Protocol Number: VS01-IIa-01

Official Title: A Phase 2a, Open-label, Randomized, Controlled, Multi-center, Proof of Concept Study, to Assess the Efficacy, Safety, and Tolerability of VS-01 on Top of Standard of Care, Compared to Standard of Care Alone, in Adult Patients with Acute-on-chronic Liver Failure (ACLF)

NCT Number: NCT05900050

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Statistical Analysis Plan (SAP)

Protocol Title:	A Phase 2a, open-label, randomized, controlled, multi-center, proof of concept study, to assess the efficacy, safety, and tolerability of VS-01 on top of standard of care, compared to standard of care alone, in adult patients with acute-on-chronic liver failure (ACLF)
Protocol Version No./Date:	12.0/12-Aug-2025
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1.0 Approvals

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(NOTE: Electronic Signatures should only be used if all parties have the ability to eSign.)

2.0 Change History

Version/Date	Change Log
1.0/28-Apr-2023	First version
2.0/07-Dec-2023	Section 4.0: Removed wording for SAP superseding the protocol due to the changes between SAP and the protocol to be listed in the following section 6.0; Section 6.1.1: Removed as protocol v8.0 and SAP are aligned in terms of pharmacodynamics analysis set; Sections 7.0, 8.x, 9.9 10.0, 11.3.2, 11.5.x, 11.6, 11.7, 11.8: Updated wording to match protocol v8.0; Sections 7.0, 9.27, 11.2, 11.5.5.11: Added MELD 3.0 score, as per protocol v8.0; Section 8.3.3: Clarified PP analysis set to consider events, in case other situations could prohibit patient being included in the analysis set; Section 9.4: Clarified unscheduled visit mapping to provide further instruction for programming; Section 9.6: Clarified age to have 1 decimal, to align throughout as CLIF-C ACLF score will use age with 1 decimal; Section 9.8: Added 'procedures' to clarify the definition of prior and concomitant; Section 9.11: Updated censoring rules for ACLF resolution and ACLF grade regression to use liver transplant and TIPS as censoring events. Further updates and clarity added to time to death through Day 28, time to death through Day 90, time to in-hospital death, time to ICU death, time to first hospital or ICU readmission for reoccurring ACLF, time to first ACLF reoccurrence; added visit window where missing; Section 9.13: Additional instructions added for CLIF-OF score derivation as further clarification for programming; Section 9.14: Additional instructions added for CLIF-SOFA score derivation as further clarification for programming; Sections 9.15, 9.20: Clarified ACLF resolution to use CLIF-OF for organ failures; Section 9.18: Updated 'discharge date' to 'planned discharge date' to be able to analyze the data the same way for all countries, due to different minimum hospitalization practices in countries; Section 9.30: Added SAAG derivation, as screening value is not coming from the central lab and must be calculated using the serum value from local lab and ascites value from the central lab; Section 11.0: Added information about missing data presentation in outputs as further clarity for programming; Section 11.1: Clarified categories to be presented in the output (included reason for screen failures and category 'randomized but not treated'); Section 11.2: Added age unit (years), removed body weight and added MedDRA version (25.1) for clarification. Added text that demographics and BL characteristics to be tabulated additionally on PP analysis set, to be able to compare the patient characteristics for PP analyses; Section 11.3.2: Added mentioning of nicotine and alcohol data collection as per protocol v8.0, and presentation of data in listings; Added text that procedures will be tabulated by system organ class and PT, as it has been deemed important to have this data summarized in tables, on top of listings. Section 11.5.1: Removed RRT as intercurrent event due to it being included in the endpoint, and missing data imputation rules updated for clarification; Section 11.5.3.2: Updated sensitivity analyses approach to remove RRT sensitivity analysis as it already is part of the endpoint; removal of imputation under MAR assumption as it is already covered in the primary analysis approach; removal of sensitivity analysis when using local lab at baseline as the primary model already includes the local labs at baseline and it has been deemed not to be useful; clarified PP sensitivity analysis approach; added sensitivity analysis of exclusion of liver transplant and TIPS patients to further explore the treatment effect; added sensitivity analysis of MAR imputation for patients who received

	TIPS or liver transplant to explore how the conclusion changes under different assumption; Sections 11.5.4.1, 11.5.4.2: Updated key secondary endpoint analysis by adding a secondary estimand and intercurrent events. Detailed description of the model and imputation method has been added; Section 11.5.4.2.2: Added sensitivity analyses for first secondary endpoint to further explore treatment effect; Sections 11.5.4.3, 11.5.5.1, 11.5.5.2: Updated mortality analysis to match the primary analysis approach and moved the mortality rates by group presentations under the section 11.5.4.3 and 11.5.4.2; Section 11.5.4.4: Added additional analysis to explore time to death when censoring for liver transplant; Section 11.5.4.5: Added analysis for ACLF grade change as per protocol v8.0; Section 11.5.5.8: Added mean and mean change plots of CLIF-C ACLF score and selected subcomponents to further visualize the data; Section 11.5.5.12.5 Clarified PHES assessment to highlight the changes in test administration time to explain why results could differ between countries; Section 11.5.5.18: Added subgroup analysis for primary and key secondary endpoint, to further explore the treatment effect within the pre-specified subgroups in an exploratory fashion; Section 11.6: Updated parameters display for further clarity of what will be analyzed; Section 11.7: Removed parameter $AUC_{0\text{-}24,dial,NH3}$ due to error in inclusion. Section 11.8.1: Updated to mention AEs related to infusion procedures are tabulated by maximum severity to give more information in the table. Removed 'not applicable' for IMP relationship as this option will not be used anymore. Section 11.8.2: Added CARPA parameters as per protocol v8.0 01Dec2023; Section 11.8.3: Removed pulse rate which is not captured on the CRF, therefore is not relevant; Section 11.8.5: Added mentioning of potentially clinically important ECG results, as the output has been requested to be added. Formatting and cosmetic changes are not listed.
3.0/31-May-2024	<u>Updates to match protocol v9.0:</u> Sections 7.0, 9.21, 11.5.5.12: Removal of tests for covert hepatic encephalopathy, such as PHES, Animal Naming Test and Stroop test assessments; removal of section 11.5.5.12.5, 11.5.5.12.6, and 11.5.5.12.7; Section 8.0: Update of the Screening window from 24 hours to up to 4 days (96 hours); other minor changes in wording; Section 11.5.3: Additional adjustment variable (delay between ACLF onset and baseline) added to the primary model; Section 11.8.2: Laboratory terminology update to reorder and rephrase for clarity. <u>Other updates:</u> Section 11.5.5.18: Age subgroups updated due to protocol update in age limit; Additional subgroup variables added to explore the treatment effect within the pre-specified subgroups in an exploratory fashion: ACLF onset (≤4 days, >4 days from BL) and geographical area (United States vs. Europe). Section 9.26: Added clarity for MELD-Na and MELD 3.0 score components; Formatting, grammar, references, abbreviations and cosmetic changes are not listed.
4.0/11-Jul-2025	Sections 6.1 and 9.1: Update treatment start time derivation for Control arm to use median treatment start time of Active arm patients; Section 7.0: Removal of reference to Health Related Quality of Life endpoints; removal of section 11.5.5.17; Addition of ACLF grade 3a;

	Section 9.2: Clarified baseline derivation when treatment start date/time is missing; Section 9.6: Added derivation of age for patients when month is missing as it is the case in Hungary; Section 9.11: Updated censoring rule for time-to-event parameters; Section 9.12: Clarified that age at ICF signature is used for CLIF-C ACLF score calculation for all timepoints; Section 9.13 and 9.14: Clarified that HE grade 'Minimal' is considered as score=0 in CLIF-C OF and CLIF-SOFA scores; Section 9.22: Clarified categories of West Haven HE grade; Section 9.26: Provide details on MELD, MELD-Na and MELD 3.0 score derivations; Section 11.2: Updated list of baseline characteristics with kidney failure and ACLF onset; Added clarification that ACLF etiology will additionally collect presence of bacterial peritonitis, presence of severe alcohol-related steatohepatitis (sASH), acute viral hepatitis, or uncontrolled autoimmune hepatitis (AIH), and presence of TIPS; Section 11.5.3.1: Added imputation for missing baseline variables; Sections 11.5.3.2.1, 11.5.4.2.2.1: Clarified when these analyses will be performed (only when >10% difference to the number of patients in Full Analysis Set); Section 11.5.3.2.2: Added clarification when tipping point approach is to be used; updated the range of tipping point; Section 11.5.3.2.4 and 11.5.4.2.2.3: Addition of sensitivity analysis excluding patients with ACLF due to sASH, acute viral hepatitis, or uncontrolled AIH at randomization, for the primary and first second endpoints; Section 11.5.3.2.5: Added Bayesian analysis as per the protocol amendment; Section 11.5.4.4: Clarified that if median and percentiles are not calculable, then p-value and hazard ratio will not be displayed; Section 11.5.4.5: Clarified that ACLF grade regression is based on central labs only; Section 11.5.5.12.4: Added an option to use Friedman's test; Section 11.8.1: Duration between AE occurrence and the most recent IP infusion will be derived and listed; Section 11.8.2 change to section 11.8.3 with addition of CTCAE grading for laboratory parameters; Clarification added for lab parameters collection timing; Insertion of the section 11.8.2: Adverse Event of Special Interest (AESI), per protocol v11.0 Section 11.8.5: Clarified that ECG abnormality assessment is based on the central read only. Formatting, grammar, references, abbreviations and cosmetic changes are not listed.
5.0/27-Aug-2025	Section 11.5.5.17: Added subgroups race, sex, and ethnicity for subgroup analyses as per FDA comment; Section 11.8.2: Clarified the collection/identification of AESI events.

3.0 Table of Contents

4.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Versantis AG Protocol VS01-IIa-01. Changes from the Protocol are detailed in section 6.1, if applicable. Any changes in the planned analyses prior to database lock will be documented through an update to the SAP and any changes after database lock will be documented in the clinical study report (CSR).

5.0 Scope

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Applicable Study Definitions
- Statistical Methods

6.0 Introduction

This SAP should be read in conjunction with the study Protocol and case report forms (CRFs). Any further changes to the Protocol or CRFs may necessitate updates to the SAP.

6.1 Changes from Protocol

In Protocol section 19.3, it is mentioned that "Treatment start date/time in Control group is defined as 11:00 on Day 1, which corresponds to the approximate timing of the administration of IMP in the Active Treatment group". However, patients in the Active Treatment group turn out to receive IMP administration on Day 1 often later than that, which has an effect on deriving baseline values. Treatment start time for Control group is to be taken as median Day 1 treatment start time of the Active Treatment group.

7.0 Study Objectives and Endpoints

Primary Objective	**Primary Endpoint**
Primary objective of the study is to evaluate the efficacy of VS-01 administered intraperitoneally (*i.p.*) daily over 4 days on top of standard of care (SOC) vs. SOC alone in patients with ACLF grades 1, 2, or 3a as measured by Chronic Liver Failure – Consortium Acute-on-Chronic Liver Failure (CLIF-C ACLF) score at Day 7.	The primary endpoint of the study is CLIF-C ACLF score at Day 7.
Secondary Objectives	**Secondary Endpoints**
To evaluate the efficacy of VS-01 administered *i.p.* daily over 4 days on top of SOC vs. SOC alone in patients with ACLF grades 1, 2, or 3a with respect to: ▪ Mortality rate; ▪ Time to Death; ▪ ACLF grade change and time to ACLF resolution;	Outcome: ▪ 90-day mortality; ▪ 28-day mortality; ▪ Time to death through Day 90; ▪ Time to death through Day 28; ▪ Change in ACLF grade through/at Days 7 and 28: o Rate of ACLF resolution;

	○ Time to ACLF resolution; ○ Rate of ≥ one ACLF grade regression; ○ Time to ≥ one ACLF grade regression;
▪ Transplant-free survival;	▪ Transplant-free survival through/at Day 90; ▪ Transplant-free survival through/at Day 28.
To characterize the safety and tolerability of VS-01 in patients with ACLF following *i.p.* administration of VS-01.	Incidence rate, severity, and relationship to VS-01 of adverse drug reactions and serious adverse drug reactions.
Exploratory Objectives	**Exploratory Endpoints**
To characterize the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of VS-01 in ACLF patients following *i.p.* administration of VS-01.	PK/PD: ▪ Plasma concentration-time profile of BL-corrected citric acid and lipids on Days 1 and 4 in patients treated with VS-01: maximum concentration (C_{max}), time to maximum concentration (t_{max}), area under the curve (AUC) (AUC_{0-3}, during 24 h [AUC_{0-24}], AUC_t, and to infinity [AUC_{inf}]), apparent elimination half-life ($t_{1/2}$), apparent clearance (CL), volume of distribution (V_d) and accumulation ratio; ▪ Change from BL through/at Days 5 and 7 in fasting plasma ammonia levels, maximum reduction in plasma ammonia ($E_{max,}$, time to normalization of plasma ammonia, and peritoneal ammonia clearance.
To evaluate the effect of VS-01 administered *i.p.* daily over 4 days on top of SOC vs. SOC alone in patients with ACLF grades 1, 2, or 3a with respect to:	Outcome:
▪ In-hospital mortality and time to in-hospital death;	▪ In-hospital mortality and time to in-hospital death;
▪ Intensive Care Unit (ICU) mortality and time to ICU death;	▪ ICU-mortality and time to ICU death;
▪ Duration of hospitalization and/or ICU stay;	▪ Duration of hospitalization and/or ICU stay;
▪ Hospital and ICU readmissions;	▪ Hospital and ICU readmission rate through/at Days 14, 28, and 90; ▪ Time to first hospital and ICU readmission for recurring ACLF;
▪ Re-occurrence of ACLF;	▪ ACLF reoccurrence rate through/at Days 14, 28, and 90; ▪ Time to first reoccurrence of ACLF;
▪ CLIF-C ACLF score (Days 5, 14, 28, and 90);	▪ Change in CLIF-C ACLF score from BL through/at Days 5, 14, 28, and 90;
▪ CLIF-C Organ Failure (CLIF-C OF) score;	Disease severity:

	▪ Change in CLIF-C OF score, number and types of organ failures from BL through/at Days 5, 7, 14, 28, and 90;
▪ CLIF- Sequential Organ Failure (CLIF-SOFA) score;	▪ Change in CLIF-SOFA score, number and types of organ dysfunctions from BL through/at Days 5, 7, 14, 28, and 90;
▪ Child-Pugh Score (CPS);	▪ Change in CPS score from BL through/at Days 5, 7, 14, 28, and 90;
▪ Model for End-stage Liver Disease (MELD), MELD-Sodium (MELD-Na), and MELD 3.0 scores;	▪ Change in MELD, MELD-Na, and MELD 3.0 scores from BL through/at Days 5, 7, 14, 28, and 90;
▪ Hepatic encephalopathy (HE);	HE: ▪ Change of West Haven grade from BL through/at Days 5 and 7; o Time to and rate of resolution of overt HE; o Time to and rate of regression of ≥ one HE grade; ▪ Change of HE grade as assessed by Hepatic encephalopathy Grading Instrument (HEGI™) from BL through/at Day 7: o Time to and rate of resolution of overt HE; o Time to and rate of regression of ≥ one HE grade; ▪ Change from BL through/at Days 5 and 7 in: o Glasgow Coma Scale;
▪ Inflammatory, metabolomic and proteomics profiles;	▪ Change in inflammatory, metabolomic and proteomic profiles from BL through/at Days 5 and 7;
▪ Renal, liver, brain, circulatory, respiratory and coagulation dysfunction and failure;	▪ Changes in renal function from BL through/at Days 5, 7, 14, 28, and 90: o Change in serum creatinine levels, creatinine clearance (Cockcroft-Gault equation), estimated glomerular filtration rate (eGFR), serum cystatin C levels, blood urea nitrogen (BUN) or urea, and uric acid; o Incidence of renal replacement therapy (RRT); ▪ Changes in liver function from BL through/at Days 5, 7, 14, 28, and 90: o Change in serum bilirubin levels; ▪ Incidence of Transjugular intrahepatic portosystemic shunt (TIPS) on Days 5, 7, 14, 28, and 90;

	▪ Incidence of liver transplant on Days 5, 7, 14, 28, and 90;

8.0 Study Design

This is a multi-center, randomized, controlled, open-label, Phase 2a proof of concept study of VS-01 in adult patients with ACLF grades 1, 2, or 3a. Approximately 60 patients will be enrolled in the study in order to achieve 54 evaluable patients for the primary endpoint at Day 7. Upon interim analysis (IA) the sample size might be increased up to approximately 120 patients (assuming a 10% drop-out rate; please refer to Protocol Section 19.8).

Patients will be randomized in a 1:1 ratio to receive either VS-01 on top of SOC (Active Treatment group) or SOC alone (Control group). Randomization will be stratified by CLIF-C ACLF score (<48 vs. ≥48) at baseline (BL, i.e., prior to dosing on Day 1) and country. Randomization will be centralized and will occur on Day 1.

Recruitment will be in a staged manner. At any time during the study, only 5 patients should be dosed with VS-01 simultaneously and dosing of subsequent patients with VS-01 should be initiated 2 days after one of the 5 patients on VS-01 treatment has received his/her last dose. This is based on the PK data from the first in human (FIH) study showing that citric acid and lipids are eliminated after 12 h, and up to 48 h post-last dose, respectively.

The study will include a Screening (SCR) period, a Treatment period, and a Follow-up period as shown in Protocol Section 13. Patients will receive the allocated treatment, either VS-01 *i.p.* daily on top of SOC or SOC alone for 4 days (Days 1 to 4) (refer to Protocol Section 11.4). Patients will be screened 1 day and up to 4 days prior to BL on Day 1.

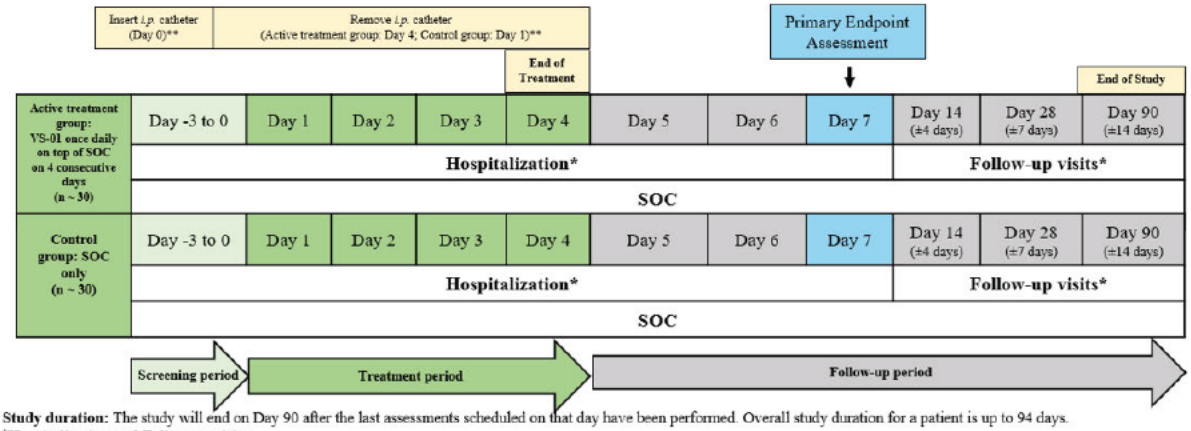
GERMANY: Patients should be hospitalized at least until Day 7, including treatment period (Days 1 to 4) and an additional 3-day post-treatment follow-up (Days 5 to 7).

ALL OTHER COUNTRIES: Patients in whom the clinical state precludes a hospital stay until Day 7, may be discharged on Day 5 after the study procedures have been completed, and no safety concerns have been raised in the Investigator's assessment, and will need to return for an out-patient study **visit on Day 7 (primary endpoint assessment)**. Day 6 assessments will only be performed in the patients who are hospitalized.

Patients will need to visit hospital for study visits on Days 14 (±4 days), 28 (±7 days) and 90 (±14 days). All patients will be followed for up to 90 days until End of Study (EOS) visit. The expected duration of the trial for each patient is up to 94 days.

The study design is summarized in **Figure *8-1* Study Design**.

Figure 8-1 Study Design



Study duration: The study will end on Day 90 after the last assessments scheduled on that day have been performed. Overall study duration for a patient is up to 94 days.
*__Hospitalization and Follow-up visits:__
FOR GERMANY: Patients should be hospitalized at least until Day 7, including treatment period (Days 1 to 4) and an additional 3-day post-treatment follow-up (Days 5 to 7).
FOR ALL OTHER COUNTRIES: Patients, in whom the clinical state precludes a hospital stay until Day 7 and no safety concerns were raised in the Investigator's assessment, may be discharged on Day 5 after the study procedures have been completed and will need to return for an out-patient study visit on Day 7. Day 6 assessments will only be performed if the patient is hospitalized.
Follow-up visits: During the follow-up period, visits may be in- or out-patient visits at the trial site
**Day 0: *i.p.* catheter insertion followed by paracentesis in all patients; Day 1: removal of *i.p.* catheter on Day 1 after randomization in the Control group; Day 4: removal of i.p. catheter after the washing step that follows the last dosing with VS-01.

Abbreviations: i.p., intraperitoneal; SOC, Standard of care.

The SCR period starts once a patient or his/her legal representative has provided written informed consent and ends on the day when the patient is randomized. After initial SCR as early as at Day -3, patient will be re-evaluated for eligibility on Day 1 and a randomization will occur.

Patients randomized to Active Treatment group will receive the VS-01 *i.p.* infusion during the Treatment period over a dwell time of 3 h (+ 30 min) on study Days 1 to 4, in addition to SOC. *Table 8-1* provides planned VS-01 doses for the current study.

Table 8-1 Planned VS-01 doses in Proof of Concept Study

Dry Body Weight Range (kg)	VS-01 Number of Kit	VS-01 Volume (mL/kg)	[redacted]
≥ 40 - < 53	1	20 – 26	[redacted]
≥ 53 - < 79	2	27 – 40	[redacted]
≥ 79 - < 105	3	30 – 40	[redacted]
≥ 105 - < 140	4	30 - 40	[redacted]

Patients will receive SOC throughout the study considering respective guidelines. SOC should be administered after each treatment session with VS-01, if applicable. At the end of each VS-01 dosing, VS-01 fluid will be removed similarly to paracentesis and a washing step will be performed by infusing 1 L of any solution suitable for *i.p.* rinse as per local standard, infused within 20 min (±10 min), followed by immediate removal by drainage under similar conditions (refer to Protocol Section 11.4). The patients will continue to receive standard of care as required after End of Treatment (EOT) on Day 4.

Patients randomized to Control group will receive standard of care throughout the study.

In all patients (both Active Treatment group and Control group), paracentesis (therapeutic or diagnostic) via the *i.p.* catheter will be performed on Day 0 (the day before randomization on Day 1) and followed by plasma volume expansion by infusing albumin (8 g/L of ascites removed) if large volume paracentesis is performed (i.e., > 5 L of ascites) (European Association for the Study of the Liver [EASL] guideline 2018 [EASL Clinical Practice Guidelines, 2018]; American Association for the Study of Liver Diseases [AASLD] guideline on the treatment of ascites, spontaneous bacterial peritonitis and Hepatorenal syndrome - acute kidney injury [HRS-AKI] [AASLD Practice Guidance, 2021]). Albumin

following paracentesis can be administered also in case of < 5 L of ascites are being removed, if this is in line with local SOC. The *i.p.* catheter should be left in place up to Day 4 only in the Active Treatment group. From Day 0 (after peritoneal fluid sampling and paracentesis) up to Day 4 (until the catheter is removed after washing step) the catheter's lock should remain closed, disconnected from any drainage system and protected with a sterile dressing except for removal of recurring ascites prior to dosing with VS-01, during the dosing procedure with VS-01 (Days 1 to 4) or upon peritoneal fluid sampling throughout the study. The catheter will be removed for the Control group on Day 1 after randomization. Refer to Protocol Section 16.2 for more information on paracentesis procedure.

All patients will be followed for up to 90 days until EOS visit. Any patients discontinuing early from the study before EOS visit should complete the early termination/withdrawal procedures defined in the Protocol Section 13.4.

A schedule for the tests and evaluations to be conducted in this study is presented in the study flow chart and study activities in Protocol Appendix 18 for patients on Active Treatment and Protocol Appendix 19 for patients in the Control group. In addition to the assessments presented in these Appendices, blood (serum and plasma; including PK and complement activation - related pseudoallergy [CARPA] samples not utilized for PK or CARPA testing), peritoneal fluid (including ascites and peritoneal fluid removed after VS-01 treatment and washing step), urine may be used for future metabolomic, proteomic and inflammatory phenotyping (see also protocol Appendix 17 for laboratory assessments).

Withdrawn patients and patients who drop out of the study will not be replaced.

An Independent Data Monitoring Committee (iDMC) is assigned by the Sponsor prior to the beginning of the study. Throughout the study, the iDMC will monitor safety parameters at regular intervals as outlined in the iDMC charter. Based on these regular reviews of emerging results, the iDMC will recommend continuation, modification, or termination of the study. The iDMC will meet after every 5 patients complete their treatment with VS-01 on top of SOC or every 4 months, whichever comes first.

8.1 Sample Size Considerations

A total sample size of 54 patients (27 per arm) is required to achieve 80% power to reject the null hypothesis of equal means when the mean difference in CLIF-C ACLF is 5.5 with a standard deviation (SD) of 7.0 for both groups with a significance level of 5% using a two-sided t-test. Approximately 60 patients will be enrolled, assuming a 10% drop-out rate. Recruitment will continue until 54 patients are evaluable with regard to the primary endpoint at Day 7.

A 5.5-point difference in CLIF-C ACLF between arms is assumed to correspond to a 35% relative reduction in mortality at Day 28 and a reduction in ≥ one ACLF grade or stable disease/avoidance of progression (see internal Statistical Report).

An Interim Analysis (IA) is planned for the purpose of sample size re-estimation (see section 10.0). The sample size re-estimation will be conducted for both the primary endpoint (CLIF-C ACLF score at Day 7) and the first secondary endpoint (90-day mortality rate). The sample size may be increased up to a total of approximately 120 patients (assuming a 10% drop-out rate).

8.2 Randomization

After initial SCR, patients will be re-evaluated for eligibility on Day 1. Patients will then be randomized to be treated with VS-01 on top of SOC or SOC alone. Patients will be randomized in 1:1 ratio, stratified by CLIF-C ACLF score at BL (<48 vs. ≥48) and country.

A centralized randomization process will be implemented to manage treatment allocation and to ensure no more than 5 patients will be administered VS-01 simultaneously. The allocation of each patient in any given study site to VS-01 on top of SOC or SOC only will strictly follow this central randomization scheme. Randomization will be done electronically.

8.3 Population Sets

8.3.1 All Screened Analysis Set

The All Screened Analysis Set will consist of all patients who provided written informed consent or whose legal representative provided written informed consent.

8.3.2 Full Analysis Set

The Full Analysis Set (FAS) will consist of all randomized patients. Patients will be included in the group they were randomized to. The FAS will be the primary population for all efficacy related endpoints and will provide an intent to treat analysis.

8.3.3 Per Protocol Analysis Set

The per protocol (PP) analysis set refers to all patients in the FAS for whom no major protocol deviations or events affecting efficacy have been observed. Protocol deviations are described in Protocol Deviation Guidance Document. Definitions of major protocol deviations will be determined prior to database closure. This analysis set will be used for providing supporting analyses of efficacy endpoints.

8.3.4 Safety Analysis Set

The Safety analysis set (SAF) will consist of all patients who received at least one dose of VS-01 on top of SOC, or SOC only (Control group). Patients will be included in the group based on the treatment received.

8.3.5 Pharmacokinetics Analysis Set

The PK analysis set will consist of all patients who received at least one dose of VS-01 with at least 3 PK measurements after dose on any PK day (i.e., on Day 1 and Day 4) and had no major protocol deviations affecting PK parameters and no deviation from protocol-defined PK sampling procedures.

8.3.6 Pharmacodynamics Analysis Set

The PD analysis set will consist of all patients who received at least one dose of VS-01 on top of SOC, or SOC only (Control group) with at least 3 PD measurements after BL and had no major protocol deviation affecting PD parameters and no deviation from protocol-defined PD sampling procedures.

9.0 Conventions and Derivations

9.1 Treatment Start Date/Time

Treatment start date/time in Active Treatment group is defined as the start date and time of the first investigational medicinal product (IMP) administration. Treatment start date/time in Control group is defined as median Day 1 treatment administration time of IMP in the Active Treatment group.

9.2 Baseline Value and Change from Baseline

Baseline is defined as the last available assessment before the treatment start date/time. If the treatment start date/time is missing for a randomized patient, baseline is defined as the last available assessment before the randomization date/time.

Change from BL is calculated as $(postbaseline\ value\ -\ baseline\ value)$.

9.3 Study Day

Study Day 1 is defined as the first day of treatment with IMP/SOC after Randomization. For dates prior to Study Day 1, the Study Day is calculated as:

$$Study\ Day\ =\ (Date\ of\ Interest/Assessment\ -\ Date\ of\ Study\ Day\ 1)$$

For dates on or after Study Day 1, the Study Day is calculated as:

$$Study\ Day\ =\ (Date\ of\ Interest/Assessment\ -\ Date\ of\ Study\ Day\ 1) + 1$$

9.4 Visit Windowing and Unscheduled Assessments

Only assessments done at specified visit window as defined in Protocol Appendix 19 and Appendix 20 are summarized in tables. Assessments that are out of visit window are listed only.

The following visits have a visit window:

- Day 14 (±4 days)

- Day 28 (±7 days)
- Day 90 (±14 days)

In case only unscheduled visit and no planned visit is conducted for particular parameters, the unscheduled visit is mapped to a scheduled visit, only if it is within the visit window as defined in the Protocol and then summarized.

If scheduled and unscheduled assessments are available for the same parameter within a single visit, then only the last scheduled assessment will be used in the analysis and unscheduled assessments will not be mapped to scheduled ones no matter if these are within or outside the visit window.

In case only unscheduled visit and no planned visit is conducted for particular parameters, the closest unscheduled visit to the target day is mapped to a scheduled visit, only if it is within the visit window as defined above.

9.5 Early Termination Visit Mapping

Early Termination visit will not be mapped to a scheduled visit.

9.6 Derivation of Age

Birthday is collected as MMM-YYYY or YYYY only with respect to local country policy (e.g. only year collection is allowed in Hungary). Day is not collected and will be imputed as 15 for age calculation used in CLIF-C ACLF score. When month is not collected, it will be imputed as June for age calculation used in CLIF-C ACLF score.

Age is rounded to 1 decimal.

9.7 Treatment Exposure and Compliance

Treatment exposure and compliance will be calculated for the investigational treatment arm only. Duration of exposure (in days) is calculated as:

$$Last\ IMP\ administration\ date - First\ IMP\ administration\ date + 1$$

Treatment compliance (%) for a particular infusion is calculated as:

$$\frac{Actual\ dose\ administered\ (mL)}{Intended\ dose\ (mL)} * 100$$

Overall treatment compliance (%) is calculated as average of the individual day treatment compliances.

9.8 Prior and Concomitant Medications and Procedures

A medication/procedure is considered to be prior if the start date and time is before the treatment start date. A medication/procedure is considered to be concomitant if the medication/procedure is ongoing at the treatment start date or started thereafter. A medication/procedure can be considered both prior and concomitant.

9.9 Method for Handling Missing Data

Patients who prematurely discontinue the treatment should continue with other study assessments as planned to collect complete information. Every effort should be made to collect complete information on patients whether or not they completed the planned treatment. Discontinuation of IMP is not a reason to discontinue from the study.

If a patient withdraws consent or is withdrawn from the study prior to EOS visit, all the procedures described for the Early Termination visit should be performed as described in the protocol section 13.4. Reason for treatment or study discontinuation must be collected on CRF.

Missing endpoint values will not be imputed for other than the primary endpoint (CLIF-C ACLF score) and first secondary endpoint (90-day mortality) analysis. Imputation methods are further detailed in section 11.5.3.1 and section 11.5.4.2.1. Effort will be made to collect withdrawn patients' survival status up to Day 90. In the case there is still missing data, data will be imputed using multiple imputation as detailed in section 11.5.4.2.1.

9.10 Date Imputation Rules

For the purpose of date imputation, Day 90/EOS or last assessment date for patients who discontinued the study is defined as the last available visit date. The following rules will be applied to impute missing or partial dates and times in appropriate data types (e.g., adverse events or concomitant medications)

Table 9-1 Table Imputation Rules for Partial Dates (D = day, M = month, Y = year, hh = hours, mm = minutes)

Parameter	Missing	Additional Conditions	Imputation
Start date	D	M and Y same as M and Y of treatment start date	Treatment start day
		Y is before or if Y same but M is before as M of treatment start date	Last day of the month
		Y is after or if Y is same but M is after as M of treatment start date	First day of the month
		Patient is not treated	First day of the month
	D and M	Y same as Y of treatment start date	Day and month of treatment start date
		Y before Y of treatment start date	Dec 31
		Y after Y of treatment start date	Jan 01
		Patient was not treated	Jan 01
	M only	If D is present and M is not, D will be treated as missing.	See imputation rule for 'D and M'
	D, M, Y	None - date completely missing	Treatment start date
Start time	mm	hh same as hh of treatment start date and date or imputed date same	mm of treatment start date
		hh not the same as hh of treatment start date	00
		Patient was not treated	00
	hh, mm	Time is missing and the date is complete and is the same as treatment start date, or the date is imputed to be treatment start date	hh:mm of treatment start date
		Time is missing but original date or imputed date is not treatment start date	00:00
		Patient was not treated	00:00
	hh only	If mm is present but hh is not, time will be regarded as missing.	See imputation rule for 'hh, mm'
Stop date	D		Earliest of the (end of study/discontinuation date, last day of month)
	D and M		Earliest of the (end of study/discontinuation date, Dec 31)
	M only	If D is present and M is not, D will be treated as missing.	See imputation rule for 'D and M'
	D, M, Y	None - date completely missing	No imputation, but assume ongoing
Stop time	mm		59
	hh, mm		23:59
	hh only	If mm is present but hh is not, time will be regarded as missing.	See imputation rule for 'hh, mm'

Note: In all cases, if an imputed start date is after a complete stop date, use the first day of the stop date month for start date.
If stop date is before a complete or imputed start date, use either a) the last day of the start day month when start date is complete, or b) assign start date same as stop date when start date is imputed.

No imputation will be performed for medications with completely missing start dates.

Medications with completely missing start dates will be listed in both prior and concomitant medications.

9.11 Time to Event Endpoints and Censoring Rules

Endpoint Description	Start Date	Event Date	Censoring Date
Time to Death Through Day 28	Treatment start date	Date of death due to any reason occurring prior or on Day 28	Day 28 (±7 days)
Time to Death Through Day 28 – Censoring Patients who Received Liver Transplant	Treatment start date	Date of death due to any reason occurring prior or on Day 28	min(Day 28 (±7 days); liver transplant date)
Time to Death Through Day 90	Treatment start date	Date of death due to any reason occurring prior or on Day 90	Day 90 (±14 days)
Time to Death Through Day 90 – Censoring Patients who Received Liver Transplant	Treatment start date	Date of death due to any reason occurring prior or on Day 90	min(Day 90 (±14 days), liver transplant date)
Time to ACLF Resolution Through Day 7	Treatment start date	ACLF resolution date occurring prior or on Day 7	min(study discontinuation date; Day 7; last assessment date; date of liver transplant or TIPS)
Time to ACLF Resolution Through Day 28	Treatment start date	ACLF resolution date occurring prior or on Day 28 (±7 days)	min(study discontinuation date; Day 28 (±7 days) visit date; last assessment date; date of liver transplant or TIPS)
Time to >= one ACLF Grade Regression Through Day 7	Treatment start date	ACLF >= one grade regression date occurring prior or on Day 7	min(study discontinuation date; Day 7; last assessment date; date of liver transplant or TIPS)
Time to >= one ACLF Grade Regression Through Day 28	Treatment start date	ACLF >= one grade regression date occurring prior or on Day 28 (±7 days)	min(study discontinuation date; Day 28 (±7 days) visit date; last assessment date; date of liver transplant or TIPS)
Transplant-Free Survival Through Day 28	Treatment start date	Transplant or death date prior or on Day 28	min(study discontinuation date; Day 28 (±7 days); last assessment date)
Transplant-Free Survival Through Day 90	Treatment start date	Transplant date prior or on Day 90	min(study discontinuation date; Day 90 (±14 days); last assessment date)

Time to In-Hospital Death	Treatment start date	Date of death due to any reason occurring in a hospital	min(study discontinuation date; Day 90 (±14 days); last assessment date; date of hospital discharge due to initial ACLF episode)
Time to ICU Death	Treatment start date	Date of death due to any reason occurring in an ICU	min(study discontinuation date; Day 90 (±14 days); last assessment date; date of ICU discharge due to initial ACLF episode)
Time to First Hospital or ICU Readmission for Reoccurring ACLF	ACLF resolution Date	Date of hospital (or ICU) readmission due to ACLF reoccurrence	min(study discontinuation date; Day 90 (±14 days) visit date; last assessment date)
Time to First ACLF Reoccurrence	ACLF resolution Date	ACLF reoccurrence date	min(study discontinuation date; Day 90 (±14 days) visit date; last assessment date)
Time to Resolution of Overt HE Through Day 5 by West Haven Criteria	Treatment start date	Overt HE resolution date occurring prior or on Day 5	min(study discontinuation date; Day 5 date; last assessment date)
Time to Resolution of Overt HE Through Day 7 by West Haven Criteria	Treatment start date	Overt HE resolution date occurring prior or on Day 7	min(study discontinuation date; Day 7 date; last assessment date)
Time to Regression of >= One HE Grade Through Day 5 by West Haven Criteria	Treatment start date	HE >= one grade regression date occurring prior or on Day 5	min(study discontinuation date; Day 5 date; last assessment date)
Time to Regression of >= One HE Grade Through Day 7 by West Haven Criteria	Treatment start date	HE >= one grade regression date occurring prior or on Day 7	min(study discontinuation date; Day 7 date; last assessment date)
Time to Resolution of Overt HE Through Day 7 by HEGI™	Treatment start date	Overt HE resolution date occurring prior or on Day 7	min(study discontinuation date; Day 7 date; last assessment date)
Time to Regression of >= One HE Grade Through Day 7 by HEGI™	Treatment start date	HE >= one grade regression date occurring prior to Day 7	min(study discontinuation date; Day 7 date; last assessment date)

Last assessment date is defined as the last assessment day for patient with missing study discontinuation information (i.e, lost to follow-up).

9.12 CLIF-C ACLF Score

CLIF-C ACLF Score is calculated by formula:

$$CLIF - C\ ACLF\ Score = 10 * [0.33 * CLIF - OF\ Score + 0.04 * age + 0.63 * ln(white\ cell\ count) - 2]$$

Age at ICF signature, derived with birthday imputed as described in section 9.6, will be used in the formula.

9.13 CLIF-C OF Score

CLIF-C OF score is calculated as sum of organ/system subscores. Subscores derivation is described in Appendix 14.0.

The worst values within a ± 90 minute time window around the time of the morning blood sample will be considered for all components of the score, except RRT use. RRT use will be based on the window of 24 hours around the time of the morning blood sample.

Use of vasopressors is to be considered when the vasopressor duration is at least 1 hour. Vasopressor given solely for HRS-AKI is not considered as circulatory failure and MAP values will be looked at instead to derive the circulatory subscore.

If patient is on mechanical ventilation (MV) due to HE, then brain subscore = 3. If reason for MV is respiratory failure, then respiratory subscore = 3. If reason for MV is both (HE and respiratory failure), then brain and respiratory subscores both equal 3. If the reason for MV is 'other', then no additional derivation will be done.

If HE grade is 'Minimal', it will be considered as subscore = 0.

Score will not be calculated and will be set to missing if any subscore values are missing.

9.14 CLIF-SOFA Score

CLIF-SOFA score is calculated as sum of organ/system subscores. Subscores derivation is described in Appendix 15.0.

The same approach applies to CLIF-SOFA score derivation as explained for CLIF-C OF score in section 9.13.

If patient had RRT, kidney subscore=4 will be assigned.

If patient is on mechanical ventilation (MV) due to HE, then cerebral subscore = 4. If reason for MV is respiratory failure, then lungs subscore = 3 or 4 according to PaO/FiO2, or SpO2/FiO2, respectively. If reason for MV is both (HE and respiratory failure), then cerebral subscore is 4 and lungs subscore is 3 or 4 according to PaO/FiO2, or SpO2/FiO2, respectively. If the reason for MV is 'other', then no additional derivation will be done.

If HE grade is 'Minimal', it will be considered as subscore = 0.

Score will not be calculated if any subscore values are missing.

9.15 Definition of ACLF Resolution

ACLF resolution is defined by ACLF grade value 'No ACLF'. Diagnosis of ACLF is made according to EASL-CLIF criteria (Protocol Appendix 2). Organ failures defining ACLF will be quantified according to CLIF-OF score.

ACLF resolution will be defined based on data from the central lab.

9.16 Definition of ACLF Grade Regression

Patients with ≥ one grade regression are those who have at least one full grade improvement, i.e., ACLF grade 3a to grade 2, grade 2 to grade 1, or ACLF grade 1 to no ACLF, or ACLF grade 2 to no ACLF by the specified time period.

ACLF regression will be defined based on data from the central lab for both initial and subsequent diagnoses.

9.17 Mortality Rate

Mortality rate is calculated as:

$$\left(\frac{Number\ of\ deaths\ in\ the\ specified\ period}{Number\ of\ patients\ at\ risk\ in\ the\ specified\ period}\right) * 100$$

In-hospital mortality rate is calculated as:

$$\left(\frac{Number\ of\ in\text{—}hospital\ deaths\ in\ the\ specified\ period}{Number\ of\ patients\ in\ hospital\ who\ are\ at\ risk\ in\ the\ specified\ period}\right) * 100$$

ICU mortality rate is calculated similarly, taking into account the deaths in ICU and total patients in ICU.

9.18 Duration of Hospitalization/ICU Stay

Duration of Hospitalization is derived from the *Hospital Stay* CRF.

Duration of hospitalization refers to any hospitalization due to initial ACLF episode. Hospital stay in days is calculated as:

$$Planned\ discharge\ date - Admission\ date + 1$$

In case of transfer during initial hospitalization (i.e, patient transferred to intermediate care unit or ICU for medical or non-medical reason), the durations are summed. Planned discharge date refers to when patient is able to be discharged from a medical perspective. Initial duration of hospitalization is considered ended when patient is able to be discharged to home (and patient has not died before discharge).

ICU-stay and intermediate care unit stay is calculated similarly, summing only the hospital stay durations due to medical reasons (non-medical reasons are excluded).

9.19 Hospital and ICU Readmissions

Elective readmission, e.g., hepatocellular carcinoma (HCC) therapy, large-volume paracentesis, and endoscopic band ligation will be excluded when defining hospital readmission.

9.20 ACLF Reoccurrence

Reoccurrence of ACLF is defined by EASL-CLIF criteria (Protocol Appendix 2). Organ failures defining ACLF will be quantified according to CLIF-OF score.

ACLF reoccurrence is defined as a patient with resolved ACLF (return to grade 0) after initial treatment with VS-01 on top of SOC or SOC only is assigned ACLF grade ≥ one. ACLF reoccurrence is defined based on data from the central lab.

ACLF reoccurrence rate is calculated as:

$$\left(\frac{Number\ of\ patients\ with\ reoccurrence\ within\ the\ specified\ period}{Number\ of\ patients\ at\ risk\ in\ the\ specified\ period}\right) * 100$$

9.21 Hepatic Encephalopathy Severity Assessment

HE grade assessment is based on West Haven criteria, HEGI™, and Glasgow Coma Scale. In case of overt HE, severity is recorded on *West Haven* Criteria *for Hepatic Encephalopathy Grade Clinical Classification* CRF.

9.22 Definition of Overt HE Resolution by West Haven Criteria

Overt HE as assessed by West Haven Criteria, is considered as grade 2, 3, or 4. Resolution of overt HE is defined as West Haven grade reduction to either grade 1, Minimal, or Unimpaired.

The rate of overt HE resolution will be calculated as:

$$\left(\frac{Number\ of\ patients\ with\ overt\ HE\ resolution\ within\ the\ specified\ period}{Number\ of\ patients\ at\ risk\ in\ the\ specified\ period}\right) * 100$$

9.23 Definition of Overt HE Resolution by HEGI™

Overt HE as assessed by HEGI™, is considered as grade 2, 3, or 4 HE episode. Resolution of overt HE is defined as no overt HE episodes (i.e., no HEGI HE grade 2, 3, or 4) have occurred.

The rate of overt HE resolution will be calculated as in section 9.22.

9.24 Definition of Regression of ≥ one HE grade by West Haven Criteria

Regression of ≥ one HE grade by West Haven Criteria is defined as any regression from higher level grade to lower level grade.

9.25 Glasgow Coma Scale Score

Glasgow Coma Scale score is derived as sum of the three items (eye opening response, verbal response, and motor response).

9.26 MELD, MELD-Na, and MELD 3.0 Scores

MELD score is calculated as follows:

$$MELD\ Score = 10 \text{ x } [(0.957 \text{ x } \ln(\text{Creatinine})) + (0.378 \text{ x } \ln(\text{Bilirubin})) + (1.12 \text{ x } \ln(\text{INR})) + 0.643]$$

- Creatinine and bilirubin in mg/dL;
- If a patient has had 2 or more hemodialysis treatments or 24 h of continuous veno-venous hemodialysis (CVVHD) in the week prior to the time of the scoring, creatinine will be set to 4 mg/dL, the maximum creatinine level allowed in the model. Patient's creatinine value greater than 4.0 mg/dL will be set to 4 mg/dL;
- If any score is <1, the MELD assumes the score is equal to 1;
- The MELD score derived from this calculation will be rounded to the tenth decimal place before multiplying by 10. The maximum MELD score is 40.

MELD-Na score is calculated as follows:

$$MELD - Na = \text{MELD} + 1.32 \text{ x } (137 - \text{Na}) - [0.033 \text{ x MELD} * (137 - \text{Na})]$$

- As MELD-Na requires MELD, all notes for MELD apply for MELD-Na
- Serum sodium value to be corrected for the range of 125-137 mEq/L
- For serum values < 125 mEq/L, MELD-Na calculations are made using 125 mEq/L
- For serum values > 137 mEq/L, MELD-Na calculations are made using 137 mEq/L

MELD 3.0 score is calculated as follows:

$$\begin{aligned} MELD\ 3.0\ Score = {} & 1.33 * (\text{if female}) + 4.56 * \ln(Bilirubin) + 0.82 * (137 - Sodium) - 0.24 \\ & * (137 - Sodium) * \ln(Bilirubin) + 9.09 * \ln(INR) + 11.14 * \ln(Creatinine) + 1.85 \\ & * (3.5 - Albumin) - 1.83 * (3.5 - Albumin) * \ln(Creatinine) + 6 \end{aligned}$$

where Sodium = Na.

- Scores are rounded to the nearest integer;
- Serum bilirubin, INR, and serum creatinine values below 1.0 are set to 1.0;
- Sodium is limited to a range of 125-137 mEq/L, and if outside of these bounds, is set to the nearest limit;
- Serum albumin is limited to a range of 1.5-3.5 g/dL, and if outside of these bounds, is set to the nearest limit;
- The following candidates receive a creatinine value of 3.0 mg/dL:
  - Those with a creatinine value greater than 3.0 mg/dL;
  - Those who received two or more dialysis treatments within 7 days prior to the serum creatinine test;
  - Those who received 24 hours of continuous veno-venous hemodialysis (CVVHD) prior to the serum creatinine test.

9.27 Definition of Treatment-Emergent Adverse Events

Treatment-emergent adverse events (TEAEs) are undesirable events which are not present prior to medical treatment, or an already present event that worsens either in intensity or frequency following the treatment start date. In reality, all adverse events (AEs) which change in severity or relationship to

IMP are assigned a new start date and captured as a new record. An AE is considered to be TEAE when the event onset date and time is on or after the treatment start date/time and until end of the study participation for the patient. In case AE with missing time is on the same day as the treatment start date, the AE is considered to be treatment-emergent.

9.28 Definition of TEAE related to IMP

TEAEs related to IMP are defined as events categorized as Definitely, Probably, or Possibly related to IMP by the investigator. If relationship is missing, the event is considered as related.

9.29 Imputation of Laboratory Values with Character Symbol

Missing laboratory data will not be imputed. However, laboratory values of the form of "< x" (i.e., below the lower limit of quantification) or "> x" (i.e., above the upper limit of quantification) will be imputed as "x" for the purpose of calculation of summary statistics and comparing to normal ranges. These values will still be displayed as "< x" or "> x" in the listings.

9.30 SAAG

Serum ascites albumin gradient (SAAG) is calculated at screening visit using the following formula:

$$SAAG = (albumin\ concentration\ of\ serum) - (albumin\ concentration\ of\ ascitic\ fluid)$$

Units of both lab parameters are mg/L.

10.0 Interim Analyses

An interim analysis (IA) is planned when approximately 50 patients have been enrolled for the purpose of an unblinded sample size re-estimation, using the promising zone method (Hsiao, 2019; Mehta and Pocock, 2011). The iDMC and select sponsor personnel will review this analysis, and results will be kept confidential from the investigators to minimize the introduction of bias. The data used for IA will be cleaned.

The sample size re-estimation will be conducted for both the primary endpoint (CLIF-C ACLF Score at Day 7) and the first secondary endpoint (90-day mortality rate). The two-sided 5% Type I error control is maintained as the sample size will be increased only if the interim analysis is promising (Hsiao, 2019). Additionally, there will be no stopping for early efficacy (i.e., the type-I error at the interim will be set at 0%), for both re-estimations. There is also no plan to stop early for futility. The initial total sample size is set at N=54 evaluable participants (N=60 accounting for 10% dropout). After approximately n_1=50 participants have been enrolled, the sample size re-estimation will be conducted. The total sample size may be increased up to a maximum of 108 evaluable participants (approximately 120 accounting for 10% dropout) based on the results of the sample size re-estimation. If increased, the total sample size will be set to the maximum sample size selected from each of the two sample size re-estimations (from primary endpoint and first secondary endpoint) and adjusted for 10% dropout.

A separate adaptive design report provides the pre-specified adaptations based on using the promising zone method (See Appendix 16.0).

While the study is an open-label study, steps to prevent the introduction of bias from information leakage following regular iDMC reviews and the IA, a robust "firewall" will be established with details documented in the iDMC charter. Composition of the iDMC, meeting structure, meeting schedule, procedures, the content, and format of iDMC reports, and other relevant details will also be detailed in a the iDMC charter.

11.0 Statistical Methods

All data collected during this study will be displayed in data listings, unless otherwise specified. Data listings will be sorted by treatment group and patient ID.

Descriptive statistics (number of observations [n], number of missing observations, mean, median, SD, quartiles Q1 and Q3, minimum, and maximum values) for continuous variables will be presented. Mean,

median, Q1 and Q3 will be presented to 1 decimal more than original data. SD will be presented to 2 decimals more than original data. Minimum and maximum will match the decimal points in the original data. In case of derived variables, the maximum number of decimal points is 4. Number of patients at the visit will be used to derive missing counts.

Unless otherwise noted, categorical variables will be summarized by number of patients with data, number of patients with missing data, frequencies and percentages of patients by categories, calculated in patients with data. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.
Tabulation done by timepoint will not include unscheduled visits. Unscheduled visit data are listed only.

All data summaries and tabulations will be prepared with SAS Version 9.4 or higher.

11.1 Patient Disposition

The number and percentage of patients screened, screen failed with reasons for failure, randomized but not treated, randomized and treated in the study will be presented, together with the number and percentage of patients included in each analysis set, on all patients who provided informed consent (all screened patients). These details will be listed as well.

A tabulation of the number and percentage of patients randomized at each country and center will be presented on FAS by treatment groups and total.

Patients who withdrew from the treatment or study prematurely and a breakdown of the corresponding reasons for withdrawal will be tabulated on FAS. End of treatment information will be listed on Safety Analysis Set, and end of study information will be listed on FAS. In case the patient discontinued prior to the first IMP infusion, a reason for withdrawal will be collected.

Details on informed consent date, protocol version, protocol amendment re-consent, re-screen, eligibility, and randomization assignments will be listed on all screened patients.

All patient visits with status and mode of contact will be listed on FAS.

11.2 Demographic and Baseline Characteristics

Demographic and BL characteristics will be collected at SCR and/or BL. The data to be presented will include sex, age (years, to one decimal point), race and ethnicity (according to the local regulation), height (cm), dry body weight (kg), Body Mass Index (BMI) (kg/m^2) calculated using dry body weight, country, ACLF grade, onset of ACLF from BL, HE grade assessed by West Haven criteria, CLIF-C ACLF score, CLIF-C OF score, CLIF-SOFA score, CPS score, MELD score, MELD-Na score, MELD 3.0 score, bilirubin, creatinine, kidney failure, International Normalized Ratio (INR), mean arterial pressure, use of vasopressors, PaO_2/FiO_2 and/or SpO_2/FiO_2, and ammonia values at screening and BL, using all available sources (local and central labs).

Medical history collected will include (but not limited to) etiology of liver disease, presence of bacterial peritonitis, presence of severe alcohol-related steatohepatitis (sASH), acute viral hepatitis, or uncontrolled autoimmune hepatitis (AIH), presence of TIPS, type of precipitating event of ACLF, and comorbidities. Medical history events will be coded using version 25.1 or later of Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized using primary system organ class, and preferred term (PT). The table will include the number and percentage of patients with the event by system organ class and PT. A patient will only be counted once within system organ class or PT. Disease etiology and precipitating events of ACLF will also be summarized descriptively.

All demographic and BL characteristics, and medical history data will be listed and tabulated for the FAS by treatment groups and the total group. In addition, demographics and BL characteristics will be tabulated on PP analysis set.

Results of pregnancy test performed at SCR for females of childbearing potential only, will be listed for the FAS.

11.3 Treatments

11.3.1 Extent of Study Drug Exposure

Patients randomized to receive VS-01 treatment will be administered the treatment *i.p.* daily for 4 days. The planned dose is decided based on the patient's dry body weight (kg) as specified in *Table 8-1*.

Exposure data, as collected on CRF, will be summarized for the VS-01 group only by time point and overall for the following parameters on SAF:

- Number of patients dosed;
- Duration of exposure in days;
- Total number of infusions administered;
- Total volume administered (individual days and overall);
- Duration of Infusion (individual days)
- Duration of dwell time (individual days)
- Number of patients with infusion interruptions;
- Reasons for infusion interruptions;
- Duration of interruptions;

Treatment compliance (% of dose administered) will be tabulated by time point and overall. Derivations are described in section 9.7.

Overall exposure (total volume administered) will be plotted by dry body weight, CLIF-C ACLF score at Day 7, age, dry body weight to number of kits ratio in scatter plots, where different style is used by number of kits received. Box plot will be done for sex and overall exposure by number of kits received.

Details of the administration of study drug including start and end date and time of administration, intended dose and administered dose, dose adjustments and interruptions, details of VS-01 recovery, and derived values such as duration of exposure and treatment compliance will be listed.

Overdoses are reported as drug related AEs and will be tabulated and listed.

11.3.2 Prior and Concomitant Medications and Procedures

Prior medications are recorded at SCR and include medications taken within 30 days of start of IMP. Concomitant medications including SOC (especially changes in medication) will be documented for each patient throughout the study, i.e., on Day 1 to EOS (Day 90) or at Early Termination/Unscheduled visits. For VS-01 treatment group, concomitant medication including SOC should be administered after the VS-01 treatment session, if possible. Necessary supportive care will be allowed.

Patients in the study should abstain from nicotine on Day 1 through Day 7, and from alcohol for the duration of the trial. Alcohol and nicotine data is listed only.

The number and percentage of patients taking prior and concomitant medications, and the number and percentage of patients using at least one medication will be tabulated based on the FAS, using World Health Organization (WHO) Drug (version: WHODrug Sep 2022 or later) coding dictionary. Medications will be classified by Anatomical Therapeutic Chemical (ATC) groups, and by preferred name within each ATC group. In addition, SOC medications will be tabulated separately.

All prior and concomitant medications data will be presented in a single listing based on the FAS.

Procedures and non-drug therapies are coded using MedDRA version 25.1 or later. Prior and concomitant procedures will be separately tabulated by number and percentage of patients with the event by system organ class and PT. A patient will only be counted once within system organ class or PT. TIPS, liver transplant, RRT, catheter insertion and removal, other procedures entered on *Procedures* CRF, and nutritional intake, will be listed.

11.4 Protocol Deviations

Protocol deviations data will be entered into a system of record, Predictivv Study Operations (PSO). The study team and the Sponsor will conduct on-going reviews of the deviation data from PSO and the resulting set of evaluable patients throughout the study, adjusting the deviation criteria as seems appropriate in the Protocol Deviations Guidance document. The evaluable patients' sets must be finalized at the post-freeze data review meeting (or earlier), prior to database lock.

Important protocol deviations will be tabulated by category on the FAS. All protocol deviations will be listed.

11.5 Efficacy Analyses

All efficacy endpoints are summarized by treatment group on the FAS, with supportive analyses on the PP analysis set, where specified.

11.5.1 Primary Endpoint Estimand and Intercurrent Events

The primary clinical question of interest is: What is the effect of VS-01 compared with standard of care on the Day 7 CLIF-C ACLF score, regardless of investigational intervention discontinuation, investigational intervention dose adjustment/interruptions and regardless of use of a prohibited medication prior to Day 7?

- Treatment condition: investigational intervention with VS-01 for 4 days, on top of standard of care as compared with standard of care only.
- Variable/endpoint: Day 7 CLIF-C ACLF Score
- Population: As described by the inclusion/exclusion criteria
- Intercurrent events:
    - Treatment discontinuation due to any reason: After a patient discontinues the treatment, they will continue in the study and further data is collected. CLIF-C ACLF score collected on Day 7 will be used in the analysis regardless how much investigational medication a patient received (Treatment Policy).
    - Death due to any reason until Day 7: Death prior to Day 7 is unlikely to occur, however if it does, then the Day 7 CLIF-C ACLF score will be set to the highest CLIF-C ACLF score observed at Day 7 in the study (Composite Strategy).
    - TIPS or liver transplant until Day 7: In case of TIPS or liver transplant prior to Day 7 (unlikely to occur), then the Day 7 CLIF-C ACLF score will be set as the highest CLIF-C ACLF score observed at Day 7 in the study (Composite Strategy).
    - Prohibited medications intake until Day 7: In case of intake of prohibited medications, the Day 7 CLIF-C ACLF score will be used regardless (Treatment Policy).
- Population-level summary measure: Treatment difference in Least Squares (LS)-Means of CLIF-C ACLF score at Day 7 between VS-01 plus standard of care and standard of care only.

Rationale for estimand: the estimand assumes investigational intervention discontinuation, investigational intervention dose adjustment/interruptions and use of prohibited medications are handled with treatment policy approach as it reflects clinical practice. It further assumes that if a patient dies, received a liver transplant or required a procedure such as TIPS, that investigational treatment or SOC was not benefiting the patient.

11.5.2 Hypothesis Testing Strategy and Multiplicity

The primary analysis is based on the CLIF-C ACLF score at Day 7.

The following null hypothesis:

H_0: $\mu_1 - \mu_2 = 0$

will be tested against the alternative hypothesis:

H_1: $\mu_1 - \mu_2 \neq 0$,

where μ_1 is the mean CLIF-C ACLF score for VS-01 treatment group and μ_2 is the mean in the Control group at Day 7. Common standard deviation is assumed to be 7.0. The hypothesis will be tested based on a two-sided significance level of 5% using an analysis of covariance (ANCOVA) test.

Multiplicity adjustment is described in section 11.5.4.

11.5.3 Primary Analysis

The primary endpoint of the study is CLIF-C ACLF score at Day 7. Score derivation is described in section 9.12.

Primary endpoint is analyzed using an ANCOVA model with treatment as a fixed effect and CLIF-C ACLF score at BL (central lab), change in CLIF-C ACLF score from SCR (local lab) to BL (local lab), and delay between ACLF onset and BL as covariates.

The difference between treatment groups will be estimated by the LS-Mean difference between VS-01 on top of SOC vs. SOC only, with two-sided 95% confidence interval (CI) and p-value. Assumptions of the model will be evaluated by diagnostic (e.g., Shapiro-Wilk test for normality) and graphical methods such as residual plots, including distribution of residuals and Q-Q plots of residuals.

11.5.3.1 Imputation Methods

As the primary endpoint is measured at Day 7, we do not anticipate any missing data, as the majority of the patients will still be in the hospital at Day 7.

If there are any deaths, liver transplants, or TIPS placement procedures occurring prior to Day 7 a patient will be assigned the maximum CLIF-C ACLF score observed at Day 7 in the study for the purpose of the primary analysis. If there is missing data for another reason (e.g., a patient was discharged prior to Day 7 and failed to return for the Day 7 visit, a laboratory sample broke, etc.) then CLIF-C ACLF score on Day 7 will be multiply imputed assuming it is missing at random (MAR). MAR means that the missingness can be explained by the observed data and missing data does not depend on unobserved data after accounting for observed factors.

Missing CLIF-C ACLF score on Day 7 will be imputed for each treatment group using multiple imputation (MI) which is assuming MAR. In case of missing data for CLIF-C ACLF score at BL (central lab) and change in CLIF-C ACLF score from SCR (local lab) to BL (central lab), missing values at SCR (local lab) and at BL (central lab) will be imputed in the same PROC MI step as Day 7 values.

Missing data pattern of CLIF-C ACLF scores on Days 1 to 7 will be explored. If the pattern of missing data is non-monotone, intermittent missing values will be imputed using a Markov Chain Monte Carlo (MCMC) method. After the missing data pattern is monotone, the regression method will be used to impute missing data (CLIF-C ACLF score SCR (local lab), CLIF-C ACLF score at BL (central lab) and CLIF-C ACLF score at Day 7). Treatment group, CLIF-C ACLF score from SCR (local lab), BL CLIF-C ACLF score (central lab), Day 2 to Day 7 CLIF-C ACLF score and time between ACLF onset and BL values will be used as factors in PROC MI procedure in SAS.

If convergence issues are encountered due to the lack of observed data, then a dynamic approach removing intermediate CLIF-C ACLF score in PROC MI of step 1.2 (appendix 17.0) will be done. In that case, the multiple imputation of missing data will be done first removing CLIF-C ACLF score at Day 3 from the imputation variables. If the model still does not converge, CLIF-C score at Day 4 will also be removed. If the model still does not converge, CLIF-C score at Day 5 will also be removed. And finally, if the model still doesn't converge, CLIF-C score at Day 6 will be removed.

Each m=20 + 1.5*[proportion of missing data (%)], where m is rounded up to the next integer value, imputed complete datasets will then be used in the same ANCOVA model, and the results are combined with PROC MIANALYZE procedure. The seed that will be used throughout the multiple imputation steps will be 165427 for reproducibility of the results.

Note that for the ANCOVA model, the change in CLIF-C ACLF score from SCR (local lab) to BL (central lab) will be calculated using the imputed values obtained from step 1.2 of PROC MI procedure (appendix 17.0), before inclusion in the model.

Other imputation methods are detailed in section 11.5.3.2 and its sub-sections.

11.5.3.2 Sensitivity Analyses

Sensitivity analyses are planned to assess the primary analysis results under different assumptions to further explore the treatment effect. The pattern of missing data will be displayed by overall, treatment group and visit. The following sensitivity analyses are planned and may be adjusted based on the extent and pattern of missing data.

11.5.3.2.1 Sensitivity Analysis 1: Analysis on PP Set

Primary analysis is repeated using the PP set, to include only the patients strictly adhering to the protocol. This analysis is performed only when >10% difference to the number of patients in FAS. Otherwise, only descriptive statistics will be provided.

11.5.3.2.2 Sensitivity Analysis 2: Tipping Point Approach

Primary analysis is repeated on FAS using the tipping point approach. This series of analyses will search for a tipping point that reverses the conclusion of a statistically significant treatment effect to p-value >0.05. The value of the shift parameter that changes the conclusion of the analysis from significant to non-significant will represent the tipping point. Tipping point approach is only implemented when the primary analysis indicates significant treatment effect.

MI analysis is performed on each chosen shift parameter in steps of 0.25 until non-significant p-value is reached. The same MI procedure will be carried out as in the primary analysis 11.5.3, except that in the step of monotone dataset imputation, PROC MI with option Missing Not at Random (MNAR) will be used, where imputed values in the Active Treatment group will be adjusted by the shift parameter. Imputed values for the Control group will not be adjusted by the shift parameter initially, unless there is no change to the study results.

The results of each tipping point analysis using a different shift parameter will be summarized in a single output showing the difference between treatment groups, 95% CI, p-value and shift parameter used. The p-value associated with the shift parameter that reverses the conclusion will be assessed whether or not it is plausible in this study. If it is plausible, the MAR assumption is questionable and additional post-hoc sensitivity analyses assuming data is MNAR will be conducted (e.g., pattern mixture model and control - based imputation).

11.5.3.2.3 Sensitivity Analysis 3: Excluding Patients who Received TIPS or Liver Transplant

To investigate the potential impact of CLIF-C ACLF score imputation in patients who received liver transplant or TIPS (i.e., replacement by the highest CLIF-C ACLF score observed at Day 7 in the study), a sensitivity analysis will be performed where these patients are excluded from the analysis. The primary analysis is repeated after excluding patients who received TIPS/liver transplant within 7 days of randomization.

11.5.3.2.4 Sensitivity Analysis 4: Excluding patients with ACLF due to sASH, acute viral hepatitis, or uncontrolled AIH at screening

To evaluate impact on the study drug efficacy assessment, a sensitivity analysis will be performed where patients with ACLF due to sASH, ACLF due to acute viral hepatitis A, B, B+D, C or E requiring antiviral treatment, and ACLF due to AIH requiring high-dose steroid treatment are excluded from the analysis. The primary analysis is repeated after excluding these patients.

11.5.3.2.5 Sensitivity Analysis 5: MAR Imputation for Patients who Received TIPS or Liver Transplant

Sensitivity analysis will be performed by repeating the primary analysis, except for patients who received TIPS or liver transplant, CLIF-C ACLF score at Day 7 will be multiply imputed assuming it is MAR.

11.5.3.2.6 Sensitivity Analysis 6: Bayesian Approach

Primary analysis will be repeated on FAS, applying a Bayesian approach using a non-informative prior distribution of the treatment effect. The Bayesian posterior distribution of the treatment effect will be calculated and used to estimate posterior probabilities that the treatment effect (mean of CLIF-C ACLF

score in VS01+SOC at Day 7 arm minus mean of CLIF-C ACLF score at Day 7 in SOC only arm) is below 3.85, 4.5 and 5.5 respectively.

11.5.4 Secondary Efficacy Analyses

The first secondary endpoint that is planned for inferential testing is 90-day mortality. To adjust for multiplicity, the primary endpoint will be tested first, and testing of the first secondary endpoint will be considered confirmatory if the primary endpoint is significant. If the primary endpoint is not significant, then testing will be considered exploratory. The remaining secondary endpoints will be analyzed without adjustment for multiplicity.

All secondary efficacy analyses are done on FAS, and repeated on PP set for the first secondary endpoint. All secondary efficacy parameters will be provided in data listings.

11.5.4.1 First Secondary Endpoint Estimand and Intercurrent Events

The secondary clinical question of interest is: What is the effect of VS-01 compared with standard of care on the 90-day mortality, regardless of investigational intervention discontinuation, investigational intervention dose adjustment/interruptions, regardless of use of a prohibited medication and having liver transplant/TIPS procedure prior to Day 90?
Secondary estimand is constructed as:

- Treatment condition: investigational intervention with VS-01 for 4 days, on top of standard of care as compared with standard of care only.
- Variable/endpoint: 90-day mortality rate
- Population: As described by the inclusion/exclusion criteria
- Intercurrent events:
    - Liver transplant or TIPS: patient survival status at Day 90 will be used regardless if they received liver transplant or TIPS procedure until Day 90 (Treatment policy).
    - Treatment discontinuation due to any reason: patient survival status at Day 90 is used regardless how much investigational product they received (Treatment Policy).
    - Prohibited medications intake until Day 90: patient survival status at Day 90 is used regardless if they had taken prohibited medications during the study (Treatment Policy).
- Population-level summary measure: Adjusted risk difference in mortality rate at Day 90 between VS-01 plus standard of care and standard of care only.

Rationale for estimand: the estimand assumes investigational intervention discontinuation, investigational intervention dose adjustment/interruptions, use of prohibited medications, and receiving liver transplant/TIPS are handled with treatment policy approach as it reflects clinical practice.

11.5.4.2 First Secondary Endpoint Analysis

The first secondary endpoint analysis is based on 90-day mortality rate, which is calculated as defined in section 9.17.

The following null hypothesis:

H_0: $p_1 - p_2 = 0$

will be tested against the alternative hypothesis:

H_1: $p_1 - p_2 \neq 0$,

where p_1 and p_2 are the 90-day mortality rates in the SOC only and VS-01 on top of SOC group, respectively.

To test the secondary hypothesis, a stratified Cochran-Mantel Haenszel (CMH) test, controlling for baseline CLIF-C ACLF score (<48 vs. ≥48) will be applied. An estimate of the unadjusted mortality rates in VS-01 on top of SOC vs. SOC only, the adjusted risk difference between the mortality rates of VS-01 on top of SOC vs. SOC only, along with the two-sided stratified Newcombe 95% CI for the risk difference; and two-sided p-value will be presented. The stratified Newcombe CI's will be estimated based on the method of Yan and Su (2010). For example SAS code of CMH test refer to section 17.0.

The treatment difference will be declared as significant if the 2-sided p-value from the stratified CMH test, controlling for CLIF-C ACLF score (<48 vs. ≥48), is <0.05.

In addition, the mortality rate at Day 90 will be calculated, as defined in section 9.17, and descriptively tabulated by ACLF Grade and West Haven HE grade at baseline.

11.5.4.2.1 Imputation Methods

Effort will be made to collect withdrawn patients' survival status up to Day 90. In the case there is still missing data, data will be imputed using multiple imputation.

Missing values are assumed to be MAR and will be multiple imputed using the MCMC method of PROC MI in SAS. The imputation model will include the following:

- Treatment arm (VS-01 on top of SOC or SOC only)
- CLIF-C ACLF score at BL (<48 vs. ≥48)
- ACLF Grade at BL
- Age at BL
- Sex

A total of m = 20 + 1.5*[proportion of missing data (%)], where m is rounded up to the next integer value, multiply imputed datasets will be created. The random number generator seed for the imputation of missing values using MCMC will be 165427 for reproducibility of the results. Each imputed dataset will be analyzed using the stratified CMH test described above. The individual results will then be combined using Rubin's rule (PROC MIANALYZE). The stratified risk difference is asymptotically normally distributed and can be combined using SAS PROC MIANALYZE directly. To obtain the combined p-value, as the CMH test statistic has a chi-square distribution, it will require transformation to normalize the CMH test statistic. The Wilson-Hilferty transformation will be used to normalize the CMH test statistic. First, the following transformation will be done:

$$wh\_cmh^{(m)} = \sqrt[3]{cmh^{(m)}/df}$$

Where $cmh^{(m)}$ is the CMH statistic computed from the *m*th imputed dataset, df is the number of degrees of freedom associated with the CMH statistic, and $wh\_cmh^{(m)}$ is the transformed value. This transformed statistic is approximately normally distributed with mean 1-2/(9 x df) and variance 2/(9x df) under the null hypothesis. This value will then be standardized to have a mean of 0 and a variance of 1 as follows:

$$st_{wh\_cmh}{}^{(m)} = \frac{\sqrt[3]{cmh^{(m)}/df} - (1 - \frac{2}{(9 * df)})}{\sqrt[2]{\frac{2}{(9 * df)}}}$$

This final transformed variable will then be passed through SAS PROC MIANALYZE to obtain the combined CMH test. The p-value of the combined CMH test is then obtained as the upper tailed p-value from the normal test produced by SAS PROC MIANALYZE on the transformed variable.

For example SAS code refer to section 17.0.

11.5.4.2.2 Sensitivity Analyses

Sensitivity analyses are planned to assess the first secondary analysis results under different assumptions to further explore the treatment effect.

11.5.4.2.2.1 Sensitivity Analysis 1: Analysis on PP Set

First secondary endpoint analysis is repeated using the PP set, to include only the patients strictly adhering to the protocol.

This analysis is performed only when >10% difference to the number of patients in FAS. Otherwise, only descriptive statistics will be provided.

11.5.4.2.2.2 Sensitivity Analysis 2: Excluding Patients who Received TIPS or Liver Transplant

The first secondary endpoint analysis is repeated after excluding patients who received TIPS/liver transplant within 7 days of randomization.

11.5.4.2.2.3 Sensitivity Analysis 3: Excluding patients with ACLF due to sASH, acute viral hepatitis, or uncontrolled AIH at screening

The first secondary endpoint analysis is repeated after excluding patients with ACLF due to sASH, ACLF due to acute viral hepatitis A, B, B+D, C or E requiring antiviral treatment, and ACLF due to AIH requiring high-dose steroid treatment patients.

11.5.4.3 28-Day Mortality

For 28-day mortality, the same CMH model will be applied as in section 11.5.4.2.

In addition, the mortality rate at Day 28 will be calculated, as defined in section 9.17, and descriptively tabulated by ACLF Grade and West Haven HE grade at baseline.

11.5.4.4 Time to Death

Time to death for any reason through Day 28, and through Day 90 will be tabulated separately using the Kaplan-Meier methodology. The number of patients who died and who were censored will be displayed. Censoring rules are defined in section 9.11. The median, 25th, and 75th percentile of the time to death and the two sided 95% CIs (Brookmeyer and Crowley method, LOGLOG transformation) will be calculated. In addition, the proportion of patients who have died at day 28 and 90 as determined by the KM method will be reported. A stratified log-rank test using BL CLIF-C ACLF score (<48 vs. ≥48) as stratification will be used to compare the two treatment groups. A stratified Cox proportional hazards model with treatment group and BL CLIF-C ACLF score (<48 vs. ≥48) will be used to estimate the hazard ratio and 95% CI (based on Wald test).

If median, 25th and 75th percentiles are not calculable, then log-rank test p-value and hazard ratio and 95% CI will not be displayed.

Kaplan-Meier plots will also be presented.

In addition, this analysis is repeated by censoring patients who received liver transplant.

11.5.4.5 ACLF Grade

ACLF grade will be assessed at SCR, BL (=Day 1), on Days 2 to 4 (pre-dose in Active Treatment group), on Days 5 to 7, 14, 28, and 90 or at Early Termination/Unscheduled visits. SCR will be assessed based on local lab, BL will be assessed based on both local and central lab results. Post baseline will be assessed based on central lab results.

ACLF Resolution

Time to ACLF resolution (by central lab) through Day 7, and Day 28 will be tabulated separately using the Kaplan-Meier methodology as described in section 11.5.4.4. Patients with resolution are defined in section 9.15. Kaplan-Meier plots will be used to display the distributions.

The number and percentage of patients with ACLF resolution (rate of ACLF resolution) at Days 7 and 28 will be tabulated. In addition, a stratified (BL CLIF-C ACLF score [<48 vs. ≥48]) Cochran-Mantel-Haenszel (CMH) test will be conducted to compare the two treatment groups.

ACLF Grade Regression

Time to ≥ one grade regression (based on the central lab) will be tabulated using the same approach as described in section 11.5.4.4. Patients with ≥ one grade regression are defined in section 9.16. Kaplan-Meier plots will be used to display the distributions.

The number and percentage of patients with ≥ one grade regression (rate of ≥ one ACLF grade resolution) through/at Days 7 and 28 will be tabulated. In addition, a stratified (BL CLIF-C ACLF score [<48 vs. ≥48]) CMH test will be conducted to compare the two treatment groups.

ACLF Grade Change

Change from BL in ACLF Grade will be summarized using a shift table. Comparisons between groups will be made using a CMH test with modified ridit scores.

11.5.4.6 Transplant-free Survival

Liver transplant-free status is checked at each visit. Transplant-free survival through/at Days 28 and 90 will be tabulated using the same approach as in section 11.5.4.4. Kaplan-Meier plots will be used to display the distributions.

11.5.5 Exploratory Efficacy Analyses

All exploratory efficacy analyses are summarized in tables and listed on the FAS.

11.5.5.1 In-Hospital Mortality and Time to In-Hospital Death

In-hospital mortality rate will be calculated as defined in section 9.17. In-hospital mortality rates will be tabulated by treatment group. Mortality rates between the two treatment groups will be compared using the same CMH test as for 90-day mortality, without missing value imputation, as in section 11.5.4.2.

Time to in-hospital death will be tabulated using the same approach as described in 11.5.4.4. A Kaplan-Meier plot will be used to display the distributions.

11.5.5.2 ICU-Mortality and Time to ICU Death

ICU mortality rate will be calculated as defined in section 9.17. ICU mortality rates will be tabulated by treatment group. Mortality rates between the two treatment groups will be compared using the same CMH test as for 90-day mortality, without missing value imputation, as in section 11.5.4.2.

Time to ICU death will be tabulated using the same approach as described in 11.5.4.4. A Kaplan-Meier plot will be used to display the distributions.

11.5.5.3 Duration of Hospitalization and/or ICU Stay

Duration of initial hospitalization and/or ICU and intermediate care unit stay will be calculated as described in section 9.18. Total duration of hospitalization and total duration in ICU (in days) due to initial ACLF episode will be tabulated descriptively, including the reasons for hospital stay and number of deaths while hospitalized.

Treatment groups will be compared using the Wilcoxon rank-sum test. Treatment difference will be expressed as the Hodge-Lehmann difference in median number of days with 95% CIs.

11.5.5.4 Hospital and ICU Readmission Rate

Any patient admitted to the hospital after the initial discharge will be counted as readmission, as defined in section 9.19. The number of readmissions through days 14, 28, and 90 will be tabulated descriptively for hospital and ICU stay.

11.5.5.5 Time to First Hospital and ICU Readmission for Reoccurring ACLF

Reoccurring ACLF is defined in section 9.20. Time to first hospital and ICU readmission for reoccurring ACLF after the initial discharge will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be displayed.

11.5.5.6 ACLF Reoccurrence Rate

ACLF reoccurrence rate, as defined in section 9.20, will be calculated through Days 14, 28, and 90, and tabulated descriptively. Reoccurrence rates between the two treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

11.5.5.7 Time to First ACLF Reoccurrence

Time to first ACLF reoccurrence, where reoccurrence is defined in section 9.20, will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be displayed.

11.5.5.8 Change from Baseline in CLIF-C ACLF Score

Change from BL in CLIF-C ACLF score will be tabulated descriptively for all time points. Change from BL to Days 5, 7, 14, 28, and 90 will be analyzed using a Mixed Model Repeated Measures (MMRM) approach with treatment, time and treatment by time interaction as fixed effects, BL as a covariate and patient as a random effect with an unstructured covariance. Missing data will be assumed to be MAR and maintained as missing. Data will be analyzed using both local and central lab results for the BL score.

Mean and mean change plot will display CLIF-C ACLF score, CLIF-C OF score, CLIF-SOFA score, creatinine, bilirubin and INR values.

11.5.5.9 Organ Failures Assessed by CLIF-C OF Score

The number and type of organ failures (kidney: serum creatinine ≥ 2 mg/dL or use of RRT; cerebral: HE West Haven Grade ≥ 3; liver: serum bilirubin ≥ 12 mg/dL; coagulation: INR ≥ 2.5; respiratory: PaO_2/FiO_2 < 200 or SpO_2/FiO_2 < 214; circulatory: use of vasopressors) will be assessed at all visits and will be summarized descriptively (based on central labs). CLIF-C OF score derivation is described in section 9.13.

The change from BL in CLIF-C OF score (based on central labs) will be tabulated for all time points. Change from BL to Days 5, 7, 14, 28, and 90 will be analyzed using the same MMRM model as in section 11.5.5.8.

11.5.5.10 Organ Dysfunctions Assessed by CLIF-SOFA Score

The number and type of organ dysfunctions (kidney: serum creatinine ≥ 1.2 – < 2.0 mg/dL; brain: HE West Haven Grade 1-2; liver: serum bilirubin ≥ 1.2 – < 12 mg/dL; coagulation: INR ≥ 1.1 – < 2.5; respiratory: PaO_2/FiO_2 > 200 - ≤ 400 or SpO_2/FiO_2 > 214 - ≤ 512; circulatory: MAP < 70 mmHg will be assessed at all visits and summarized descriptively (based on central labs). CLIF-SOFA score derivation is described in section 9.14.

The change from BL in CLIF-SOFA score will be tabulated for all time points (based on central labs). Change from BL to Days 5, 7, 14, 28, and 90 will be analyzed using the same MMRM model as in section 11.5.5.8.

11.5.5.11 CPS, MELD,MELD-Na, and MELD 3.0 Scores

CPS, MELD, MELD-Na, and MELD 3.0 scores will be assessed at SCR, on Day 1 BL (pre-dose in Active Treatment group), Days 5, 7, 14, 28, 90 or at Early Termination/Unscheduled visits. MELD, MELD-Na, and MELD 3.0 score derivation is described in section 9.26. The change from BL (based on central labs) in CPS total score, MELD, MELD-Na, and MELD 3.0 scores will be summarized descriptively by time point.

The change from BL to Days 5, 7, 14, 28, and 90 will be analyzed using the same MMRM model as in section 11.5.5.8.

11.5.5.12 Hepatic Encephalopathy

HE assessment is based on West Haven criteria, HEGI™, and Glasgow Coma Scale. BL and Day 7 HE assessments will be performed by a rater blinded to the study arm. HEGI™ will be assessed at BL and Day 7 / Early Termination directly after HE grade assessment according to West Haven criteria. HE grade by West Haven criteria will be used for randomization.

11.5.5.12.1 West Haven Criteria

West Haven criteria is assessed at all study visits and it is used to grade the severity of HE based on the level of impairment of autonomy, changes in consciousness, intellectual function, behavior, and the dependence on therapy. Refer to Protocol Appendix 5 for additional information on the test.

The number and percentage of patients by West Haven grade at each time point will be summarized descriptively. Change from BL will be summarized using a shift table.

Resolution of Overt HE

Overt HE resolution, as assessed by West Haven Criteria, is defined in section 9.22. Time to resolution through/at Days 5 and 7 will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be used to display the distributions.

The rate of resolution will be calculated as defined in section 9.22, for Days 5 and 7, and summarized descriptively. The rates between the treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

Regression of ≥ One HE Grade

Time to regression of at least one grade will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be displayed.

The rate of regression will be calculated for Days 5 and 7 and summarized descriptively. The rates between the treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

11.5.5.12.2 HEGI™

HEGI™ was developed in accordance with United States Food and Drug Administration (FDA) guidance, based on the West Haven criteria but modified to be easier to apply and more objective, to assess the severity of HE as an endpoint (refer to Protocol Appendix 5).

HE grades according to West Haven and HEGI™ will be listed side-by-side for each time point. The number and percentage of patients by HEGI™ HE grade at each time point will be summarized descriptively. Change from BL will be summarized using a shift table.

Resolution of Overt HE

Overt HE resolution, as assessed by HEGI™, is defined in section 9.23. Time to resolution through/at Day 7 will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be displayed.

The rate of resolution will be calculated as defined in section 9.23, and summarized descriptively. The rates between the treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

Regression of ≥ One HE Grade

Time to regression of at least one grade through day 7 will be analyzed similarly as in section 11.5.4.4. Kaplan-Meier plot will be displayed.

The rate of regression will be calculated and summarized descriptively. The rates between the treatment groups will be compared using a stratified CMH test, with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

11.5.5.12.3 West Haven and HEGI™ Comparison

West Haven and HEGI™ HE grades will be summarized side-by-side by time point and treatment group. West Haven grades <2 will be combined as HEGI does not have such granularity.

Primary model will be repeated to further explore the effect of HEGI™ assessment on the primary endpoint. CLIF-C ACLF score will be analyzed where HEGI™ assessment of HE grade is used instead of West Haven in the CLIF-OF score for BL and Day 7. In case HEGI™ grade is <2, the corresponding West Haven grade will be used (if available).

Repeated measures correlation (rmcorr: Repeated Measures Correlation) analysis will be performed by transforming the HE grades to numeric values and presenting the correlation statistic and rmcorr plot.

11.5.5.12.4 Glasgow Coma Scale

Glasgow Coma Scale is a neurological scale which provides a reliable and objective way of recording the conscious state of a person. The scale is composed of three tests requiring eye, verbal, and motor

responses and is recorded at Day 1, Day 5, Day 7 and Early Termination/Unscheduled visits. Three test scores are summed to derive the Glasgow Coma Scale score.

Absolute values and changes from BL of the Glasgow Coma Scale scores will be summarized descriptively by time point. The change from BL of Glasgow Coma Scale score at Day 5 and Day 7 will be analyzed using the same MMRM model as section 11.5.5.8. In case the data is not approximately normally distributed, a nonparametric test (Wilcoxon rank-sum test or Friedman test) will be performed.

The proportion of patients with severe (<9), moderate (9-12) and mild (>12) score will be summarized descriptively by time point.

11.5.5.13 Inflammatory, Metabolomic, and Proteomic Profiles

Serum inflammatory markers (interleukins and interferons, C-Reactive Protein, procalcitonin and white blood cells) and plasma lactate samples will be taken at BL (Day 1), Day 5, Day 7, and Early Termination/Unscheduled Visit.

Absolute values and change from BL to Days 5 and 7 will be summarized. Individual patient profile plots and box plots will be displayed.

Univariate and multivariate logistic regression analysis will be done for CRP, white blood cells and plasma lactate to check for predictors of mortality at Day 28 and Day 90. In univariate logistic regression model, the response variable is patient outcome (died, alive) at Day 28, and marker (CRP, WBC or lactate) is the dependent variable, adjusted for treatment group. The marker values at Day 1, Day 5 and Day 7 are of interest. In multivariate logistic regression model, dependent variables are the three markers (at Day 1, Day 5, and Day 7), adjusted for treatment group. The proportions, treatment difference and 95% confidence intervals will be reported.

Peritoneal fluid will be collected for future inflammatory, metabolomic, and proteomic phenotyping. Analysis is optional and described as such in the TFL shells.

11.5.5.14 Renal Function

Renal function biomarkers (serum creatinine levels, creatinine clearance (Cockcroft-Gault equation), eGFR (Modification of Diet in Renal Disease [MDRD] and Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI]), serum cystatin C levels, BUN or urea and uric acid) will be assessed as part of laboratory analysis and will be performed daily for Active Treatment and Control groups at SCR, Days 1 to 4 (twice daily for the Active Treatment group only; pre-dose and at 6 h [±1 h] post-dose based on infusion start), Days 5 to 7, Day 14, Day 28 and Day 90 or at Early Termination/ Unscheduled visits.

Absolute values and change from BL will be summarized for all time points. As an exploratory endpoint, change from BL to Days 5, 7, 14, 28, and 90 are of interest and will be analyzed using a Mixed Model Repeated Measures (MMRM) approach with treatment, time and treatment by time interaction as fixed effects, BL as a covariate and patient as a random effect with an unstructured covariance. Missing data will be assumed to be MAR and maintained as missing. Box plots will be displayed by time point.

Incidence of RRT

RRT will be recorded as a continuum throughout the study up to Day 90 or Early Termination Visit. The number of patients who received RRT up to Day 5, 7, 14, 28, and 90 will be summarized. Incidence rates between the two treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

11.5.5.15 Liver Function

Liver function biomarker (serum bilirubin) will be assessed as part of laboratory analysis at the same time points as specified in section 11.5.5.14.

Absolute values and change from BL of serum bilirubin will be summarized for all time points. As an exploratory endpoint, change from BL to Days 5, 7, 14, 28, and 90 are of interest and will be analyzed using MMRM approach with treatment, time and treatment by time interaction as fixed effect, BL as covariate and patient as a random effect with and unstructured covariance. Missing data will be assumed to be MAR and maintained as missing. A Box plot will be displayed by time point.

11.5.5.16 TIPS and Liver Transplant

TIPS and liver transplant procedures will be recorded as a continuum throughout the study up to Day 90 or Early Termination Visit.

The number of patients who received TIPS or liver transplant up to Day 5, 7, 14, 28, and 90 will be summarized. Incidence rates between the two treatment groups will be compared using a stratified CMH test with BL CLIF-C ACLF score (<48 vs. ≥48) as a stratification variable.

The clinical outcome after surgery/procedure will be included in a listing.

11.5.5.17 Subgroup Analyses

The subgroups of interest are:

- Age (<65, ≥65, ≥75)
- Race
- Sex
- Ethnicity
- ACLF grade at BL
- CLIF-C ACLF score (median CLIF-C ACLF score at BL as the cutoff value; two groups)
- HE grade at BL
- Kidney failure at BL
- ACLF onset (≤4 days, >4 days from BL)
- Geographical area (United States vs. Europe)

Subgroup analysis will be performed to further explore the treatment effect.

The primary analysis of the primary endpoint (CLIF-C ACLF score at Day 7) is repeated by adding subgroup and subgroup*treatment interaction as fixed effects. Forest plot will be done showing p-values for the interaction term, LS-Mean estimates and 95% CI for the treatment difference in each subgroup.

The first secondary endpoint (90-day mortality) and 28-day mortality analysis will be repeated by adding the subgroup as a stratification factor. Forest plot will be done showing p-values for the Breslow Day test, treatment difference and 95% CI for the treatment difference in each subgroup.

Descriptive summary will be provided, and if the number of patients/events in a specific subgroup do not allow a subgroup analysis, a descriptive summary will be provided only.

Other subgroups may be identified based on clinical findings. Subgroup analysis will have an exploratory nature.

11.6 Analysis of Pharmacokinetic Concentrations and Parameters

PK sampling of plasma citric acid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and cholesterol concentrations will be done in VS-01 treatment arm only on Day 1 and Day 4 at pre-dose, and at 1 h (±15 min), 2 h (±15 min), 3 h (±15 min) and 6 h (±30 min) post-dose (based on infusion start time on each day); on Day 2 (24 h post dose Day 1, pre-dose Day 2) and Day 3 (pre-dose); on Days 5, 6, and 7 at approximately 24 h, 48 h, and 72 h post-dose on Day 4, respectively; or at Early Termination Visit. Pre-dose samples on Days 1, 2, 3 and 4 are taken pre-dose to their respective day's dose.

Peritoneal fluid citric acid, DPPC and cholesterol concentrations will be measured on Days 1 and 4 at pre-dose, and at 1 h (±15 min), 2 h (±15 min), 3 h (±15 min) post-dose (based on infusion start time on each day); on Day 2 (24 h post dose Day 1, pre-dose Day 2) and Day 3 (pre-dose); on Days 5 to 7 or at Early Termination Visit upon paracentesis only if clinically indicated. Samples on Days 2 and 3 are taken consistently early in the morning.

PK parameters for citric acid, DPPC, and cholesterol will be estimated using non-compartmental methods with WinNonlin®. The plasma and peritoneal fluid PK parameters will be estimated from the concentration-time profiles using the linear-log trapezoidal method. In estimating the PK parameters,

Below the Quantification Limit (BQL) values at the beginning of the profile will be set to zero. BQL values that occur after the first quantifiable point will be considered missing. Values that are embedded between BQLs, or quantifiable values occurring after two or more BQLs, will be set to missing at the discretion of the pharmacokineticist. For endogenous compounds (i.e., with measurable 0h-concentrations), BQL concentrations before or after dosing are set to ½ of the Lower Limit of Quantification (LLOQ) or to missing if they are flanked by 2 measurable concentrations.

Actual times [in hours rounded to three decimal places and negative pre-dose times set to zero (t=0)] rather than scheduled times will be used in the calculation of PK parameters. Where actual time is not available, scheduled PK sampling time will be used.

Baseline adjustment is performed for endogenous compounds by subtracting BL concentration value [C(0) on Day 1] from all concentrations. If BL value is missing, then it is set as ½ of LLOQ. If the adjusted value is negative, then it is set as ½ of LLOQ.

All individual patient plasma and peritoneal concentrations data as well as PK parameters will be listed.

The following PK parameters for citric acid, DPPC and cholesterol will be evaluated for Day 1 and Day 4, where applicable, in the BL-adjusted / weight and BL-adjusted plasma:

Parameter	Description	Analyte	SAS Programming Notes
C_{max}	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units.	Citric acid DPPC Cholesterol	Cmax from WinNonlin® (WNL)
C_{max}/Dose	Dose Normalized C_{max}	Citric acid DPPC Cholesterol	Cmax_D from WNL
T_{max}	Time to maximum plasma concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	Citric acid DPPC Cholesterol	Tmax from WNL
AUC_{0-3} AUC_{0-24}	Area under the concentration-time curve (time 0 to 3h or 24h). Calculated by linear/log trapezoidal method	Citric acid DPPC Cholesterol	AUC0-3, AUC0-24 from WNL
AUC_{0-24}/Dose	Dose Normalized AUC_{0-24}.	Citric acid DPPC Cholesterol	AUC_D from WNL
AUC_{0-inf}	AUC calculated until infinity using the linear/log trapezoidal. AUC_{0-t}+ C_{last}/λ_z. For endogenous compounds in case of a flat line (relative to baseline) at the end of the PK profile AUC_{0-24} may be used for the calculation of AUC_{0-inf}.	Citric acid DPPC Cholesterol	AUCINF_obs from WNL If AUC_%Extrap_obs >20% then parameter is flagged, but not excluded from the statistics
λ_z	Terminal phase elimination rate constant. λz will be calculated by least squares linear regression of the log(ln)-linear disposition phase of the concentration-time curve.	Citric acid DPPC Cholesterol	Lambda_z from WNL If Rsq ≤ .80 then parameter is flagged, but not excluded in the descriptive statistics
$t_{1/2}$	Terminal phase half-life expressed in time units. r^2 greater than 0.80 is required to obtain a reliable $t_{1/2}$.	Citric acid DPPC Cholesterol	HL_Lambda_z from WNL If Rsq ≤ .80, AUC_%Extrap_obs >20% then parameter is

			flagged, but not excluded from the descriptive statistics
CL/F	Apparent total clearance of the drug from plasma after administration. Dose / $AUC_{0\text{-}inf}$	Citric acid DPPC Cholesterol	CL_F_obs from WNL If Rsq ≤ .80, AUC_%Extrap_obs >20% then parameter is flagged, but not excluded from the descriptive statistics
V_z/F	Apparent volume of distribution during the terminal phase after drug administration, Dose / (λ_z x $AUC_{0\text{-}inf}$)	Citric acid DPPC Cholesterol	Vz_F_obs from WNL If Rsq ≤ .80, AUC_%Extrap_obs >20% then parameter is flagged, but not excluded from the descriptive statistics
Accumulation Ratio (R)	$AUC_{0\text{-}24}$,Day 4/$AUC_{0\text{-}24}$,Day 1	Citric acid DPPC Cholesterol	SAS

The following PK parameters for citric acid, DPPC, and cholesterol will be evaluated for Day 1 and Day 4, where applicable, in the weight and BL-adjusted peritoneal fluid:

Parameter	**Description**	**Analyte**	**SAS Programming Notes**
$C_{max,dial}$	Maximum peritoneal fluid concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units.	Citric acid DPPC Cholesterol	Cmax from WNL
$T_{max,dial}$	Time to maximum peritoneal fluid concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	Citric acid DPPC Cholesterol	Tmax from WNL
$AUC_{0\text{-}3,dial}$	Area under the peritoneal fluid concentration-time curve (time 0 to 3 h). Calculated by linear/log trapezoidal method.	Citric acid DPPC Cholesterol	AUC0-3 from WNL
$AUC_{0\text{-}3,dial}/AUC_{0\text{-}3}$	Relative exposure dialysate versus plasma	Citric acid DPPC Cholesterol	SAS

Additional PK parameters may be estimated at the discretion of the pharmacokineticist.

Descriptive statistics (number of patients, number of missing values, mean, geometric mean, SD, coefficient of variation, geometric coefficient of variation, median, Q1, Q3, min, and max) will be used to summarize the calculated PK parameters and concentrations by analyte and actual time point. For t_{max}, only median, min and max will be presented. BL-adjusted concentrations and PK parameters will be summarized by number of kits received. Weight and BL-adjusted PK parameters will be summarized.

Plasma and peritoneal fluid concentration-time curves will be provided by analyte, and number of kits received, presenting each patient as a separate line on linear and log-linear scales. In addition, the mean concentration time curve will be provided on the same plot.

Plasma and peritoneal fluid PK parameters (C_{max} and AUC_{last}) will be plotted against dose (mg/kg) on linear and log-scales for each analyte.

Safety analysis set (VS-01 arm) will be used to summarize PK concentrations, and PK analysis set will be used to summarize PK parameters.

11.7 Analysis of Pharmacodynamic Parameters

PD sampling of ammonia will be done in both VS-01 treatment and SOC only patients. Blood sampling for VS-01 arm is done on Day 1 and Day 4 at pre-dose (fasting), and at 1 h (±15 min), 2 h (±15 min), 3 h (±15 min) and 6 h (±30 min) post-dose (based on infusion start time on each day); on Day 2 and Day 3 at 24 h (±1 h fasting) and 48 h (±1 h fasting) post-Day 1 BL samples, respectively (however pre-dose to their respective day's dose); on Days 5 to 7 (±1 h) respective to Day 1 BL sample (fasting) or at Early Termination Visit (fasting). Fasting samples are taken consistently early in the morning. For patients in the control group, ammonia plasma concentrations will be measured at BL, and once daily on Days 2 to 7 or at Early Termination Visit in fasted state. Samples will need to be done early in the morning and timing should be consistent across Days 2 to 7 (i.e., ±1 h from the time of BL collection on Day 1).

Peritoneal fluid sampling for VS-01 arm is done on Day 1 and Day 4 at pre-dose (fasting), and at 1 h (±15 min), 2 h (±15 min), 3 h (±15 min) post-dose (based on infusion start time on each day); on Day 2 and Day 3 at 24 h (±1 h fasting) and 48 h (±1 h fasting) post-Day 1 BL samples, respectively (however pre-dose to their respective day's dose); on Days 5 to 7 or at Early Termination Visit in a fasted state only if re-puncture for paracentesis is clinically indicated. Fasting samples are taken consistently early in the morning. For patients in the control group, peritoneal fluid samples will be collected on following visits other than BL, only in case of re-puncture: on Days 2 to 4 early in the morning and consistently across days (i.e., ±1 h from the time of BL collection on Day 1); upon paracentesis, only if clinically indicated (fasting) on Days 5 to 7 or at Early Termination Visits.

Actual times [in hours rounded to three decimal places and negative pre-dose times set to zero (t=0)] rather than scheduled times will be used in the calculation of PD parameters. Where actual time is not available, scheduled PD sampling time will be used.

If BL concentration is missing, then it is set as ½ of LLOQ.

The following PD parameters of plasma ammonia will be evaluated for Day 1 and Day 4, where applicable:

Parameter	Description	Analyte	SAS Programming Notes
$C_{min,NH3}$	Minimum concentration that ammonia achieves in plasma over dosing interval. Obtained directly from the experimental data without interpolation, expressed in concentration units.	Ammonia	Cmin from WNL
$T_{min,NH3}$	Time to minimum plasma concentration. First observed time to reach minimum analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	Ammonia	Tmin from WNL
$AUC_{0-3,NH3}$	Area under the plasma concentration-time curve (time 0 to 3 hours post-dose). Calculated by linear/log trapezoidal method.	Ammonia	AUC0-3 from WNL
$AUC_{0-24,NH3}$	Area under the plasma concentration-time curve (time 0 to 24 hours post-dose). Calculated by linear/log trapezoidal method. If the 24 h-concentration is missing, $AUC_{0-24,NH3}$ is set to missing.	Ammonia	AUC0-24 from WNL
$TE_{max,NH3}$	Time to normalization of plasma ammonia	Ammonia	TEmax from WNL
$E_{max,NH3,dwell}$	Maximum reduction in plasma ammonia from baseline, within the 3-hour dwell time	Ammonia	Emax_dwell from WNL

	on Day 1 and Day 4 (pre-dose, 0, 1, 2 and 3 h post-dose).		
$E_{max,NH3,overall}$	Maximum reduction in plasma ammonia from baseline, across study visits based on trough concentrations.	Ammonia	Emax_overall from WNL

The following PD parameters of ammonia in peritoneal fluid will be evaluated for Day 1 and Day 4, where applicable:

Parameter	**Description**	**Analyte**	**SAS Programming Notes**
$C_{max,dial,NH3}$	Maximum concentration that ammonia achieves in peritoneal fluid over dosing interval. Obtained directly from the experimental data without interpolation, expressed in concentration units.	Ammonia	Cmax from WNL
$T_{max,dial,NH3}$	Time to maximum peritoneal fluid concentration. First observed time to reach maximum analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	Ammonia	Tmax from WNL
$AUC_{0-3,dial,NH3}$	Area under the peritoneal fluid concentration-time curve (time 0 to 3 hours post-dose). Calculated by linear/log trapezoidal method.	Ammonia	AUC0-3 from WNL
$CL_{dial,NH3}$	Ammonia clearance in the peritoneal fluid. $Amount_{dial,NH3}/AUC_{0-3,dial,NH3}$, where $Amount_{dial,NH3}$ is ammonia plasma concentration at 3h x weight (volume) of peritoneal fluid recovered.	Ammonia	Cl_F from WNL
$AUC_{0-3,dial,NH3}$/ $AUC_{0-3,NH3}$	Relative extent of exposure *i.p.* versus plasma	Ammonia	SAS

Additional PD parameters may be estimated at the discretion of the pharmacokineticist.

All individual patient ammonia concentration data and PD parameters will be listed. All PD parameters will be summarized by number of kits (1, 2, 3, 4, all kits) and time point in a descriptive manner (number of patients, number of missing value, mean, geometric mean, SD, coefficient of variation, geometric coefficient of variation, median, Q1, Q3, min, and max). For concentration data summaries, if 50% or more of the patients show concentration values '<LLOQ' at any time point, descriptive statistics are not calculated and will be presented as N.C (not calculated). If <50% if patients had concentration values '<LLOQ', descriptive statistics will be calculated by substituting 0 for values '<LLOQ' and the geometric mean is not calculated.

PD parameters will also be presented by West Haven HE grade and separately by HEGI™ HE grade at BL.

Plasma and peritoneal fluid concentration-time curves will be provided by number of kits received and treatment group, presenting each patient as a separate line. In addition, mean concentration time curves will be provided on the same plot, presenting a separate line for each group.

Plasma and peritoneal fluid ammonia concentrations on Day 1, Day 5, and Day 7 will be summarized in box plots by West Haven HE grade and HEGI™ HE grade at BL.

Safety analysis set is used to summarize PD concentrations, and PD analysis set will be used to summarize PD parameters.

Additional analysis to assess the influence of weight may be conducted.

11.8 Safety Analyses

Safety analysis is done using the safety analysis set and all data will be listed.

11.8.1 Adverse Events

AEs are collected from providing informed consent up to EOS on Day 90. All summaries of AEs will be based on TEAEs (see definition in section 9.27). The number and percentage of patients experiencing AEs will be summarized by system organ class and PT. The number of events will also be presented.

Summaries by maximum severity and relationship to overall study treatment, and summary of infusion procedure related AEs by maximum severity will also be provided. Severity is categorized by National Cancer Institute Common terminology criteria for AEs (NCI-CTCAE) V5.0 grading criteria (grade 1 to grade 5). Relationship to IMP is categorized as definitely related, probably related, possibly related, unlikely related, or not related Patients with multiple events within a particular system organ class or PT will be counted under the category of their most severe event or most relatedness within that system organ class or PT.

Serious adverse events (SAEs) and AEs leading to discontinuation from the study will be presented by system organ class and PT.

All AEs, both serious and non-serious, will be tabulated and reported by severity or intensity and the relationship of the AE to the IMP (VS-01). AEs will be tabulated based on the day of the study when they were reported and overall. Start and stop dates and outcomes will be recorded. Duration between AE occurrence and the most recent IP infusion will be derived and listed. AE outcome will be classified into fatal, not recovered/not resolved, recovered/resolved, recovered/resolved with sequelae, recovering/resolving, and unknown.

The following tables will be produced by system organ class and PT:

- All TEAEs
- TEAEs by maximum NCI-CTCAE grade
- TEAEs by relationship to IMP
- Serious TEAEs
- Serious TEAEs by relationship to IMP
- Serious TEAEs leading to discontinuation from the study
- TEAEs leading to discontinuation from the study
- TEAEs by day of occurrence
- TEAEs by outcome
- TEAEs leading to discontinuation from the IMP
- TEAEs leading to death
- TEAEs by West Haven HE Grade at Baseline
- TEAEs related to infusion procedure by maximum NCI-CTCAE grade
- TEAEs by reporting day category (until Day 7, Day 8 to Day 28, Day 29 onwards)

All AEs and SAEs (including non-TEAEs), and deaths will be listed.

11.8.2 Adverse Event of Special Interest

Adverse events of special interest (AESI) are AEs identified for enhanced monitoring due to their potential scientific or medical interest specific to the Sponsor's product or program. Peritonitis has been designated as an AESI in this study.

AESIs are collected in the CRF for patients enrolling under Protocol version 11.0 or later. For patients who enrolled prior to Protocol version 11.0, AESIs are manually identified.

The number and percentage of patients experiencing at least one AESI, and the corresponding number of events, in each of the following categories will be summarized:

- All AESIs
- Serious AESIs
- Treatment-emergent AESI
- Serious treatment-emergent AESI
- Treatment-emergent AESIs leading to discontinuation from the IMP
- Treatment-emergent AESIs leading to death

The number and percentage of patients experiencing at least one AESI, and the corresponding number of events, will also be summarized by system organ class and PT.

All AESIs will be listed.

11.8.3 Laboratory Data

For full list of parameters see Protocol Appendix 17.

Biochemistry, hematology and coagulation tests ("Safety Lab") will be performed daily for Active Treatment and Control groups at SCR and/or BL (to central and local laboratory), Days 1, 2, 3, and 4 (twice daily for the Active Treatment group only: at BL on Day 1 prior to randomization and at 6 h [±1 h] post-dose based on infusion start, on Days 2 to 4 pre-dose and at 6 h [±1 h] post-dose based on infusion start), Days 5 to 7, Day 14, 28, and 90, or at Early Termination/Unscheduled visits (to central laboratory). See Protocol Appendix 17 for a complete list of biochemistry, hematology, and coagulation analyses.

Complement activation - related pseudoallergy (CARPA) parameters as part of "Safety Lab" will be analyzed at BL for both treatment arms, and as soon as possible when a suspected infusion reaction to VS-01 occurs on Day 1 to Day 4 in the Active Treatment group.

Organ failure and/or dysfunction as part of "Safety Lab" will be assessed using the parameters bilirubin, creatinine, INR, HE grade (once according to West Haven Criteria, once according to HEGI™), Mean Arterial Pressure (MAP), Partial pressure of oxygen (PaO_2)/Fraction of inspired oxygen (FiO_2) or Oxygen saturation (SpO_2)/FiO_2 according to CLIF-C OF and CLIF-SOFA scores on their respective timepoints. Analysis is described under sections 11.5.5.9 and 11.5.5.10.

Organ functions as part of "Safety Lab" will be assessed using parameters (refer to Protocol Appendix 19 for patients on Active Treatment and in Protocol Appendix 20 for patients on Control for time points): Serum creatinine, creatinine clearance [Cockcroft-Gault equation], serum cystatin C and eGFR (MDRD and CKD-EPI, see Protocol Appendix 14), BUN or urea and uric acid will be used to assess the renal function. Serum bilirubin will be used to assess the liver function. Coagulation failure and/or dysfunction will be assessed using INR on the following days: Day 5, 7, 14, 28, 90, or at Early Termination/ Unscheduled visits (See Protocol Appendix 17 for a complete list of coagulation analyses).

Pregnancy test (to local laboratory) will be done in serum or urine for women of child-bearing potential (see Protocol Appendix 17).

Urinalysis (to central and/or local laboratory) will be performed if urine is available, at SCR, daily on Days 1 to 7 (prior to randomization on Day 1 and pre-dose on Day 4 for Active Treatment group) or at Early Termination/Unscheduled visits. See Protocol Appendix 17 for a complete list of analyses.

Peritoneal fluid tests (to central laboratory) will be performed at SCR for both Active Treatment group and Control group; for Active Treatment group daily on Days 1 to 4 (prior to randomization on Day 1 and pre-dose on Days 2 to 4), or in case of clinical requirement for a re-puncture after catheter removal on Days 5 to 7 or at Early Termination/Unscheduled visits; for Control group in case of re-puncture then at the same time points (other than SCR) as for Active Treatment group. Peritoneal fluid tests include albumin gradient (for serum ascites albumin gradient [SAAG]), ammonia, total protein concentration, glutamine, and pH.

Peritoneal fluid safety test (to local laboratory) includes neutrophil count. Timing of the test is the same as for other peritoneal fluid tests.

Peritoneal fluid for microbial culture (to central laboratory) will be performed at SCR for both Active Treatment group and Control group; for Active Treatment group on Day 4 pre-dose. If neutrophil count is positive in the peritoneal fluid safety test on Days 1, 2 or 3, additional microbial culture test should be also performed on the respective days. In case of clinical requirement for a re-puncture after catheter removal, on Days 5 to 7 or at Early Termination/ Unscheduled visits, a sample of ascitic fluid may be taken to rule out the presence of spontaneous bacterial peritonitis. For Control group, peritoneal fluid samples will be collected on above-mentioned visits other than SCR, only if in case of re-puncture.

VS-01 Dwell Safety lab (to local laboratory) includes venous blood gas analysis (vBGA) to measure pH, bicarbonate (HCO_3^-) (mmol/L), base excess (BE), PaO_2, and partial pressure of carbon dioxide ($PaCO_2$) to monitor acid-base status, as well as ionized Ca (Ca_{ion}) and lactate. Additionally, total calcium (Ca_{tot}) and Ca_{tot}/Ca_{ion} ratio (derived) are measured to monitor citrate accumulation, as well as Activated partial thromboplastin time (aPTT) to monitor coagulation. These will be assessed on Days 1 to 4 at directly pre-dose, during VS-01 administration (dwell time) at 1 h (+30 mins) and 3 h (+30 mins) post-dose based on infusion start.

Serum inflammatory markers (interleukins and interferons, C-Reactive Protein, procalcitonin and white blood cells) and plasma lactate will be used to assess inflammation. Analysis is described in sections 11.5.5.13, 11.5.5.14, and 11.5.5.15.

Biobanking samples for future metabolomic, proteomic and inflammatory phenotyping: In addition, blood (serum and plasma; including PK and CARPA samples not utilized for PK or CARPA testing), peritoneal fluid (including ascites and peritoneal fluid removed after VS-01 treatment and washing step), and urine may be used for future metabolomic, proteomic and inflammatory phenotyping (see Protocol Appendix 17).

COVID-19 tests will be performed as per local standards and the data will be listed only.

Albumin, pH, and total protein measured in ascites at SCR will be used for descriptive analysis only in case parameters are measured with the use of a non-validated method for ascites (this will be mentioned in the CSR).

Absolute values and changes from BL will be presented descriptively using International System of Units (SI) unless otherwise stated, for central lab results and safety local laboratory test results.

The number and percent of lab parameters by maximum CTCAE grade will be summarized by parameter and time point, for all applicable parameters.

Shift tables of BL assessment to postbaseline assessment by reference range (low, normal, high) will be presented for all continuous parameters from central lab and safety local laboratory tests.

Shift tables of BL assessment to each post-baseline visit assessment will be presented for categorical parameters from central lab.

All continuous central laboratory parameters and safety local laboratory test results will be plotted in a box plot by time point.

All data and data outside of the study-specific reference ranges will be listed.

11.8.4 Vital Signs

Vital signs are assessed at SCR; twice daily on Day 1 to Day 4 (pre-dose and post-dose [based on infusion start] after VS-01 removal and washing step for Active Treatment group); daily on Day 5 to Day 7, Day 14, 28, and 90 or at Early Termination/Unscheduled visits. Vital signs will include body temperature (°C), SpO_2 (%), FiO_2, systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (beats/minute), respiratory rate (breaths/min) and MAP (mmHg). MAP is calculated using the formula provided in Protocol Appendix 7.

Vital signs absolute values and changes from BL will be summarized by time point.

11.8.5 Physical Examinations

Full physical examination will be performed at SCR and Day 7 or Early Termination/Unscheduled visits and a targeted physical examination will be performed on the rest of the visits.

The full physical examination should include general appearance, skin, neck, eyes, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological examinations. Subsequent targeted physical examinations should include body systems as clinically appropriate at additional patient encounters. A targeted physical examination will include heart, lungs, abdomen, extremities plus central nervous system (CNS) assessment, based on the investigator's decision. For each body system, result (normal, abnormal, not done), abnormal findings and whether or not the findings are clinically significant will be collected. The results will be tabulated by body system, finding (normal, abnormal not clinically significant, abnormal clinically significant, not done), and time point.

Height (cm) and dry body weight (kg) are measured at SCR. Dry body weight is also measured at EOS or Early Termination/Unscheduled visits. Dry body weight is defined as the weight measured after removing ascites completely and emptying urinary bladder. The results will be tabulated by time point and listed together with vital signs.

11.8.6 Electrocardiogram

Electrocardiogram (ECG) for central reading will be performed at SCR, daily on Days 1 and 4 (twice daily for Active Treatment group only; at BL on Day 1 prior to randomization and at 6 h [±1 h] post-dose based on infusion start, on Day 4 pre-dose and at 6 h [±1 h] post-dose based on infusion start), daily on Days 2 and 3 (pre-dose before VS-01 administration for Active Treatment group), daily on Days 5 and 7 or at Early Termination/Unscheduled visits.

The parameters to be measured are heart rate (HR), PR, QRS, QT and QTcF intervals. Absolute values and changes from baseline will be summarized descriptively. Potentially clinically important ECG results will be summarized. Abnormality assessment is per the central read, and clinical significance assessment as per the investigator. The number and percent of patients with postbaseline abnormal results (abnormal, clinically significant) and findings as assessed by investigator will be tabulated.

12.0 References

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6. rmcorr: Repeated Measures Correlation. https://CRAN.R-project.org/package=rmcorr

13.0 Glossary of Abbreviations

Glossary of Abbreviations:	
AASLD	American Association for the Study of Liver Diseases
AE	Adverse event
AESI	Adverse event of special interest
ACLF	Acute-on-chronic liver failure
AIH	Autoimmune hepatitis
ANCOVA	Analysis of covariance
aPTT	Activated partial thromboplastin time
ATC	Anatomical Therapeutic Chemical
AUC	Area under the curve
AUC_{0-3}	Area under the curve during 3 h
AUC_{0-24}	Area under the curve during 24 h
AUC_{inf}	Area under the concentration-time curve to infinity
BE	Base excess
BL	Baseline
BMI	Body Mass Index
BUN	Blood urea nitrogen
Ca_{ion}	Ionized calcium
CARPA	Complement activation - related pseudoallergy
Ca_{tot}	Total calcium
CI	Confidence interval
CL	Apparent clearance
CLDQ	Chronic Liver Disease Questionnaire
CLIF-C	Chronic Liver Failure Consortium
CLIF-C ACLF	Chronic Liver Failure – Consortium Acute-on-Chronic Liver Failure
CLIF-OF	Chronic Liver Failure Consortium - Organ Failure
CLIF-SOFA	Chronic Liver Failure Consortium - Sequential Organ Failure Assessment
C_{max}	Maximum concentration
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMH	Cochran-Mantel-Haenszel
CNS	Central nervous system
CPS	Child-Pugh Score
CRF	Case Report Form
CRP	C-reactive protein
CSR	Clinical Study Report
CVVHD	Continuous Veno-Venous Hemodialysis

DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
EASL	European Association for the Study of the Liver
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
$E_{max,NH3}$	Maximum reduction in plasma ammonia
EOT	End of Treatment
EOS	End of Study
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	First-in-human
FiO_2	Fraction of inspired oxygen
HCC	Hepatocellular carcinoma
HCO_3^-	Bicarbonate
HE	Hepatic encephalopathy
HEGI™	Hepatic encephalopathy Grading Instrument
HR	Heart rate
HRS-AKI	Hepatorenal syndrome – acute kidney injury
HRQoL	Health-Related Quality of Life
IA	Interim Analysis
ICU	Intensive care unit
iDMC	Independent Data Monitoring Committee
IMP	Investigational medicinal product
INR	International Normalized Ratio
i.p.	intraperitoneal
ITT	Intent-to-treat
LS	Least Squares
MAP	Mean Arterial Pressure
MAR	Missing at random
MCMC	Markov chain Monte Carlo
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-stage Liver Disease
MELD-Na	MELD-Sodium
MI	Multiple imputation
MMRM	Mixed model repeated measures
MNAR	Missing not at random
MV	Mechanical ventilation

NCI-CTCAE	National Cancer Institute Common terminology criteria for AEs
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PD	Pharmacodynamic
pH	Potential of Hydrogen
PK	Pharmacokinetic
PP	Per Protocol
PT	Preferred Term
RRT	Renal Replacement Therapy
SAAG	Serum ascites albumin gradient
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
sASH	Severe alcoholic steatohepatitis
SCR	Screening
SD	Standard deviation
SI	International system of units
SOC	Standard of Care
SpO_2	Oxygen saturation
$t_{1/2}$	Apparent elimination half-life
t_{max}	Time to maximum concentration
TEAE	Treatment-emergent adverse event
TIPS	Transjugular portosystemic shunts
US	United States
vBGA	Venous Blood Gas Analysis
V_d	Volume of distribution
VAS	Visual Analog Scale
WHO	World Health Organization

Appendix

14.0 CLIF-C OF Score

Organ/system	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin <6 mg/dl	Bilirubin ≥6 mg/dl and <12 mg/dl	Bilirubin ≥12 mg/dl
Kidney	Creatinine <2 mg/dl	Creatinine ≥2 mg/dl and <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	INR ≥2.0 and <2.5	INR ≥2.5
Circulatory	MAP ≥70 mmHg	MAP <70 mmHg	Use of vasopressors
Respiratory PaO_2/FiO_2 or SpO_2/FiO_2	 >300 or >357	 ≤300 and >200 or >214 and ≤357	 ≤200# or ≤214#

The shaded area describes criteria for diagnosing organ failures.
*HE, hepatic encephalopathy; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; SpO_2, pulse oximetric saturation.
**Patients submitted to Mechanical Ventilation (MV) due to HE and not due to a respiratory failure were considered as presenting a cerebral failure (cerebral subscore = 3).
#Other patients enroled in the study with MV were considered as presenting a respiratory failure (respiratory subscore = 3).

Sources:

1. Score Calculators | EF CLIF | European Foundation for the study of chronic liver failure

2. Jalan R, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. J of Hepatol 2014; 61; 1038–1047.

15.0 CLIF-SOFA Score

Organ/System	0	1	2	3	4
Liver (Bilirubin, mg/dL)	<1.2	≥1.2 to <2.0	≥2.0 to <6.0	≥6.0 to <12.0	≥12.0
Kidney (Creatinine, mg/dL)	<1.2	≥1.2 to <2.0	≥2.0 to <3.5[a]	≥3.5 to <5.0[a]	≥5.0[a]
Cerebral (HE grade)[b]	No HE[c]	I	II	III[d]	IV[d]
Coagulation (INR)	<1.1	≥1.1 to <1.25	≥1.25 to <1.5	≥1.5 to <2.5	≥2.5 or platelets ≤20 × 10^9/L
Circulation (MAP, mmHg)	≥70	<70	Dopamine ≤5 or dobutamine or terlipressin	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Lungs PaO2/FiO2 or SpO2/FiO2[e]	>400 >512	>300 to ≤400 >357 to ≤512	>200 to ≤300 >214 to ≤357	>100 to ≤200 >89 to ≤214	≤100 ≤89

Abbreviations: CLIF-SOFA = Chronic Liver Failure-Sequential Organ Failure Assessment; FiO2 = fraction of inspired oxygen; HE = hepatic encephalopathy; INR = international normalized ratio; MAP = mean arterial pressure (1/3 systolic blood pressure + 2/3 diastolic blood pressure); PaO2 = partial pressure of oxygen; SpO2 = oxygen saturation.

The shaded area describes criteria for diagnosing organ failure.

a Kidney failure is defined as a serum creatinine level of ≥2.0 or use of renal replacement therapy
b Cerebral function is assessed using West Haven criteria
c Minimal HE corresponds to "No HE" (Grade 0)
d Patients submitted to mechanical ventilation due to HE and not due to a respiratory failure were considered as presenting a cerebral failure (cerebral subscore =3)
e If PaO_2/FiO_2 is not available, SpO_2/FiO_2 can be used as a surrogate

The CLIF-SOFA score includes subscores ranging from 0 to 4 for each of 6 components (liver, kidneys, brain, coagulation, circulation, and lungs), with higher scores indicating more severe organ impairment. Aggregated scores range from 0 to 24 and provide information on overall severity. The text in bold indicates the diagnostic criteria for organ failures.

Sources:

1. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with Acute Decompensation of cirrhosis. Gastroenterology 2013;144:1426–1437.

2. Jalan R, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. J of Hepatol 2014; 61: 1038–1047.

3. Score Calculators | EF CLIF | European Foundation for the study of chronic liver failure

16.0 Adaptive Design Report

Details on the adaptive study design are described in a separate document Adaptive Design Report. This document is managed outside of this SAP.

17.0 Example SAS Code

The example SAS code can be modified as necessary without any update needed in the SAP.

```
/* 1. Imputation under MAR assumption */
/* 1.1 Impute intermittent missing values */
proc mi data=<in_dataset> out=<imp_dataset1> seed=165427 nimpute=m;
    by treatment;
    var screening baseline day2 day3 day4 day5 day6 day7;
    MCMC chain=multiple initial=em nbiter=200 impute=monotone;
run;

/* 1.2 Impute monotone missing values */
proc mi data=<imp_dataset1> out=<imp_dataset2> seed=165427 nimpute=1;
    by _imputation_;
    class treatment;
    var treatment screening baseline day2 day3 day4 day5 day6 day7 <other
variables>;
    monotone regression;
run;

/* 1.3 Perform ANCOVA for each imputed dataset*/
proc mixed data = <imp_dataset2>;
    by _imputation_;
    class treatment;
    model day7 = treatment baseline <other variables> / solution;
    lsmeans treatment / diff = control('SOC') cl;
run;

/* 1.4 Combine statistics*/
proc mianalyze data=<proc mixed output>;
    class treatment;
    modeleffects <value>;
run;
```

```
/* 2. Tipping point approach */
/* 2.1 Impute intermittent missing values, same as in step 1.1 above */
/* 2.2 Imputation of monotone missing values by MNAR and shift parameter in
active treatment group*/
/* Loop over the range of shift parameters 0 to 6 in steps of 0.25 */
proc mi data=<imp_dataset1> out=<imp_dataset2> seed=165427;
    class treatment;
    monotone regression;
    mnar adjust(day7 / shift=&shift adjustobs=(treatment='VS01'));
     var treatment baseline day2 day3 day4 day5 day6 day7;
run;

/* 2.3 Perform ANCOVA for each imputed dataset, same as in step 1.3 above*/
/* 2.4 Combine statistics, same as in step 1.4 above */
/* End loop*/

/*3. Imputation for 90-day mortality endpoint */

/* 3.1 Impute missing values using MCMC approach, including specific
parameters in the imputation model */

proc mi data=<in_dataset> out=<imp_dataset1> seed=165427 nimpute=m;
      class mortality90;
      var treatment BL_CLIF_C_ACLF BL_ACLF_Grade BL_Age sex mortality90;
      MCMC chain=multiple initial=em nbiter=200;
run;

/* 3.2 Perform CMH test for each imputed dataset*/

proc freq data=<imp_dataset1>;
    by _imputation_;
    tables BL_CLIF_C_ACLF*treatment*mortality90/ cmh riskdiff(common
cl=newcombe);
run;

/* 3.3 Wilson-Hilferty transformation to be done for CMH test statistic;
Not needed for risk difference */


/* 3.4 Combine statistics*/
proc mianalyze data=<proc freq output>;
      class treatment;
      modeleffects <value>;
run;
```