results of the fluid cultures should be documented in the medical notes. Microbiology cultures will be performed at local labs and the results will be captured in the subject's source documents but will not be the entered into the eCRF. All new infections will be recorded as AEs and recorded in subject's source documents and the eCRF. Additional samples will be obtained upon clinical suspicion.
- Microbiological tests (Cytomegalovirus [CMV] polymerase chain reaction [PCR], and galactomannan antigen index)
- Record ACLF grade (Table 2-1)
- Illness severity scores: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups
- Record prior (30 days prior to screening) and concomitant medications
- Record AEs

Subjects with ACLF-1b, ACLF-2, or ACLF-3a will be included at diagnosis of the syndrome. Subjects with ACLF-1a will be followed up and included if they deteriorate to ACLF-1b, ACLF-2, or ACLF-3a within the 10-day Screening Period. Subjects with ACLF-3b will be followed up and included if they improve to ACLF-3a, ACLF-2, or ACLF-1b within the 10-day Screening Period.

Evaluation of cardiac function at screening will be optional in subjects without vascular failure but mandatory in those with shock. This evaluation will be performed according to local guidelines.

7.2.1.2 Treatment Period (Days 1 to 17)

All screening laboratory results and assessments must be available and all inclusion and exclusion criteria must have been satisfied prior to initiating treatment. The Treatment Period is from Day 1 until Day 17. For subjects in the SMT+PE-A 5% treatment group, PE-A 5% treatment may stop early depending on clinical response (see Section 6.1.1, Table 6-1, and

Table 6-2). Subjects who remain in the hospital will continue to have Treatment Period assessments performed up through Hospital Discharge.

7.2.1.2.1 BASELINE/TREATMENT DAY 1

- Randomization (after confirmation of subject eligibility and prior to treatment)
- Record vital signs (SBP, DBP, HR, RR, T) (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation) (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
  - Plasma biomarker retains for subjects in the SMT+PE-A 5% treatment group (plasma removed will be collected from the plasma bag after the first 15 minutes and the last 15 minutes of the PE-A 5% session)
- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- PE-A 5% (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

7.2.1.2.2 TREATMENT DAYS 2, 4, 6, 8, 10, 12, 14, 16

- Record vital signs (SBP, DBP, HR, RR, T) measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group
- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight

- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation) (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Serum albumin concentration (measured immediately prior to and immediately after the end of the PE-A 5% session in the SMT+PE-A 5% treatment group)
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
  - Plasma biomarker retains for subjects in the SMT+PE-A 5% treatment group (plasma removed will be collected from the plasma bag after the first 15 minutes and the last 15 minutes of each PE-A 5% session)
- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4).
- PE-A 5% (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

7.2.1.2.3 TREATMENT DAYS 3, 5, 7, 9, 11, 13, 15, 17

Record vital signs (SBP, DBP, HR, RR, T) on days when IVIG is administered, measured immediately prior to IVIG administration

- Ascites assessment according to the ICA criteria (Appendix 10)
- Record weight
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Infection surveillance if positive result obtained at Screening Visit using microbiology (cultures) including blood, urine, ascetic fluid, sputum, and pleural fluid. Day 3 sample will be collected if positive result at screening. Additional samples will be obtained upon clinical suspicion. All new infections will be recorded as AEs.
- Record ACLF grade (Table 2-1)
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, MELD score (Appendix 4)

- IVIG 200 mg/kg infusion administered after every 2 PE-A 5% sessions (Days 3, 7, 11, or 15) (SMT+PE-A 5% treatment group only). See Table 6-1 and Table 6-2.
- IVIG 100 mg/kg infusion administered following the last, odd-numbered PE session on either days 9, 13, or 17 (SMT+PE-A 5% treatment group only). See Table 6-1 and Table 6-2.
- Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups (Days 3, 7, and 17 only)
  - Blood sample will be collected before IVIG administration (SMT+PE-A 5% treatment group only)
- Record prior and concomitant medications
- Record AEs

7.2.1.3 Follow-Up Period

7.2.1.3.1 HOSPITAL DISCHARGE

- Record vital signs (SBP, DBP, HR, RR, T)
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 10)
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for (Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
  - Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups
- Illness severity scoring: CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Record ACLF grade (Table 2-1)
- Record prior and concomitant medications
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death

Subjects who remain in the hospital will continue to have treatment period assessments performed up through Hospital Discharge. Follow-up assessments for Days 21, 28, 60, and 90 will be performed for all subjects.

7.2.1.3.2 FOLLOW-UP VISITS (DAY 21 [+1 DAY], AND DAYS 28 [+ 2 DAYS], 60 [+ 2 DAYS], 90 [+ 2 DAYS])

- Record vital signs (SBP, DBP, HR, RR, T)
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 10)
- HE assessment (Appendix 9)
- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
  - Blood biomarker retains (Days 28, 60, and 90) for subjects in the SMT and SMT+PE-A 5% groups
- Illness severity scoring: CLIF-C OF score, , CLIF-C ACLF score, Child-Pugh score, and MELD score(Appendix 4)
- Record ACLF grade (Table 2-1)
- Record prior and concomitant medications
- Record AEs
- Record data on liver transplantation, subject survival, and cause of death on Days 28 and 90

7.2.1.3.3 FOLLOW-UP FOR EARLY DISCONTINUED SUBJECTS WITHDRAWN FROM THE STUDY

Subjects who discontinue from the study early should have all Day 90 procedures performed at the time of withdrawal (preferably within 1 week of withdrawal) including the following:

- Record vital signs (SBP, DBP, HR, RR, T)
- Full physical examination (excluding breast and genitourinary examination)
- Ascites assessment according to the ICA criteria (Appendix 10)
- HE assessment (Appendix 9)

- Hemodynamic assessment (MAP)
- Peripheral oxygen saturation (SpO_2) assessment
- Blood sample collection for (see Table 7-1):
  - Clinical laboratory assessments (eg, hematology, chemistry, coagulation)
  - Serum albumin concentration
  - Systemic inflammation assessment (CRP [in chemistry panel], WBC [in hematology panel], and procalcitonin)
  - Blood biomarker retains for subjects in the SMT and SMT+PE-A 5% groups
- Illness severity scoring:, CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (Appendix 4)
- Record ACLF grade (Table 2-1)
- Record prior and concomitant medications
- Record AEs
- 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, b]	Hemoglobin, hematocrit, platelets, red blood cell count, white blood cell count with differential	Central
Chemistry[a,b]	Sodium, potassium, creatinine, blood urea nitrogen (BUN), calcium, magnesium, lactate dehydrogenase (LDH), aspartate Aminotransferase (AST), alanine Aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline Phosphatase (ALP), glucose, total bilirubin, direct and indirect bilirubin, CRP, lactate, serum albumin	Central
Coagulation[a,b]	INR, fibrinogen, platelet count, prothrombin time	Central
Urine pregnancy test	Qualitative urine β-HCG will be performed at screening	Local
Systemic inflammation assessment	Procalcitonin, WBC (hematology panel), CRP (chemistry panel)	Central
Microbiology tests	CMV, PCR, and galactomannan antigen index	Central
Biomarker retains[c]	Blood biomarker	Central
	Post-PE-A 5% plasma biomarker obtained from plasma removed during the first 15 minutes and the last 15 minutes of the PE-A 5% session	

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

[a] Laboratory tests required for determination of illness severity scores are presented in Appendix 4. Local results used for illness severity scores will be entered into the eCRF and the subject's source documents.

[b] To be collected before and after each PE session for the SMT+PE-A 5% treatment group.

[c] Blood biomarker retains will be drawn for potential analysis of cytokines (interleukin [[IL]] 6 (IL-6), IL-8, IL-10, and tumor necrosis factor alpha found in the blood which are significantly correlated with disease activity. Blood samples and post-PE-A 5% plasma samples will be stored for possible future analysis of other biomarkers that may be identified throughout the length of the clinical study and beyond. This will help identify biomarkers during the critical period involved in the development and clinical course of ACLF in patients admitted to the study with AD of cirrhosis and dynamics of ACLF development which could impact future treatments and target the disease in its earliest stages. 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.

7.2.3 Drug Concentration Measurements

As indicated, serum albumin concentration (clinical chemistry laboratory assessment) will be sequentially measured during the clinical study and before and after each PE-A 5% session up to Day 90 visit.

8 ASSESSMENT OF SAFETY

8.1 Safety Variables

- The number of suspected ADRs, including adverse reactions (ARs), and incidence rate of subjects with suspected ADRs during the treatment period and within 72 hours after PE-A 5% completion (or after PE-A 5% 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 PE-A 5% session.
- Clinical laboratory test parameters (blood cell counts, platelet count, INR, fibrinogen, etc.) will also 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 (Day 90 visit 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 and Appendix 2. 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's 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 and decompensated liver disease inherent in subjects with ACLF, 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 subject.

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 Events and Intercurrent Illnesses

8.3.1 Warnings/Precautions

For complete information on Albutein 5% refer to the IB.

Plasma Exchange is a safe technique that may induce very well-known and therefore preventable and controllable AEs, according to the guidelines provided below. Considering the special vulnerability of the subjects studied, a number of precautions (in addition to the habitual measures) will be taken to minimize the risks of the procedure:

- PE will be carried out by specialized certified personnel, under direct and continuous supervision by the specialized physicians (intensivists, hepatologists, and hematologists).
- Vital signs and laboratory test parameters will be monitored more frequently than usual.

There are AEs that may be expected during the replacement procedure, due to the process of apheresis, the replacement fluid used, or as a result of the subject disease or idiosyncrasy. These AEs include, but not limited to, hypocalcaemia (manifesting as paresthesias, headache, vision alterations, nausea, cramps and chest oppression), anaemia and coagulation disorders secondary to coagulation factor dilution (mainly hypofibrinogenemia, with possible minor bleeding associated to severe thrombocytopenia), hypotension (accompanied by paleness, perspiration, bradycardia, nausea, vomiting, syncope, sphincter relaxation and seizures) (though the risk is minimal, since the infusion amount is equivalent to the extracted amount), allergic reactions with urticaria, dyspnea, wheezing, hypotension, tachycardia, facial reddening and palpebral edema, and AEs related to vascular access. Other events may be of psychological origin or may be inherent to the process, such as anxiety, headache, nausea, immunoglobulin level decreased, anemia, or pain after remaining immobile for several hours. However, these AEs will still be required to be reported.

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 treatment.

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 the 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 deterioration 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, gastrointestinal (GI) bleeding, portal hypertension, ascites, spontaneous bacterial peritonitis, HRS, and associated organ/system failure(s) (eg, liver, kidney, brain, coagulation, circulation, and/or lung) (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.

Definitely 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 "definitely 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/Package Insert)for any serious ADRs (potentially related 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 ACLF hospital discharge, hospitalization is to be considered only for hospital stay equal to 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 ACLF
- 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 (eg, 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 [Day 90 visit] 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 pre-existing AE that gets worse regarding intensity or frequency, it should be indicated.

For AEs that occur during a PE-A 5% session, 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 (if applicable)

There are no events of special interest related to Albutein 5%.

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 investigational product 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 of ACLF includes, but not limited to: HE, gastrointestinal (GI) bleeding, portal hypertension, ascites, spontaneous bacterial peritonitis, HRS, and associated organ/system failure(s) (eg, liver, kidney, brain, coagulation, circulation and/or lung), which may develop at any time during the course of the disease.

The development of sequelae of ACLF and associated organ/system failure(s) 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 sequelae of ACLF and associated organ/system failure(s) 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 or prolonged 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

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 [Day 90 visit] 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 the study (Day 90) 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 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] (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 subjects 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 General

Categorical variables will be summarized by frequency of each category and the corresponding percentages. Continuous data will be summarized by the mean (SD) or the median (range) according to the approximate normality of data distributions. Depending on the nature of each variable, univariate analyses will compare treatment groups using Student's t tests, Mann-Whitney U-tests, Fisher's Exact test, or Chi-square test. Survival probabilities will be estimated by means of Kaplan-Meier curves and treatment groups will be compared by the log-rank test. More details will be provided in the SAP.

9.1.2.2 Primary Endpoint

This is a study to demonstrate superiority of SMT+PE-A 5% to SMT alone on 90-day overall survival. The null hypothesis is that the hazard functions for 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 death are not the same for the 2 treatment groups, or equivalently, the hazard ratio is not equal to 1. The effects of SMT+PE-A 5% treatment on the 90-day overall survival after study 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 (EU or NA) and the 3 ACLF grades (ACLF-1b, ACLF-2, or ACLF-3a). 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 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.

The primary 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).

9.1.2.3 Secondary Endpoints

The effect of SMT+PE-A 5% treatment on 90-day transplant-free survival and on 28-day overall survival will be assessed by carrying out the same analyses specified for the primary efficacy variable described above.

The analysis of the 2 secondary endpoints will be performed using the ITT population.

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 + PE-A 5% versus SMT alone for the secondary efficacy endpoints will only be tested if the superiority for the primary efficacy endpoint is demonstrated at the two-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 transplant or death through Day 90 after randomization of SMT+PE-A 5% versus SMT alone.
2. Time to death through Day 28 after randomization of SMT+PE-A 5% versus SMT alone.

9.1.2.4 Exploratory Endpoints

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

The analyses of the primary and secondary endpoints will be repeated for each sub-group of subjects with ACLF-1b, ACLF-2, or ACLF-3a for exploratory purposes. Additional sub-group analyses by age group, sex, race, and region (EU versus NA) may be performed for the primary endpoint.

All exploratory efficacy analyses related to in-patient hospital stay (including ICU stay) overall and transplant-free survival, and 28-day transplant-free survival will be assessed by carrying out the same analyses specified for the primary efficacy variable described above. These analyses will also be repeated for each sub-group of subjects with ACLF-1b, ACLF-2, and ACLF-3a for exploratory purposes.

Changes from baseline (last measurement taken prior to the start of study treatment on Day 1), during and after the treatment period and at the end of hospitalization (or before liver transplantation or death) in organ function parameters and sub-scores and in CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score and systemic inflammation parameters will be compared between treatment groups by means of analysis of covariance (ANCOVA) with 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 baseline subjects' characteristics, they will be accounted for to adjust treatment effects in the

ANCOVA models for each of these exploratory variables. 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. CLIF-SOFA score and ESR assessments were removed in protocol Version 3.0, and any data collected prior to this protocol version may be listed but will not be subject to any summaries or formal statistical analyses.

The number and percentage of subjects showing a specific ACLF course (resolution, improvement, steady course, or worsening) or a specific organ failure as well as the number and percentage of new infections at the end of hospitalization will be compared between treatment groups by means of Fisher's exact or Chi-square test. As sensitivity analyses, treatment effects will be adjusted for clinically and statistically significant imbalances in baseline characteristics by fitting appropriate Logistic Regression model or by means of the Cochran-Mantel-Haenszel (CMH) test.

Time-to-hospital discharge will be estimated by the Kaplan-Meier method and compared between treatment groups by the Log-rank test stratified by region and the 3 ACLF grades.

Albumin level in serum will listed for each subject and summarized by treatment group.

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 definitely or possibly related, the event will be defined as a suspected ADR. A suspected ADR with a causal relationship of "definitely 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 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 (including suspected ADRs) temporally associated with PE-A 5% (ie, those occurring during a PE session or within 72 hours after the completion of a PE session) will be summarized by presenting 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, SBP, and DBP) will be listed for each subject and summarized by treatment group. In case a subject presents a clinically significant abnormality of vital signs during a PE session, the event will be flagged and reported as an AE temporally associated with the PE session.

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 an AE.

9.2 Determination of Sample Size

Based on the results from the CANONIC study, the overall 90-day mortality rate is assumed to be about 50% in the whole study population (13,22). Assuming a potential drop-out rate of 10% and 5% type-1 error for a 2-sided Chi-square test, a global sample size of 380 subjects (190 per treatment group) will allow 80% statistical power to detect an absolute reduction of 15% (a 35% 90-day mortality rate) in subjects treated with SMT+PE-A 5%.

Alternatively, the sample size was calculated based on the log-rank test as follows. Assuming the 90-day mortality rate is 50% for the control group, or equivalently, the survival rate is

50%, which translates to a hazard rate of 0.0077 assuming an exponential distribution. Using a log-rank test at the two-sided significance level of 5%, a total of 141 events would provide at least 80% power to detect an absolute reduction of 15% in mortality rate (a 35% 90-day mortality rate, or equivalently, a 65% survival rate) in subjects treated with SMT + PE-A 5%. This absolute difference in mortality rates translates to a hazard ratio of 0.6215, or an approximate 38% risk reduction. With all subjects followed for the entire 90-day duration of the study, a total of 332 subjects would be required to obtain 141 events. Assuming a dropout rate of 10%, a global sample size of 370 subjects (185 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 a protocol amendment(s) and/or final clinical study report.

9.7 Subject Populations 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 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 and receive at least the first 2 PE-A 5% sessions (SMT+PE-A 5% treatment group) or survive more than 2 days following randomization (SMT control group). 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 subjects who receive at least one PE-A 5% 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. 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, multi-center publication of the study results from all appropriate study centers. If such a multi-center 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).
- 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' 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.

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17 APPENDICES

Appendix 1 Schedule of Study Procedures and Events

Procedures and Evaluation / Study Period / Study Day	Screening	Treatment Period[a]															
	-10 to -1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Inclusion/exclusion criteria	X																	
Medical history & demographics	X																	
Chest X-ray	X																	
ECG	X																	
Abdominal ultrasound	X																	
Urine pregnancy test	X																	
Physical exam[b]	X																	
Randomization		X																
Vital signs[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ascites assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HE assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemodynamics assessment[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral oxygen saturation (SpO_2) assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical laboratory assessments[f]	X	X	X		X		X		X		X		X		X		X	
Infection surveillance[g]	X			X														
Microbiology tests[h]	X																	
Record ACLF grade[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Illness severity scores[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Systemic inflammation assessment[k]		X	X		X		X		X		X		X		X		X	
Blood biomarker retains[l]	X			X				X										X
Post-PE-A 5% biomarker retains[m]		X	X		X		X		X		X		X		X		X	
PE-A 5% (1.2 plasma vol)[n]		X	X		X		X		X		X		X		X		X	
IVIG infusion[o]				X				X		X		X		X		X		X
Prior and concomitant medications[p]	X[n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[a] Depending on clinical response, subjects may not complete all treatment days. For subjects in the SMT+PE-A 5% treatment group, PE-A 5% treatment may stop early depending on clinical response (see Section 6.1.1, Table 6-1, Table 6-2, and Figure 4-1). For all subjects, if a subject receives a liver transplant, study treatment will be discontinued. All subjects who remain in the hospital will continue to have Treatment Period assessments performed up through Hospital Discharge. Follow-up assessments for Days 21, 28, 60, and 90 will be performed for all subjects (see Appendix 2).

[b] Full physical examination (excluding breast and genitourinary examination).

[c] Vital signs include systolic blood pressure, diastolic blood pressure, heart rate, body temperature, and respiratory rate. For subjects in the SMT+PE-A 5% treatment group, vital signs will be measured immediately before and immediately after each PE-A 5% session.

[d] HE assessment as per Appendix 9.

[e] Hemodynamics (MAP) will be monitored.

[f] Clinical laboratory assessments will include hematology, chemistry, and coagulation (see Table 7-1). For subjects in the SMT+PE-A 5% treatment group, assessment will be performed immediately before and immediately after each PE-A 5% session. For subjects in the SMT group, clinical laboratory assessments will be performed on Days 1 and 2, followed by every other day.

[g] Infection surveillance if positive result obtained at Screening Visit using microbiology (cultures) including blood, urine, ascetic fluid, sputum, and pleural fluid. If a positive result occurs at screening, a Day 3 result will be collected. If a thoracentesis or paracentesis has been performed within the last 5 days prior to screening, results of the fluid cultures should be documented in the medical notes. Microbiology cultures will be performed at local labs and the results will be captured in the subject's source documents but will not be the entered into the eCRF. All new infections will be recorded as AEs and recorded in subject's source documents and the eCRF. Additional samples will be obtained upon clinical suspicion.

[h] Microbiology testing includes cytomegalovirus, polymerase chain reaction, and galactomannan antigen index.

[i] ACLF grade (Table 2-1)

[j] Illness severity scores include CLIF-C OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score criteria (see Appendix 4 for illness severity scores).

[k] Systemic inflammation assessment includes testing for procalcitonin, WBC (part of hematology panel), and CRP (part of chemistry panel).

[l] For subjects in the SMT+PE-A 5% treatment group, blood biomarker retain samples will be collected prior to IVIG infusion (Screening, Days 3, 7, and 17). See Table 7-1 for additional information.

[m] Applies to subjects in the SMT+PE-A 5% treatment group only. Post-PE-A 5% plasma biomarker retains will be collected after each PE-A 5% session from the plasma bag on Days 1, 2, 4, 6, 8 10, 12, 14, 16.

[n] Applies to subjects in the SMT+PE-A 5% treatment group only.

[o] IVIG 200 mg/kg will be administered every 2 PE sessions on Day 3, 7, 11, or 15 (see Section 6.1.1.3). IVIG 100 mg/kg will be administered 1 day after the last, odd-numbered PE session on Days 9, 13, or 17 (see Section 6.1.1.3).

[p] Record prior (30 days prior to screening) and concomitant medications.

Appendix 2 Schedule of Study Procedures and Events (Hospital Discharge through Day 90)

Study Period	Hospital Discharge[a]	Follow-Up Period			
Procedures and Evaluation **Study Day**		**Day 21**	**Day 28[a]**	**Day 60**	**Day 90[a,b]**
Vital signs[c]	X	X	X	X	X
Physical exam[d]	X	X	X	X	X
Ascites assessment	X	X	X	X	X
HE assessment	X	X	X	X	X
Hemodynamics assessment[e]	X	X	X	X	X
Peripheral oxygen saturation (SpO_2) assessment	X	X	X	X	X
Clinical laboratory assessments[f]	X	X	X	X	X
Record ACLF grade[g]	X	X	X	X	X
Illness severity scores[h]	X	X	X	X	X
Systemic inflammation assessment	X	X	X	X	X
Blood biomarker retains[i]	X		X	X	X
Prior and concomitant medications[j]	X[i]	X	X	X	X
Adverse events	X	X	X	X	X

[a] On Hospital Discharge, Day 28, and Day 90, data on liver transplantation, subject survival, and cause of death will be recorded either by a medical visit or by telephone contact.

[b] Subjects who discontinue from the study early should have all Day 90 study procedures performed at the time of withdrawal (preferably within 1 week of withdrawal).

[c] Vital signs include systolic blood pressure, diastolic blood pressure, body temperature, heart rate, respiratory rate.

[d] Full physical examination (excluding breast and genitourinary examination).

[e] Hemodynamics (mean arterial pressure) will be monitored.

[f] Clinical laboratory assessments will include hematology, chemistry, and coagulation (see Table 7-1).

[g] ACLF grades (Table 2-1).

[h] Illness severity scores include CLIF-OF score, CLIF-C ACLF score, Child-Pugh score, and MELD score (see Appendix 4 for illness severity scores).

[i] Blood biomarker retains. See Table 7-1 for testing and handling.

[j] Record prior (30 days prior to screening) and concomitant medications.

Appendix 3 Nadler's Formula

Nadler's formula is used to calculate the total blood volume (TBV) of a human being based on gender, height, and weight.

1. Male: $0.3669 \times \text{Height}^3 + 0.03219 \times \text{Weight} + 0.6041$
2. Female: $0.3561 \times \text{Height}^3 + 0.03308 \times \text{Weight} + 0.1833$

Height in meter, body weight in kilogram

Note: the TBV calculated by the formula above is in liters (L). To convert volume from L to milliliters (mL), use the following formula:

1 L = 1000 mL

Appendix 4 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 <6mg/dL	6 ≤ Bilirubin <12mg/dL	Bilirubin ≥12mg/dL
Kidney	Creatinine <2mg/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.# Other patients enrolled in the study with MV were considered as presenting a respiratory failure (respiratory sub-score=3).*

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]

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)			

MELD Score

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

Appendix 5 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 $\leq$5 cm, or up to 3 tumors each with diameter $\leq$3 cm
- No extra-hepatic involvement
- No major vessel involvement

Appendix 6 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 New York Heart Association (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.

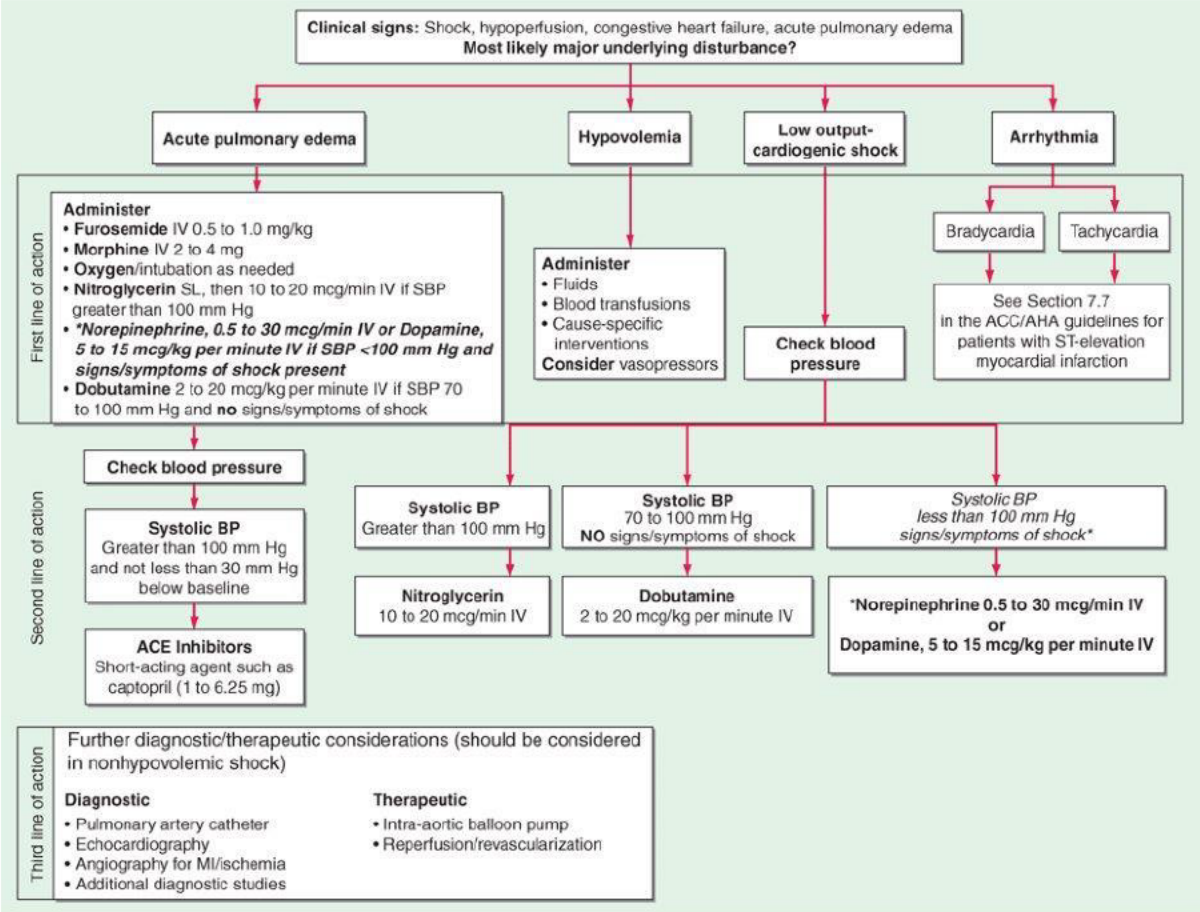
Appendix 7 GOLD Staging System for COPD Severity

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 FEV1/FVC <0.7 in spirometry, as sign of the airflow limitation that is not fully reversible.

Stage	Description	Findings (based on postbronchodilator FEV1)
0	At risk	Risk factors and chronic symptoms but normal spirometry
I	Mild	FEV1/FVC ratio less than 70 percent FEV1 at least 80 percent of predicted value May have symptoms
II	Moderate	FEV1/FVC ratio less than 70 percent FEV1 50 percent to less than 80 percent of predicted value May have chronic symptoms
III	Severe	FEV1/FVC ratio less than 70 percent FEV1 30 percent to less than 50 percent of predicted value May have chronic symptoms
IV	Very severe	FEV1/FVC ratio less than 70 percent FEV1 less than 30 percent of predicted value Or FEV1 less than 50 percent of predicted value plus severe chronic symptoms

GOLD = Global Initiative for Chronic Obstructive Lung Disease; COPD = chronic obstructive pulmonary disease; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity

Appendix 8 Algorithm For Cardiogenic Pulmonary Overload



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Appendix 9 West Haven Criteria for Hepatic Encephalopathy

WEST HAVEN CRITERIA OF ALTERED MENTAL STATUS IN HEPATIC ENCEPHALOPATHY			
Stage	**Consciousness**	**Intellect and Behaviour**	**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 behaviour	Muscular rigidity and clonus; Hyperreflexia
3	Somnolent but arousable	Gross disorientation; bizarre behaviour	Muscular rigidity and clonus; Hyperreflexia
4	Coma	Coma	Decerebrate posturing

Appendix 10 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: (47)

Appendix 11 Summary of Changes from Version 5.0 to Version 6.0

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

Sections	**Change From:** **(Version 5.0, dated 08-Jul-2020)** (Strikethrough is added to highlight deleted text)	**Change To:** **(Version 6.0)** (Underline is added to highlight new text)	**Rationale:**
Title page (Sponsor Signatories)	[redacted] MD [redacted] Grifols Therapeutics LLC Email address: [redacted] Telephone number: [redacted]	[redacted] [redacted] Grifols Therapeutics LLC Email address: [redacted] Telephone number: [redacted]	Due to change in clinical assessment monitor.
Investigator Signature Page	Removed Investigator Signature Page from the protocol amendment	No 'Investigator Signature Page' is required within the protocol.	Template updated to remove 'Investigator Signature Page' from protocol body text.
Title page (Sponsor Signatories)	[redacted] [redacted] ~~Grifols Bioscience Industrial Group~~ ~~Email address:~~ [redacted] ~~Telephone number:~~ [redacted]	[redacted] [redacted] Scientific & Innovation Office Grifols Email address: [redacted] Telephone number: [redacted]	Due to organization structural change.
Synopsis (Overall Study Design and Description), 4.2	In case of hepatic encephalopathy (HE), written informed consent will be obtained from a relative or a legally authorized representative ~~(surrogate)~~.	In case of hepatic encephalopathy (HE), written informed consent will be obtained from a relative or a legally authorized representative if the subject is considered incompetent to consent.	To clarify that signatures from a relative or a legally authorized representative can only be obtained **if** the subject is not competent to provide his/her consent.
Synopsis (Diagnosis and Main Criteria for Inclusion), 5.1	~~4.~~ In case of HE, informed consent will be provided by a relative or a legally authorized representative ~~(surrogate).~~	4. In case of HE, informed consent will be provided by a relative or a legally authorized representative if the subject is considered incompetent to consent.	To clarify that signatures from a relative or a legally authorized representative can only be obtained **if** the subject is not competent to provide his/her consent.

Sections	**Change From:** **(Version 5.0, dated 08-Jul-2020)** (Strikethrough is added to highlight deleted text)	**Change To:** **(Version 6.0)** (Underline is added to highlight new text)	**Rationale:**
Synopsis (Study Assessments and Procedures), 7.2.1.1	Documentation of demographics: ~~date~~ of birth, gender, race, and ethnic origin	Documentation of demographics: <u>year</u> of birth, gender, race, and ethnic origin	To align protocol wordings with the current legislation.
8.3.11.1	FAX (back-up only): [redacted] ~~(USA/Canada)~~	See change in the previous column.	Administrative change.
Appendix 1, Schedule of Study Procedures and Events	Deleted 'X' marked under 'Day 5, Day 7, Day 9, Day 11, Day 13, Day 15, and Day 17 for 'Infection surveillance' to match with footnote 'g' and protocol body text. It was an error in the assessment table.	See change in the previous column.	To correct internal inconsistencies.