Title:	A multi-centre, randomised controlled study, to evaluate the safety and performance of The DIALIVE Liver Dialysis Device (LDD) in alcohol related cirrhosis patients with Acute on Chronic Liver Failure (ACLF) versus standard of care (SOC).
Short Title:	DIALIVE in ACLF
Clinical Investigation Plan Number:	Final Version v7.0 of 10 October 2019
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Sponsor:	Yaqrit Limited
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LIST OF ABBREVIATIONS

ALF	Acute Liver Failure
ACLF	Acute on Chronic Liver Failure
AD	Acute Decompensation
ADE	Adverse Device Effect
AE	Adverse Event
AKI	Acute Kidney Injury
AV	Acceptable Value
BP	Blood Pressure
CA	Competent Authority
CI	Chief Investigator
CIA	Clinical Investigation Agreement
CIP	Clinical Investigation Plan
CRF	Case Report Form
CRRT	Continuous Renal Replacement Therapy
CRO	Contract Research Organisation
CRP	C-Reactive Protein
CT	Computed Tomography
CVS	CardioVascular System
CVVH	Continuous veno-venous haemofiltration
CVVHD	Continuous veno-venous haemodialysis
CVVHDF	Continuous ven-venous haemodiafiltration
CXR	Chest X-ray
DCF	Data Clarification Form
DD	Device Deficiency
DIBD	Developmental International Birth Date
DIC	Disseminated intravascular coagulopathy
DM	Data Manager
DNACPR	Do Not Attempt Cardio-Pulmonary Rescuscitation
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
EAA	Endotoxin Activity Assay

EC	European Commission
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
ER	Essential Requirements
EU	European Union
FBC	Full Blood Count
GCP	Good Clinical Practice
GDPR	General Data Protection Regulations
GI	Gastro-Intestinal
HCC	Hepatocellular Carcinoma
HD	Haemodialysis
HDF	Haemodiafiltration
HDU	High Dependency Unit
HE	Hepatic Encephalopathy
HF	Haemofiltration
HIT	Heparin Induced Thrombocytopaenia
HMA	Human mercaptalbumin
HNA	Human non-mercaptalbumin
IB	Investigator Brochure
ICF	Informed Consent Form
ICU	Intensive Care Unit
INR	International Normalised Ratio
LDD	Liver Dialysis Device
LFT	Liver Function Tests
MAP	Mean Arterial Pressure
MDD	Medical Devices Directive (93/42/EEC)
Mg	milligrams
MHRA	Medicines and Healthcare products Regulatory Agency
mL	milliliters
Mm	millimeters
mmHG	millimeters of Mercury
MRI	Magnetic Resonance Imaging

NCA	National Competent Authority
NHS	National Health Service
NHS R&D	National Health Service Research & Development
NICE	National Institute of Clinical Excellence
PI	Principal Investigator
PIS	Participant Information Sheet
PT	Prothrombin Time
PTT	Partial thromboplastin time
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
REC	Research Ethics Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SBP	Spontaneous Bacterial Peritonitis
SDV	Source Data Verification
sCr	Serum Creatinine
SIV	Site Initiation Visit
SOC	Standard of Care
SOP	Standard Operating Procedure
TMG	Trial Management Group
TV	Target Value
U&Es	Urea and Electrolytes
UADE	Unanticipated Adverse Device Effect
UCL	University College London
UGIB	Upper Gastric Intestinal Bleed
UK	United Kingdom
USADE	Unanticipated Serious Adverse Device Effect

1. OVERALL SYNOPSIS OF THE CLINICAL INVESTIGATION

Title	A multi-centre, randomised controlled study, to evaluate the safety and performance of The DIALIVE Liver Dialysis Device (LDD) in alcohol related cirrhosis patients with Acute on Chronic Liver Failure (ACLF) versus standard of care (SOC).
Study Design	The design is a multi-centre, randomised, controlled, study to generate data for the evaluation of safety (as measured by the percentage of subjects who experience at least one serious adverse event (SAE), and performance (as measured by the lowering of the plasma endotoxin concentrations, and improved albumin function) of the DIALIVE in 30 evaluable patients with Acute on Chronic Liver Failure (ACLF) on the background of alcoholic cirrhosis versus standard of care (SOC).
Hypothesis	The hypothesis is that DIALIVE will significantly improve the prognosis of ACLF patients by modulating systemic inflammation.
Target Population	Men and Women ≥18 years, ≤81yr. Patients with a background of alcoholic cirrhosis who have either: 1. ACLF grade 1, ACLF grade 2 or grade 3a[1] as per CLIF-OF scoring system; OR 2. .Serum bilirubin > 20 mg/dL (342 µmol/L) as single organ failure[2]; OR 3. Stage 1b Acute kidney injury[3]. Treatment will be undertaken in an intensive care (ICU) or renal dialysis unit setting if the patients are randomised to the DIALIVE treatment arm. For patients randomised to the 'Standard of care' arm, the location of treatment (ICU or general ward) will be determined by their clinical need and will be decided by the site Principal Investigator.

[1] ACLF grade 3a patients, i.e. with 3 organ failures only, are included based on a favorable safety report by the Data Safety Monitoring Board following review of safety data on 12 ACLF grade 1 and 2 patients studied so far. ACLF grade 1 patients who have poor prognosis (28 day mortality > 15%) according to the more recent evidence, but were not identified within the original CANONIC study.

[2] Rapidly progressive hyperbilirubinaemia with serum bilirubin rising to > 20 mg/dL (342 µmol/L)in ACLF is associated with high mortality, equivalent to ACLF 1.

[3] Recent data on Acute Kidney Injury (AKI) in decompensated cirrhotics suggest that even milder degrees of renal dysfunction (AKI 1b defined as serum Cr > 1.5 mg/dL or 134 µmol/L) are associated with high mortality. AKI 1b does not constitute 'kidney failure' in the CLIF-OF scoring system.

Inclusion Criteria	• Male or female subjects aged ≥18 years ≤81yr • Subject is able to provide informed consent to participate in the study, otherwise written informed consent must be obtained on behalf of the subject by next of kin or a legal representative in accordance with local ethical and legal requirements. • History indicative of alcohol-related cirrhosis based on clinical, radiological and/or histological evidence. • History of an acute decompensation event (including but not limited to ascites, gastrointestinal bleeding, hepatic encephalopathy and/or acute bacterial infections), occurring within ≤6 weeks of screening. • Subject with : o ACLF Grade 1, 2 or 3a defined per the CLIF-C OF scoring system OR o single hepatic organ failure for serum bilirubin > 20 mg/dL (342 µmol/L) at screening and randomization, OR o AKI-stage 1b (sCr > 1.5 mg/dL or 134 µmol/L). • Where a subject has received corticosteroids as specific therapy for alcohol hepatitis and is deemed unresponsive to steroids after 7 days of treatment (lack of response defined as Lille score of > 0.45 or steroids stopped before 7 days due to any complication such as infection). This refers to the first course of corticosteorid therapy. Further courses of corticosteroids during the course of illness must not be used to determine steroid responsiveness.

Exclusion Criteria	• Co-infection with HIV and AIDS defining illness[4]
	• Subjects with acute or sub-acute liver failure without underlying cirrhosis.
	• Subjects with severe thrombocytopaenia, as defined by the platelet count of <40,000/mm^3 (at screening/randomization) or rapid reduction in platelet count (> 50% reduction) in the previous 24 hrs prior to randomisation (day 0)
	• Subjects with International Normalised Ratio (INR) > 3
	• ACLF 3b patients, i.e. ACLF with more than 3 organ failures.
	• Subjects with cirrhosis who develop decompensation at any time in the post-operative period following partial liver resection or major non-liver surgery.
	• Subjects with uncontrolled infection. Patients may be entered into the study provided antimicrobials have been administered for at least 48 hours with an appropriate response observed prior to randomization.
	• Subjects with respiratory organ failure (as per CLIF-C OF scoring: $PaO_2/FiO_2 \leq$ 200 mmHg or 27 kPa or $SaO_2/FiO_2 \leq 214$).
	• Subjects with haemodynamic instability:
	  ○ i) persistent hypotension (mean arterial pressure < 65 mmHg) with evidence of tissue hypoperfusion, not responsive to volume resuscitation and/or low dose vasopressor support;
	  ○ ii) a norepinephrine dose of > 0.2 µg/kg/min, or a second pressor (terlipressin for variceal haemorrhage and/or hepato-renal syndrome does not count as pressor, unless it is specifically used to treat systemic hypotension) at screening or randomization. Patients can be reconsidered for study inclusion after at least a 24 hour period of norepinephrine requirement < 0.2 µg/kg/min.
	• Subjects not considered appropriate for full active treatment including organ support or those with a Do Not Attempt Cardio-Pulmonary Resuscitation order (DNACPR).
	• Subjects with active, or with a history of non hepatic malignancy unless adequately treated or in complete remission for five or more years.
	• Patients with HCC outside Milan criteria.
	• Significant systemic or major illness other than liver disease, including coronary artery disease, cerebrovascular disease, pulmonary disease, renal failure, serious psychiatric disease, that, in the opinion of the Investigator would preclude the subject from participating in and completing the study.
	• Subjects who have received any investigational drug or device within 30 days of dosing or who is scheduled to receive another investigational drug or device in the course of the study; concomitant observational studies are allowed.
	• Evidence of unncontrolled seizures.
	• Subjects diagnosed with Creutzfeldt-Jakob disease.
	• In females: known pregnancy or lactating.
	• Subjects weighing less than 30 kg (as per contra-indication of oXiris and septeX)
	• Where subjects present with a known allergy to heparin or have type II thrombocytopaenia caused by heparin (HIT syndrome type II).
	• In the opinion of the investigator, it is unsafe for the patient to be considered for the study.

[4] A patient can be HIV +ve as long as they are clinically stable and **not presenting** with any AIDS defining illness i.e.
a CD4+ T-cell count below 200 cells/µL
a CD4+ T-cell percentage of total lymphocytes of less than 15%
or one of the defining illnesses such as PCP, Kaposi's sarcoma, CMV, Candidiasis etc

	Objectives	Outcomes Measures
Primary End Point	To evaluate the safety of the DIALIVE in patients with Acute on chronic liver failure (ACLF)	The percentage of subjects who experience at least one (1) serious adverse event (SAE) between study Day 1 and Day 10; especially the incidence rate of SAE in both arms of the study at Day 1 and Day 10. The percentage of subjects who discontinued DIALIVE due to a serious adverse device event (SADE) between Day 1 (first day of treatment) and Day 10 (DIALIVE arm only).
Secondary End Point(s)	To evaluate the impact of biochemical performance outlined hereunder towards clinical benefit for patients receiving DIALIVE treatment plus Standard of Care vs Standard of Care alone	• Mortality: 28-days and 3 months • Change in ACLF Grade • Change in CLIF-ACLF score • Improvement in individual organ function: brain (HE), kidney (serum creatinine), CVS (mean arterial pressure), pulmonary (P/F ratio or S/F if arterial blood gas info is not available), liver (serum bilirubin) and coagulation (INR and platelet levels). • Status of ICU and Hospital Discharge (including discharge to a hospice for palliative care). • Lengths of stay in ICU and hospital at D28 and D90. • ICU and hospital re-admissions with another episode of ACLF up to 90 days post randomization.

Secondary end Point(s)	Objectives	Outcomes Measures
	To evaluate the performance of the DIALIVE in patients with ACLF	• Change in Plasma endotoxin level (endotoxin activity and endotoxin concentration) between; 1. Study D1,D3, D5 and D10 across SOC arm 2. Study D1, D3, D5 and D10 across DIALIVE arm 3. Start and end of a treatment session with DIALIVE on D1, D2 and D3 4. Study D1, D3, D5 and D10 between SOC and DIALIVE arms for patients with the same grade of ACLF at the time of inclusion in the study Target-Value (TV): 40% reduction; Acceptable-Value (AV): 20% reduction Change in Albumin function (Human non-mercapt albumin -2 (HNA-2) / Human marcapt albumin (HMA) ratio: 5. Study D1, D3, D5 and D10 across SOC arm 6. Study D1, D3, D5 and D10 across DIALIVE arm 7. Start and end of a treatment session with DIALIVE on D1, D2 and D3. 8. Study D1, D5 and D10 between SOC and DIALIVE arms for patients presenting with the same grades of ACLF TV: 40% reduction; AV: 20% reduction

Exploratory end points	**Objectives**	**Outcomes Measures**
	To study other exploratory biological and clinical effects of the DIALIVE in patients with ACLF compared with the Standard of Care group.Some of these parameters are centre-specific in line with local expertise, research interest and the capacity to perform these tests.	**Organ Function and Biomarkers** • Liver: Changes in MELD score, plasma/serum cCK18/M30 and flCK18/M65 (markers of liver cell death). • Kidney: Changes in serum creatinine and urinary NGAL (marker of kidney injury). • Brain: West Haven Criteria to assess changes in severity of hepatic encephalopathy. • Immune function: Incidence of Infection. Changes in white cell count, and assessment of neutrophil and monocyte function. • Assessment of Coagulation and haemostasis: Incidence of thrombotic or bleeding complications, assessment of pro- and anti-coagulant clotting factors and complement activation.

Screening/ Baseline Assessments	Screening assessments will commence following informed consent. See schedule of assessments.
Additional Study Assessments (per schedule of events)	The treatment phase will occur within the window period of 10 days to achieve one successful treatment cycle with DIALIVE. See schedule of assessments.

Sample size/Statistical Analysis	30 evaluable subjects will be randomized to receive either standard of care (SOC) or treatment with DIALIVE (1:1 randomisation). All statistical analyses will be carried out on the safety population, defined as the subset of randomized patients who receive at least one treatment[5]. No imputation of missing values will be implemented. Only available data will be analysed. In addition, a sensitivity analysis will be carried out to check the main safety results using SAE-data of all evaluable patients No statistical tests will be performed. All final statistical analyses will be descriptive. The categorical variables will be summarised by means of the count and percentages corresponding to each variable category, while continuous variable will be summarized by the mean and Standard Deviation of each distribution or the median and min-max values in case of those variables non-normally distributed. For each treatment arm, treatment-emergent AE's, SAEs, SADEs, ADEs, USADEs and DDs will be summarised by organ system and treatment causality. The number and percentage of patients discontinuing the study due to Aes, SAEs, SADEs or other stopping criteria confirmed by the Data Safety Monitoring Board (DSMB) will be summarized for each arm. Secondary and exploratory parameters (changes from baseline at each study visit) will be descriptively analysed by treatment arm by means of the appropriate statistics. Statistical analyses of safety data will be run when each study cohort is completed and the corresponding results will be communicated to the DSMB. Data extraction at interim time points do not serve study analyses.
Number of Visits	Patients will undergo the following Screening assessment (up to 72hrs)[6] Study Day 0-randomisation (DIALIVE treatment may commence on this day if all day 1 parameters have been met) Study Day 1 assessments[7] ***Study Days 1-10 treatment phase commences and completes*** Study Day 5 assessments Study Day 10 assessments Study Day 14 assessments Study Day 28 assessments Study Day 90 assessments

[5] One treatment will be defined as ***any*** duration of exposure to the DIALIVE.

[6] Screening period (post consent) has been set up to 72 hours to allow sufficient time to gather all relevant clinical information for the subject to be eligible to progress to randomisation. However, if this information becomes available sooner, then randomisation can proceed after that time (without having to wait for 72 hours). For further clarity, the plan for gathering the most recent and relevant clinical information, yet not delaying the start of the study is as follows:

a. Thorough clinical examination of the patient and assessment of FBC, U&E, LFT, Coagulation must be repeated during this period. If randomised to the DIALIVE arm FBC, U&E, LFT, Coagulation results from the screening period may be used if performed up to 24 hours prior to starting treatment unless patients' clinical status has changed.
b. Microbiology results from samples sent in and up to the previous 72 hours (at commencement of screening) need not be repeated. On the day of screening, if the patient is not septic clinically as determined by the PI, then consent can proceed without having to wait for microbiology results. Likewise, if a patient is deemed to be septic based on clinical grounds without positive microbiological cultures, the PI will determine if it is safe to continue with consent and randomization, and this will be reviewed daily.
c. Viral serology results from up to 8 weeks before the screening period will be accepted

Per Patient Duration/Total Trial Duration	3 months per patient /39 months for completion of the study. Proposed start Feb 2017. Proposed finish Dec 2019
Applicable legislation	National and/or local legislation is application, including the new GDPR regulation, and especially in view of the inclusion of patients who might become or are unconscious and on the involvement of a legal representatives – if appropriate/allowed.
Legal Represenative	The Legal Representative, appointed by the sponsor YAQRIT Ltd, is: FAKKEL-bvba Mr Jaak Minten Groenendael 43/001 3400 Landen Belgium

d. Liver imaging such as liver ultrasound, doppler studies or CT/MRI scan up to 8 weeks prior will be acceptable unless there has been a significant change in the clinical status dictating repeat imaging.

*Some or all of the above tests may need to be repeated during the screening period should there be any significant change in the clinical status of the patient as assessed by the clinical team.

[7] May concur with study Day 0 if all assessment parameters (as per the schedule of assessments) have been met. DIALIVE treatment must commence before the end of study day 1.

2. IDENTIFICATION AND DESCRIPTION OF THE INVESTIGATIONAL DEVICE

2.1 Summary description of the investigational device and its intended purpose.

The device under investigation is the DIALIVE Liver Dialysis Device (LDD) comprising of the Baxter (previously Gambro®) SepteX™ Hamodialyser, the Gambro® oXiris Haemodialyser. The novelty of the DIALIVE lies in the synergistic effect of using commercially available filters on a haemofiltration platform (Prismaflex) to control and monitor the *ex-vivo* circulation of subject blood. The LDD acts as one unit that removes dysfunctional albumin and endotoxins, infuses fresh functional albumin and specifically targets systemic inflammation. The Prismaflex is a standard dialysis pump system with a software driven control unit. As per the Instructions for Use of septeX and oXiris, a minimum version 4.0 of the software is to be installed[8].

The available current knowledge and the evidence of the role of albumin and of endotoxins in the pathophysiology of liver failure have led to the formulation of a hypothesis that albumin removal and replacement, in conjunction with endotoxin removal may be beneficial in the treatment of subjects with liver failure.

It is predicted that DIALIVE will reduce the death rate of hospitalised ACLF patients, allow intensive care ACLF patients to return to the ward and home and reduce readmission rates to ICU and the hospital. In those patients that do not recover, DIALIVE will bridge them to liver transplantation. Thus DIALIVE will improve both mortality and quality of life (QOL).

All components of the system are commercially available and individually CE Marked for haemofiltration applications. The filters will be purchased directly from Baxter.

Replacement human serum albumin is administered during each treatment session at a rate of 5 g/hr of dialysis. The amount of replacement albumin to match the amount removed by the DIALIVE is extrapolated through results from previous animal studies and the in-vitro study of albumin clearance by the device. This equates to albumin replacement of 40 grams for an 8-hour and 60 grams for a 12- hour completed treatment session. The aim is to maintain post-treatment serum albumin level as close to the baseline as possible. The re-infused fresh albumin will be administered through the Hepalbin™ filter (Albutec GmbH). Albumin for other indications should occur as per standard practice in both SOC and DIALIVE arms.

The Hepalbin™-Adsorbent is a nano-structured carbon filter that removes up to 95% of the industrial stabilisers octonoic acid (caprylate) and N-acetyltryptophanate from commercially available pharmaceutical albumin preparations. These stabilisers are associated with the binding sites of albumin, thus reducing albumin functionality and binding capacity. While in healthy individuals, these stabilisers are metabolized by the liver and have a very short half-life, this is significantly prolonged in liver disease potentially causing accumulation of these materials. The use of Hepalbin™-Adsorbent for albumin transfusion indicated that there may be clinical benefit to stabilizer removal in patients with decompensated liver disease in a small controlled study [16].

[8] In the majority of hospitals most recent versions of both Prismaflex and software are available. In Austria the Model ref: 60 23014700 can be in used in Graz; the software version installed is version 8.1
SepteX and oXiris filters are the brandnames of the filters and have no further model specification.

2.2 Definitions.

Screen failures:

1. The patient does not meet one or more criteria, which are assessed after the patient (and/or consultee if applicable) signed the Informed Consent; patient can re-enter the study later if at that moment all inclusion criteria and no exclusion criteria are met. Patient informed consent remains valid until end of the study or until withdrawal from the study.
2. Patient who did not start dialysis before the end of DAY 1 if randomized to the DIALIVE treatment arm, is considered a screen failure; such a patient does not have to resign a consent form, but can re-enter the study via de novo demonstration of compliance with the in- and exclusion criteria.

Patient withdrawal: a patient (or the consultee if applicable) can withdraw consent at any time. No further clinical data can be collected as of that moment; the data collected prior to that moment, remain part of the clinical study.

Patient exclusion: a patient is considered excluded from the study (and from re-entering into the study):

1. Upon demonstration of more than 2 interuptions of more than 24 hrs between subsequent dialysis sessions and within the first 10 day time period after randomization (DIALIVE arm);
2. If the clinical condition of a patient meets one or more study stopping criteria.

Patients considered to be a 'screen failure' or 'excluded' will remain in study for a minimum follow up period of 28 days.

Standard definitions: definitions for DIALIVE organ failure and ACLF grading per the CLIF-C OF scoring system, Lille score, and others, are given in Appendix 1-2.

Hepatic Encephalopathy (HE) : determination of HE as part of ACLF grading will be based upon clinical assessment and graded as per the West Haven criteria. However, if there is ambiguity or observer variability, an EEG may be sought by the PI for supportive evidence. This is particularly relevant for grade 1 HE which may not be consistenly reproducible using the West Haven criteria, and as such is now included as part of the covert HE spectrum along with minimal HE often requiring psychometric tests or EEG for diagnosis

Specific therapy such as corticosteroids for alcoholic hepatitis can be used if a patient is already within the study and these have not been trialed previously and as long as clinical justification and rationale is clearly documented.This applies to patients in both SOC and the DIALIVE arm. For other indications of steroid use such as potential relative adrenal insufficiency, it should precede a short synacthen test, particularly in patients who are haemodynamically stable, to establish this diagnosis.

Standard of Care (SOC) is defined as the standard medical and nursing management of patients with ACLF Grades 1 and 2 as per local practices. Medical intervention is to be guided by the treating physician taking into account the overall clinical picture, precipitating event, and the nature and severity of organ dysfunction. The interventions may include (but not limited to) the supportive management with fluid therapy, including albumin therapy where indicated, antibiotics for suspected or confirmed infections, nutrition, bowel management, thromboprohylaxis, and organ system support including the use of terlipressin as splanchnic vasopressor and for cardiovascular support where indicated, oxygen and non-invasive respiratory support, laxatives and non-absorbable antibiotics for hepatic encephalopathy, and dialytic therapy for renal failure. SOC may also include urgent liver transplantation as per local practices.

Patients presenting with, or developing during the study period, severe actue renal failure requiring renal support, will receive continuous renal replacement therapy (CRRT) as per standard local practices. This would apply to subjects in both SOC and DIALIVE arms. For those in the DIALIVE arm, the same vascular catheter which is used for DIALIVE treatment may be used for CRRT following the completion of the DIALIVE treatment session. It is however envisaged

that increased intensity of dialysis component in the DIALIVE system (from 1lit/hr to 1.5lit/hr) and allowance for fluid removal in the new protocol may delay or remove altogether the need for CRRTin these situations. The decision for additional CRRT would depend on clinical situation and will be taken by the site PI.

DIALIVE arm consists of standard of care (defined above), plus DIALIVE treatment.

A DIALIVE treatment session denotes treatment for the day, whereas A DIALIVE treatment cycle refers to the total number of treatment days.

A DIALIVE treatment **session** = A minimum, cumulative duration of 8 to 10 hrs (± 30 min) of the DIALIVE treatment, thus allowing for breaks which may be planned (for example patients needing to go for imaging) or unplanned (device clotting, issues with vascular access or other any other unplanned reasons). A break within a treatment session (consisting of a cumulative time longer than 4hrs), or a cumulative duration of less than 8 hrs of dialysis during one day constitutes a session failure for that day. A session failure will require to restart the dialysis session within 24 hrs after the end of the previous session and will reset the start of the dialysis cycle.

A DIALIVE treatment **cycle**= Consists of 3 consecutive days of 8-10 hrs of DIALIVE treatment sessions on each day within the 10 day window period. A break of more than 24hrs between each treatment session will require a new baseline (i.e. a new treatment cycle commenced). A second failed treatment session within the 10 day window period would result in patient exlusion.The patient must have completed one successful treatment cycle within the 10 day window period to be considered evaluable.

Day of randomization constitutes study Day 0.

The first treatment session (DIALIVE arm only) must commence before the end of study Day 1. As such, dialysis might also start on the day of randomization which might created confusion as 'the first day of dialysis' then concurs with DAY 0 of the study schedule.

Commencement of DIALIVE treatment session i.e. connection of patient to the Prismaflex machine = time point 0, can occur at any time before the end of study Day 1 subject to availability of all relevant information required for treatment to commence. All blood samples should be measured from this time point. Any pre samples required should be taken just before connection.

Nuts and bolts of DIALIVE Treatment: a ready reference

-*Before starting treatment*

The patient to be moved to an intensive care unit or similar once randomised to DIALIVE.
Appropriate monitoring needs to be instituted including the following:

- Insertion of an arterial line for continuous invasive blood pressure monitoring and blood sampling
- Insertion of a vascular catheter for DIALIVE treatment
- Insertion of a central venous catheter for prompt treatment with vasopressors if required

(Lines should be inserted under USS guidance. Correction of clotting abnormalities, if any, should follow local protocols. The line positions should be confirmed through appropriate means; e.g. CXR for neck lines).
* NB: Majority of ACLF grade 3 patients are likely to be already in the ICU with the above monitoring in situ.

Additional tests (Baseline samples for FBC, U/E, LFT, coagulation, septic screen will already have been collected to check eligibility) prior to commencing DIALIVE therapy will be done:

- Arterial blood gas analysis

- Haemolysis screen (FBC including reticulocyte count, blood film, unconjugated and conjugated bilirubin, LDH, haptoglobin, urinary free haemoglobin) – to be perfomed only on D1, before and after therapy
- DIC screen, if clinically indicated
- And, Endotoxin Activity Assay (EAA) analysis (start of day 1, 2 and 3)
- Blood to be collected as part of biobank samples.(start of day 1, 2 and 3)

Fluid pre-loading of up to 500mls over 1-2 hours is implemented, the volume and speed of fluid therapy to be determined on a clinical basis, and the choice of fluid would preferably be albumin (4.5 or 5% solution as per local availability).

- *during dialysis session*

- Anticoagulation with unfractionated heparin and/or prostacycline analogues (the dose as per local protocols)

- Blood flow rates on DIALIVE device to be started at 50 mls/min and increased gradually over 1-2 hours to up to a maximum of 250 mls/min

- Replacement fluid (pre-dilution solution) to start at a rate of 1000 ml/hr and increased gradually to up to 3500 mls/hr (50mls/kg/hr for a 75 kg person) subject to haemodynamic stability. Concomitant dialysis component to run at 1500 mls/hr (25 ml/kg/hr for a 75 kg person).

- Albumin is exchanged simultaneously with DIALIVE treatment. Infusion of fresh albumin will be through the HepAlbin filter at a rate of 5 g/hr (to replace albumin removed by the device). This equates to an infusion of 20% human albumin solution at a rate of 25mls/hr.

- Mean arterial pressure is to be continuously monitored, and MAP is maintained at 90% or higher of pre-treatment MAP or above 65 mmHg, whichever is higher.

- 2-hourly blood gas analysis (including lactate), and 4 hourly other bloods (FBC, U/E, LFT, coagulation) at 0, 4 and 8-12 hrs (end of treatment) should be performed.

- A senior staff member will closely supervise the dialysis procedure during the first few hours of treatment, with a clear internatl escalation plan available, and discuss with study CI where necessary

- Effluent of dialysis fluid from the effluent line AND waste collection bag to be collected a 0-1, 4, 8-10 hrs (end of treatment) as part of biobank samples.

- *Following completion of treatment session* :

- Blood within the circuit is to be returned back to the patient, unless the filter is clotted.

- Repeat Haemolysis screen (at end of session of D1 only)

- EAA-analysis (end of days 1, 2 and 3)

- Ensure treatment data is stored in the device and retrieved at a later date

- Blood to be collected as part of biobank samples. (end of days 1, 2, 3)

2.3 Details concerning the manufacturer and traceability of the investigational device.

Membrane[9]:		**septeX™ Haemofilter**		**oXiris® Haemofilter**
Manufacturer:		Baxter, USA		Baxter, USA
Indications:		Continuous renal replacement therapy for subjects with acute renal failure		For subjects who have acute renal failure, fluid overload or both.
Intended use:		Continuous veno-venous haemodialysis (CVVHD) in conditions where the removal of plasma components with molecular weights up to a value of 45 kDa, such as substances that mediate inflammation, is indicated		Veno-venous therapies: slow continuous ultrafiltration (SCUF); continuous veno-venous haemofiltration (CVVH); continuous veno-venous haemodialysis (CVVHD); continuous veno-venous haemodiafiltration (CVVHDF)
Contraindications:		There are no contraindications to CVVHD with high cut-off membrane		Where subjects present with a known allergy to heparin or have type II thrombocytopaenia caused by heparin (HIT syndrome type II)
MDD Classification:		IIb		III (incorporates heparin)

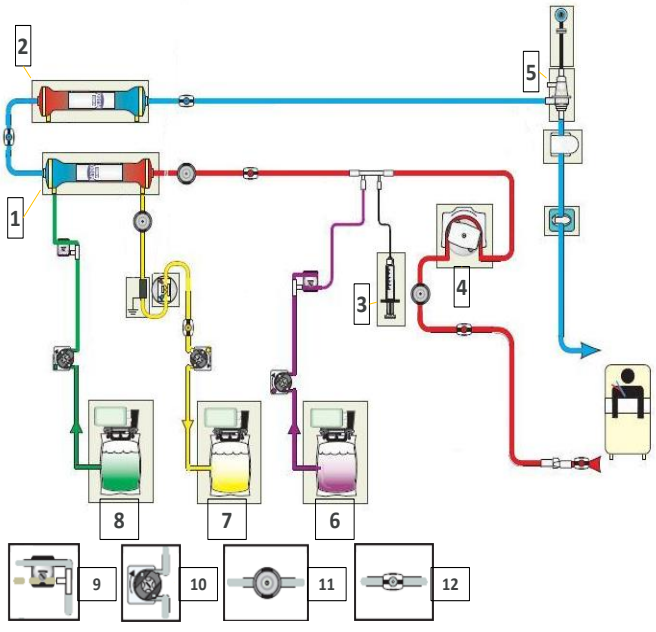
[9] Both the septeX and oXiris filters are brandnames from Gambro; products are not further specified with model number and are distributed by Baxter as commercial devices to support dialysis in kidney failure.

2.4 Device materials that will be in contact with tissues or body fluids

Membrane		septeX™ Haemofilter		oXiris® Haemofilter
Housing		Polycarbonate		Polycarbonate
Potting compound		Polyurethane		Polyurethane
Tubing		Plasticized polyvinyl chloride		Plasticized PVC
Cartridge plate		Polyethylene terephthalate glycol-modified (PETG)		PETG

Fig 1. DIALIVE Liver Dialysis Device (LDD) as incorporated into the extracorporeal circuit. Filter set 1 + 2 + interconnecting tubing constitutes the DIALIVE unit, rest is standard dialysis equipment

1. Gambro Septex – Albumin Removal (Allocated "FILTER SET 1")
2. Gambro oXiris– Bacterial Endotoxin Removal (Allocated "FILTER SET 2")
3. Heparin Syringe pump
4. Blood pump
5. Bubble trap
6. Pre-Diluter Solution bag
7. Effluent bag
8. Dialysate bag
9. Safety valve
10. Pump
11. Pressure sensor
12. Sampling site



DIALIVE

Albumin replacement during DIALIVE session via the Hepalbin™ filter

Extracorporeal Support Equipment	Regulatory Status
Prismaflex Monitor	CE 0086
Extension Tubing	CE 0086
Accessories	CE 0086

2.5 Summary of the necessary training and experience needed to use the investigational device.

The main end users of the LDD are expected to be the Hepatologists, Intensive care physicians, Renal physicians and the Liver transplant surgeons, all of whom have extensive experience in using dialysis and haemofiltration equipment. Training on the DIALIVE device set up will be provided to each site by Yaqrit (sponsor) who has experience in the DIALIVE delivery. The set up and Instructions For Use (IFU) are outlined in the Investigator's Brochure and a separate IFU and will be provided to each site.

2.6 Description of the specific medical or surgical procedures involved in the use of the investigational device.

Patients randomised to receive DIALIVE liver dialysis treatment will be treated in a high dependency setting (HDU), or Intensive Care Unit (ICU) to ensure provision of expertise in the use of the Liver Dialysis Device and provision of continuous monitoring whilst the patient is receiving DIALIVE liver dialysis treatment. Please see § 2.2. Definitions.

2.7 Current Standard of Care and Need for Improvement

Two-hundred and sixty epidemiological studies from 8 different European countries between 2008 and 2013 [1] estimated that liver cirrhosis affected 0.1% of the total EU population with an incidence of 14- 26 new cases per 100,000 inhabitants and about 170,000 deaths per year [1]. Although the overall mortality rate for liver cirrhosis slightly declined in Europe (as well as in USA) from 1980 to 2010 due to better management and control of liver diseases and alcohol consumption; it increased in Eastern European countries, the United Kingdom and Ireland [2]. The only available treatment for cirrhosis and liver failure patients is liver transplantation, and with the shortage of donors world-wide about 1 million patients die from liver failure each year.

The number of liver transplants performed each year is limited by organ availability and over the past 15 years, the number of transplants in Europe has plateaued at about 6000/year (European Liver Transplant registry; ELTR.org). A recent analysis of the Eurotransplant data of over 16,000 patients listed for liver transplantation in Europe, 27.8% died while waiting for a suitable organ for transplantation become available [3]. Consequently, there is a clear need to find regimes which can be used to maintain and improve the condition of such patients requiring transplant [4].

Liver transplantation is the only treatment for patients with liver failure and an alternative to this remains an unmet need. In Acute Liver Failure (ALF), the ideal LDD would replace the functions of the failing liver in order to permit spontaneous recovery as the liver's powerful regenerative potential obviates the need for transplantation. In patients with Acute on Chronic Liver Failure (ACLF), an LDD would ideally support hepatic function during a period of acute deterioration until spontaneous recovery from the episode of decompensation. It would allow time for drugs to address the precipitating event or the underlying the liver disease. In patients with decompensated cirrhosis, a LDD could support hepatic function until liver transplantation. In addition, LDDs may have potential to treat the multi-organ failure that accompanies liver failure including hepatic encephalopathy, renal failure and immune dysfunction.

Acute decompensation (AD) or cirrhosis complicated with organ failure (ACLF) has a mortality rate of 30-40% and death is mostly related to multiple organ dysfunction [5]. The condition is widespread, affecting about 30% of all hospitalised cirrhotic patients and has a 28-day mortality of about 30% [6, 7]. Furthermore, ACLF occurs in young patients (mean age 52 years) and about 50% have no knowledge of their underlying cirrhosis. Indeed, in about 40% patients, no precipitating event can be identified. A prospective observational study of hospitalized patients with cirrhosis in 2013 by the European Association for the Study of the Liver-Chronic liver failure (EASL-CLIF) Consortium, the EASL-CLIF Acute-on-Chronic Liver Failure in Cirrhosis (CANONIC), the largest and most comprehensive registry on ACLF to date, showed a 28 day

mortality of 22%, 32% and 78% for ACLF grades 1, 2 and 3, respectively. [6]. The diagnosis of ACLF is made using the CLIF Consortium organ failure scores (CLIF-C OFs), the prognosis of these patients using the CLIF Consortium ACLF score (CLIF- C ACLF), and ACLF severity grades defined using the CLIF-OF organ failure score [8], In the CANONIC study two organs, the kidneys and the brain, were found to be of particular significance in determining short term mortality. More recent studies suggest that even milder degrees of renal impairment in cirrhosis, not in the 'failure' range of the CLIF-OF scoring system, may be associated with development of ACLF and significant mortality. This refers to the subgroup(1b) of stage 1 AKI defined by a serum Creatinine (sCr) at presentation of above 1.5 mg/dL [9]. As for brain dysfunction, mild hepatic encephalopathy (HE) particularly grade 1 HE may not be consistently reproducible using the West Haven criteria, and as such is now included as part of the covert HE spectrum along with minimal HE [10] often requiring psychometric tests or EEG for diagnosis.

Additioally, post-hoc analysis of CANONIC data has identified rapidly progressive hyperbilirubinaemia with a serum bilirubin level greater than 20 mg/dL at the time of presentation as a single hepatic organ failure being associated with poor prognosis. This criterion was also used in the RELIEF study, the only randomized controlled trial to date of MARS therapy in ACLF [11].

2.8 Rationale for utilising DIALIVE device for ACLF patients

The novelty of DIALIVE is that it incorporates albumin removal (and replacement) and endotoxin removal, which has not been applied before into one device. DIALIVE has powerful and selective toxin elimination (using an endotoxin-specific filter adsorber, oXiris®, and replacement of defective albumin (SepTex™ filter plus fresh albumin reinfusion) functions while preserving other important plasma proteins. The two filters used are already CE-marked and have been on the market since 2010 to treat sepsis, Acute Kidney Injury (AKI) and Continuous Renal Replacement Therapy (CRRT).

The currently available Liver Dialysis Devices (LDDs) are based on the principal of removal of protein bound and water soluble substances (blood purification) by albumin dialysis, by plasma separation and filtration or by therapeutic plasma exchange. Devices based solely on the removal of water soluble substances (blood detoxification) have not shown any benefit in survival, possibly because of the limited, non-specific absorptive capacity of chemical adsorbents. Recent clinical trials of MARS [11] and Prometheus [12], devices based on the principles of albumin dialysis, in patients with ACLF failed to demonstrate a survival benefit compared to standard medical treatment. In these clinical trials the oxidized fractions of human mercaptalbumin (HMA, non-oxidized), human nonmercaptalbumin-1 (HNA1, reversibly oxidized) and human nonmercaptalbumin- 2 (HNA2, irreversibly oxidized), were markedly increased. Both MARS and Prometheus treatments resulted in a shift of HNA1 to HMA while HNA2 was not significantly affected. This shift in albumin fraction was transient and disappeared within 24 hours after treatment. There were no significant differences between MARS and Prometheus treatments with respect to the redox state of albumin.

From the pathophysiological perspective, ACLF is characterised by systemic inflammation and endotoxaemia. The severity of neutrophil dysfunction has also been shown to correlate with the risk of death of ACLF patients [13]. The mechanism of this immune failure is unclear but studies have clearly demonstrated that the defects in neutrophil function can be reversed if the patient's neutrophil is incubated with normal plasma. In addition, incubation of normal neutrophils with patient plasma makes them dysfunctional, assuming the patient phenotype [13, 14]. These observations suggest that the plasma can transmit the neutrophil functional defect. Further studies showed that this substance in plasma was an 'endotoxin- like substance' suggesting that removing this from the plasma could prevent immune failure and possibly consequent infection in ACLF patients. This rapidly evolving cycle of inflammation / hepatocyte cell death / immune failure / exacerbation of liver failure is referred to as the ACLF spiral. The data support the hypothesis that targeting inflammation may prevent cell death, and endotoxin removal may prevent immune failure thereby reducing the risk of infection and

mortality.

DIALIVE targets the ACLF spiral with a combination of albumin removal and replacement and, endotoxin removal; thereby reducing systemic inflammation. The results of these studies led to the hypothesis that a combination of albumin removal and replacement and, endotoxin removal may be useful to treat patients with liver failure. In both cases, achieving an improvement by 20% would result in improved survival.

Importantly, all current LDDs have limited effect on inflammation and do not restore albumin function. DIALIVE addresses these deficiencies and replaces irreversibly destroyed albumin and removes endotoxin.

The success of the DIALIVE observed in animal models is related to its ability to efficiently remove endotoxin and irreversibly oxidized albumin (human non-mercapt albumin-2; HNA-A2). Thus the combination of endotoxin and damaged-albumin removal followed by fresh albumin infusion improved liver function and mostly importantly markedly increased the survival of pigs.

2.9 Findings of pre clinical work

Pre-clinical data (animal)
Two animal studies were performed (n=29 pigs); 13 in study 1 and 16 in study 2 in clinically-relevant, fully monitored pigs with paracetamol-induced acute liver failure that were managed in an intensive care unit setting with full supportive therapy to generate proof of concept data. This model was chosen because it exhibits the key pathophysiological features of ACLF [15]
a. Multiorgan dysfunction manifested by severe encephalopathy, renal failure and consequent mortality.
b. Biochemical evidence of liver injury, coagulopathy and lactatemia.
c. The classical pathophysiological factors the DIALIVE device is designed to address and is found in patients with liver failure:
(i) albumin dysfunction
(ii) endotoxemia and consequent neutrophil dysfunction

Animal Study 1: [Funded by DoH, HTD-420]: Performed in Tubingen, Germany. N=13 pigs were randomised to standard of care or device treatment, using an early version of DIALIVE (using non-CE marked filters). The results showed safety and biocompatibility of the device with significant improvement in the key variables such as the severity of endotoxemia, albumin and neutrophil dysfunction. Difference in survival between the two groups using a log-rank test was, p=0.02. The hazard ratio (Control:Device) was 5.45 (95% CI 1.1-26.4). This device however, will not be CE marked and it was thought that the results were too variable for translation into man. Therefore, additional studies were performed using an improved device design, which can be directly translated into man (*Animal Study 2, see below*).

Animal Study 2: [Funded by MRC-DPFS G09221]: Was performed using the current version of the device to be CE marked. In the first part, the data showed that an improved biological and clinical effect with the use of both filters together, producing a synergistic benefit. In the second part, n=16 pigs were studied in a randomised, blinded clinical trial to receive standard of care or treatment with DIALIVE [5].

Briefly, the results showed:
a. Safety, biocompatibility and ease of use with a set up time of less than 2 hours.
b. Difference in survival between the two groups using a log-rank test was, p=0.02. The hazard ratio (Control:Device) was 3.81 (95% CI 1.2-12.4).
c. Improved clinical condition: The animals required significantly less fluid support and had better respiratory function in the DIALIVE group.
d. The severity of endotoxemia, neutrophil and albumin dysfunction was attenuated in the DIALIVE animals.

The optimized DIALIVE used in animal study 2 was easy to use with a set-up time of 1.6-2 hours. No device failures were observed, and circuit clotting requiring change of filter was also not observed. Device related complications such as embolism, bleeding or DIC were not observed in any of the animals studied. The vascular catheters used were the same as that used in humans for haemofiltration, and no device related issues were observed.

The results of the above animal studies demonstrated that DIALIVE was safe and increased survival of the pigs treated with the device compared with untreated controls.

Pre-clinical data (in-vitro study)

A further laboratory based study was devised to investigate and quantify the removal of a range of biological markers, including endotoxin, from whole blood *in vitro* and when using the DIALIVE system as configured in the clinical trial, i.e. oXirs plus septeX plus connecting tubing mounted onto the Prismaflex. This preliminary data will be used to support the continuing European multi-center, first into man, clinical investigation, which is currently underway. The methods, results and conclusions are listed in the IB v 3.0. The main results and conclusions from the *in vitro* tests are:

- The system demonstrated effective clearance of small molecules as expected;
- The progressive removal over time of albumin across the filters was demonstrated: total albumin removal amounted to 6.5g/L, or the equivalent of 1.1g/L/hr.
- Consistent removal of endotoxin was demonstrated using the chromogenic LAL assay.

We feel the results seen from the previous animal studies and the results obtained from the recent *in vitro* study, continues to support the hypothesis for the ongoing first into man clinical investigation which is currently underway.

3 RISKS AND BENEFITS OF THE INVESTIGATIONAL DEVICE AND CLINICAL INVESTIGATION

3.1 Anticipated clinical benefits

It is anticipated and hypothesised that a clinically effective Liver Dialysis Device (LDD) could reduce deaths from liver failure, allow earlier discharge from intensive care and in a proportion of patients, prolong survival without transplantation.

Patient groups that could benefit from the device include:

- Patients with ALF and ACLF: preventing need for transplantation or serve as a bridge to transplantation in ALF, and aiding recovery to pre-event level of liver function in ACLF;
- Patients with decompensated cirrhosis who are waiting for a transplant: preventing clinical deterioration while on a waiting list thereby reducing mortality;
- Patients with decompensated cirrhosis not considered suitable for transplantation: long term dialysis to improve survival;
- Patients with intractable pruritus and/or fatigue: improve quality of life and prevent need for transplantation.

The following patients may also benefit:

- Multiple organ dysfunction in intensive care – almost all patients with critical illness have endotoxemia which contributes to inflammation;

- Alzheimer's disease and stroke – albumin is dysfunctional in these conditions where plasmapheresis has been found to be beneficial;

- Intoxication from protein bound substances – protein bound toxins such as drugs and biological weapons may be treatable with albumin exchange;

- Cholestatic syndromes: such as porphyria, in-born errors of metabolism, drug related cholestasis and graft versus host disease;

- Severe iron accumulation – treatment of heart failure due to iron overload as albumin can bind iron.

3.2 Anticipated adverse device effects associated with DIALIVE

The procedural and device risks associated with the indicated use of the septeX™ and oXiris® filters in summary are:

Risk	Mitigation
Blood loss, e.g. leading to organ failure – exsanguination from extracorporeal blood circuit – exsanguination via haemofilter to dialysing fluid circuit – coagulopathy associated with extracorporeal therapies	- CVVHD/HF/HDF via CVC is long established - septeX™ sets are indicated for CVVHD, and cytokine removal - oXiris® sets are indicated for CVVHDF, and endotoxin adsorption and cytokine adsorption - UCL studies of LDD in a porcine PALF model (series septeX™ and oXiris® using Prismaflex platform) showed experimental arrangement to be compatible, biocompatible and safe - CIP specifies ICU/HDU monitoring particularly monitoring: -liver function -cardiovascular function -cerebral function -renal function -pulmonary function -albumin and immune function
Air embolism, eg leading to ischaemia, congestion, metabolic dysfunction, reperfusion injury *et sequelae*: – air introduced via extracorporeal blood circuit – air introduced via haemofilter membrane from dialysing fluid circuit – air introduced via anticoagulant infusion	Prismaflex control unit, indicated for CVVHD, HF and CVVHDF, controls and monitors extracorporeal circuit: o physiological flow rates o air ingress o blood loss

Thromboembolism, e.g. leading to congestion and organ failure: – particulates – degradation products	Prismaflex control unit, indicated for CVVHD, HF and CVVHDF, controls and monitors extracorporeal circuit: o physiological flow rates o air ingress o blood loss septeX™ and oXiris® sets MDD CE'd for CRRT applications
Haematological risks: – haemolysis (mechanical and chemical lysis) – anaemia – leucopenia – thrombocytopenia – altered function of cells – hypoalbuminemia – thrombosis – platelet activation – complement system activation (inflammation)	septeX™ , oXiris® sets and extension tube are: o assured compatibility with blood (ISO 10993-4) o CE marked (93/42/EEC) o Complaints data do not identify issue
Material medicated toxaemia, including those arising from leachables, particulate, degradation products and incompatibility with packaging: – cytotoxicity – hypersensitisation – irritation – systemic toxicity – genotoxicity – carcinogenicity – immunotoxicity – reproductive/developmental toxicity – pyrogenic fever	– septeX™ and oXiris® sets MDD CE'd for CRRT applications
Contamination – production, transport and storage induced infection (transmissible agents may include bacteria (and endo/exo toxins), mould, yeast, parasites and unclassified pathogens: – fever – septicaemia – lupus – organ failure	– septeX™ , oXiris® sets and extension tube are: - terminally sterilized EtO (ISO11135-1) - validated primary packaging - CE marked (93/42/EEC) unadulterated - single-use only – interconnection and priming uses aseptic technique

Performance degradation – adsorption of plasma proteins, lipids, calcium – adhesion of platelets, leukocytes or erythrocytes – potential mechanical haemolysis	
Misuse of the LDD by clinical staff not qualified to deal with; - adverse events - exsanguination - thromboembolism - clotting - exacerbation of symptoms and complications of liver failure death resulting from delivery of liver dialysis support	The DIALIVE is only available: - to the named investigator - exclusively for clinical investigation - under approved CIP (SAFETY Clinical Investigation) potential future benefits outweigh CIP risks Set-up and Priming – septeX™ barcode removed so that treatment parameters can be set manually – septeX™ set loaded as normal – oXiris® dialyzer aseptically connected using extension tube – septeX™ and oXiris® set primed in series (auto prime followed by manual prime) – oXiris® blood return line substituted on Prismaflex for septeXTM blood return line – air removal of oXiris® dialyser finished by hand – complete by clamping off oXiris® dialysing fluid-effluent circuit Operation – Prismaflex control, monitoring and safety features all engaged – CVVHDF proceeds as indicated except blood path series connection of septeX™ and oXiris® –ICU/HD monitoring per CIP and institutional standard of care – Restitution of all extracorporeal blood during

	and at end of session, except in case of filter clothing.
Circumventing of Prismaflex monitoring and control features: - LDD adverse events - exanguination - thromboembolism - clotting - exacerbation of symptoms and complications of liver failure - death	Functional compatibility of Prismaflex, septeX™ set + oXiris® set verified in porcine model Prismaflex pre-programmed sequential procedures mostly eliminate slips, lapses and mistakes Prismaflex control, monitoring and safety features engaged Prismaflex CE marked for CVVHDF Investigator familiarity with CVVHDF Use is restricted to qualified investigator and supervised qualified support familiar with aseptic technique and liver failure patients
Blood loss due to venous cannulation	- Undertaken by experienced staff trained in the procedure - Standard clinical procedures are followed - Coagulation results reviewed prior to cannulation - CIP specifies ICU/HDU monitoring particularly monitoring: - liver function - cardiovascular function - renal function
Hypotension induced by DIALIVE, especially in patients who may be intravascularly depleted because of fluid restriction, diuretic therapy, inadequate oral intake and anemia, or those with evidence of vasoplegia.	- perform fluid preloading - simultaneous replacement of lost albumin - stepwise increase of blood flow rate through the DIALIVE - stepwise increase of replacement fluid - closely monitor the patient

3.3 Residual risks associated with participation in the clinical investigation.

The risks associated with intermittent haemodialysis (HD) and continuous haemodiafiltration (HF) are well established and understood. Similarly the risks associated with intravenous albumin delivery for replacement of blood loss are well known. Both procedures constitute normal SOC. Risks associated with the use of an extracorporeal circulation, known as the "first use syndrome", is not specifically related to the DIALIVE but rather a risk associated with any type of extracorporeal circulation exposure. Well established protocols exist to adjust the condition by experienced medical staff.

3.4 Possible interactions with concomitant medical treatments

Heparin and/or Epoprostenol will be administered to keep the circuit open and the amount used will be dependent upon the clotting profile of the patient determined by their platelet count, prothrombin time, partial thromboplastin time and fibrinogen. Clotting may be corrected by the administration of clotting products as required prior to insertion of the central line and will be decided on a case by case basis.

There is a potential for elimination of albumin bound and water soluble drugs, but there are well established protocols for dose adjustments during haemofiltration particularly for drugs such as antibiotics.

Terlipressin: Should only be administered in patients diagnosed with hepatorenal syndrome according to the ICA guidelines. In case a patient has a variceal bleed, terlipressin can be administered as per protocol. The use of terlipressin for these indications are for splanchnic vasoconstriction and not as a pressor for systemic hypotension.

Lactulose and Rifaximin: Should only be commenced in patients if they have clinical evidence of hepatic encephalopathy. They may be continued on Lactulose and Rifaximin if they were receiving it at the time of randomization.

Albumin: The use of albumin should be restricted for evidence-based indications. These include: a diagnosis of spontaneous bacterial peritonitis, hepatorenal syndrome and large volume paracentesis (>2 litres). Risks associated with albumin administration are well known and appropriately taken care of by medical staff.

Beta-blockers: A). Should be continued in patients receiving it at the time of randomization unless the patients is hypotensive in which case the dose either be reduced or the drug stopped If this is stopped completely, alternative medications such as octreotide may be used per local policies. B) should not be started if the patient is not receiving it at the time of screening.

3.5 Steps that will be taken to control or mitigate the risks

All hazards associated with the intended use of DIALIVE including single fault conditions where applicable, have been identified and evaluated. The process of risk reduction has not introduced any new risks that have not been overlooked and further risk control measures are judged to be unnecessary.

On the basis of the risk management report, the technological solutions and organisational measures for qualifying and monitoring subjects implemented in the proposed protocol, including compliance with relevant safety, performance and clinical care standards, any residual risk are judged to be very low or as low as reasonably practicable and acceptable when weighed against the anticipated patient benefits as elicited from the clinical investigation.

Regular safety review and recommendations will be performed by individual expert investigators and by the members of the TMG and DSMB to mitigate any potential risks associated with DIALIVE therapy. New recommendations will be implemented at appropriate iterations of the protocol versions.

3.6 Risk-to-benefit rationale

The series connection of the septeX™ and oXiris® dialyser sets extends the risks addressed in section 3.2 without the introduction of new risks in respect of:

- infection;
- blood loss;
- air ingress.

The risk analysis, risk-to-benefit assessment and risk control is described in detail in the Investigator's Brochure (IB). After risk mitigation, no hazards remain with an unacceptable risk level.

4 OBJECTIVES AND HYPOTHESES OF THE CLINICAL INVESTIGATION

4.1 Objectives, primary and secondary

Primary Objective

To evaluate the safety of the DIALIVE in patients with alcohol related cirrhosis with Acute on Chronic Liver Failure Grades 1, 2 and 3a (ACLF) compared with the group treated with standard of care.

Secondary Objective

To evaluate the performance of the DIALIVE in the above mentioned patient group. To evaluate clinical benefit in patients receiving DIALIVE treatment plus Standard of Care (SOC) versus SOC alone.

4.2 Hypotheses and claims

The underlying hypothesis is that DIALIVE will significantly improve the prognosis of patients with ACLF by i) exchanging irreversibly damaged native albumin, which accumulates during this disease process and is ineffective in its function, with fresh albumin, and ii) removal of endotoxins, inflammation and endotoxaemia being the central pathogenetic feature of this illness.

This is a first into man study of a novel Liver Dialysis Device. No specific hypothesis is to be statistically assessed in this first feasibility study. Randomization of patients is implemented to obtain unbiased clinical data appropriately balanced between SoC and DIALIVE treated patients to provide fundamental learning data on the outcome of treatment in both study arms, in line with the recommendations of the DSMB (see appendix 7). To this end, safety data, are regularly reviewed (i.e. after each cohort of patient enrollment) but also other clinical parameters are reviewed, to learn and adjust study design when necessary and as appropriate.

Based on this perspective it is the goal to obtain an adequate number of observations in each study arm, with emphasis on the learning from DIALIVE treatment. Future trials will be defined to assess the efficacy of DIALIVE treatment to the appropriate extend.

4.3 Risks and anticipated adverse device effects that are to be assessed

The oXiris® and septeX™ dialysers are going to be clinically assessed for their safety and performance in relation to the intended goal of removing oxidized albumin and endotoxins in a combined method. For this, the described system will be applied in a specific patient population, i.e. Acute-On-Chronic Liver Failure patients.

The following elements are addressed in the clinical Investigation to mitigate the risk of use:

- The system is only used by a few, selected, qualified and trained investigators already familiar with the haemodialysers and with the Prismaflex;

- The CIP specifies further ICU/HDU monitoring requirements: liver function, haematology, coagulation factors, platelet count, cardiovascular function, cerebral function, renal function, pulmonary function; albumin function, immune function;

- The IB and IFU provides further set-up and priming instructions for the DIALIVE system and additional information will be provided to the investigators as clinical experience will demonstrate to optimize the patient's treatment;

- The CIP is designed to minimize risk of exacerbating symptoms and in line with standard of care, i.e. there are no specific clinical trial procedures to be applied other than haemodialysis while there are multiple health checks build in to monitor the patient's condition;

- The CIP provides organisation control for the patient, i.e. patient treatment on the HDU or /ICUunit per local standard medical practice but with adequate monitoring capabilities.

5 DESIGN OF THE CLINICAL INVESTIGATION

5.1 General

This is a European, multi-centre, randomised, controlled, study to generate data for the evaluation of safety (as measured by the percentage of subjects who experience at least one serious adverse event (SAE)) and performance (as measured by plasma endotoxin concentrations and albumin function) of the DIALIVE in 30 evaluable patients with Acute on Chronic Liver Failure (ACLF) versus standard of care (SOC). The study will therefore be open label. The design is appropriate for the study as the primary outcome is to assess safety of the device on a small group of patients.

5.1.1 Measures taken to minimize or avoid bias

The trial is being conducted by investigators who have no direct association with the inventor or the sponsor to ensure no bias is introduced.

5.1.2 Primary Endpoints

The goal is to evaluate the percentage of subjects who experience at least one (1) serious adverse event (SAE) between study Day 1 and Day 10; especially the incidence rate of SAE between the study groups occurring in the period of Day 1 to Day 10.

The percentage of subjects who discontinued DIALIVE due to a serious adverse device event (SADE) between Day 1 (first day of treatment) and Day 10 (DIALIVE arm only)[10].

5.1.3 Secondary End Points

To evaluate the performance of the DIALIVE in patients with ACLF. This is measured by;

- Change in Plasma endotoxin level (endotoxin activity and concentration) between;

  D1, D3, D5 and D10 across SOC arm
  D1, D3, D5 and D10 across DIALIVE arm

[10] In case a patient's treatment is discontinued, the follow up period will remain 28 days from the moment of randomization.

Start and end of a treatment session (on D1, D2 and D3) with DIALIVE
D1,D3, D5 and D10 between SOC and DIALIVE arms for patients presenting with the same grades of ACLF (i.e. Grade 1 ALCF compared with Grade 1 ACLF)

Target-Value (TV): 40% reduction; Acceptable-Value (AV): 20% reduction at end of treatment cycle.

- Change in Albumin function (Human non-mercapt albumin -2 (HNA-2) / Human marcapt albumin (HMA) ratio:

  D1, D3, D5 and D10 across SOC arm
  D1,D3, D5 and D10 across DIALIVE arm
  Start and end of a treatment session (on D1, D2, D3) with DIALIVE
  D1,D3, D5 and D10 between SOC and DIALIVE arms for patients presenting with the same grades of ACLF (i.e. Grade 1 ALCF compared with Grade 1 ACLF)

TV: 40% reduction; AV: 20% reduction at end of treatment cycle.

- Mortality: 28-days and 90 days
- Change in ACLF Grade
- Change in CLIF-ACLF score
- Improvement in individual organ function: Brain (HE), Kidney (creatinine), CVS (mean arterial pressure), Pulmonary (P/F ratio, or S/F if patient is not intubated), Liver (serum bilirubin) and coagulation (INR and platelets).

- Status at ICU and Hospital Discharge (including discharge to a hospice for palliative care).

- Lengths of stay in ICU and Hospital at D28 and D90

- ICU and Hospital re-admissions with another episode of ACLF up to 3 months after randomisation.

5.1.4 Exploratory End points

- To study other exploratory biological and clinical effects of the DIALIVE in patients with ACLF compared with the Standard of Care group.Some of these parameters are centre-specific in line with local expertise, research interest and the capacity to perform these tests. Liver: Changes in MELD score, plasma/serum cCK18/M30 and flCK18/M65 (markers of liver cell death).

- Kidney: Changes in serum creatinine and urinary NGAL (marker of kidney injury).

- Brain: West Haven Criteria to assess changes in severity of hepatic encephalopathy.

- Immune function: Incidence of Infection, changes in white cell count, CRP and assessment of neutrophil and monocyte function. .

- Assessment of Coagulation and haemostasis: Incidence of thrombotic or bleeding complications, assessment of pro- and anti-coagulant clotting factors and complement activation.

5.1.5. Methods and timing for assessing, recording, and analysing variables

All data will be recorded real-time in the subject source documents and entered into an electronic Case Report Form (eCRF) by the investigator and/or his/her delegates within a reasonable period following the study visit. The Sponsor/appointed DM centre will manage and maintain the study database throughout the Investigation. At the conclusion of the Investigation, the database will then be locked and analysed. A final copy of the database will be provided to the study site. Where data is transferred electronically, this will be in accordance with EU data protection Directive (Directive 95/46/EC) and applicable national legislation requirements. There will be a documented record of data transfer and measures in place for the recovery of original information after transfer.

Statistics: A qualified statistician will conduct data analyses, and this will be done independently of data entry. Given the small sample size in this safety study, analyses will be primarily descriptive in nature and will include analyses of both the primary and secondary outcomes and hypothesis. All treated subjects entered into the study will be included in the analyses. A sensitivity analysis will be conducted including all evaluable patients. All serious adverse events will be reported in full detail. Please refer to the Statistical Analysis Plan (SAP) for further details.

5.1.6. Equipment to be used for assessing the clinical investigation

All equipment except for the DIALIVE device, the EAA luminometer for endotoxin analysis, and the Primsmaflex platform[11] will be the property of the Investigator(s) and/or institutions and maintained and calibrated according to institutional guidelines. Records of maintenance and calibration will be made available at the Sponsor's request.

5.1.7. Replacement of subjects.

30 evaluable patients will be recruited across all sites to receive either treatment using the DIALIVE or SOC. Screen failures will be replaced to achieve an even balance in each cohort. The patient numbers will be increased and replaced if there are dropouts during the treatment period to allow 30 evaluable patients to be included.
Dropouts can be i) patients who withdraw their consent, ii) patients who are considered 'not evaluable' because of an incomplete dialysis cycle (for DIALIVE patients) or iii) if a patient dies in the 10-day period following randomization to the SOC-arm, or iv) patients who are excluded based on a stopping rule.
A dropout rate of 17% is anticipated on the basis of observations made during execution of this study in the first 2 cohorts of patients. Patients who drop-out for reasons of an incomplete dialysis cycle are followed up for a duration of 28 days post-randomization.

5.2. Investigational device exposure

For the randomization an Interactive Wireless Randomization System (IWRS) is used, which is linked to the study data base. Randomization is automatically assigned through the IWRS system. Patients are randomised to either the DIALIVE or SOC arm in a 1:1 ratio.

[11] The prismaflex platform will be provided by Yaqrit to those sites that do not have access to such haemofiltration machines. The machine will remain the property of Yaqrit Ltd during and after the study. A mainenance agreement in such circumstances will be established with each institution.

Patients will be assigned into five (5) Cohorts. Each cohort will consist of six (6) patients (3 DIALIVE: 3 SOC). If there are dropouts in any of the following cohorts during the study period, patients will be replaced in each of the cohorts described below to reach the appropriate number of 6 evaluable patients in each cohort.

Cohort 1: including 3 control and 3 DIALIVE treated patients.
Cohort 2: including 3 control and 3 DIALIVE treated patients.
Cohort 3: including 3 control and 3 DIALIVE treated patients.
Cohort 4: including 3 control and 3 DIALIVE treated patients.
Cohort 5: including 3 control and 3 DIALIVE treated patients.

5.3. Subjects

5.3.1. Inclusion criteria

- Male or female subjects aged ≥18 years ≤81yr
- Subject is able to provide informed consent to participate in the study, otherwise written informed consent must be obtained on behalf of the subject by next of kin or a legal representative in accordance with local ethical and legal requirements.
- History indicative of alcohol-related cirrhosis based on clinical, radiological and/or histological evidence.
- History of an acute decompensation event (including but not limited to ascites, gastrointestinal bleeding, hepatic encephalopathy and/or acute bacterial infections), occurring within ≤6 weeks of screening.
- Subject with :
    - ACLF Grade 1, 2 or 3a defined per the CLIF-C OF score system OR
    - single hepatic organ failure for serum bilirubin > 20 mg/dL (342 µmol/L) at screening and randomization, OR
    - AKI-stage 1b (sCr > 1.5 mg/dL or 134 µmol/L)[12].
- Where a subject has received corticosteroids for alcohol hepatitis and is deemed unresponsive to steroids after 7 days of treatment (lack of response defined as Lille score[13] of > 0.45 or steroids stopped before 7 days due to any complication such as infection). This refers to the first course of corticosteorid therapy. Further courses of corticosteroids during the course of illness must not be used to determine steroid responsiveness.

5.3.2. Exclusion criteria

- Co-infection with HIV and AIDS defining illness[14]
- Subjects with acute or sub-acute liver failure without underlying cirrhosis.
- Subjects with severe thrombocytopaenia, as defined by the platelet count of <40,000/mm^3 (at screening) or rapid reduction in platelet count (> 50% reduction) in the previous 24 hrs prior to randomisation (day 0)
- Subjects with International Normalised Ratio (INR) > 3.
- ACLF 3b patients, i.e. ACLF with more than 3 organ failures.
- Subjects with cirrhosis who develop decompensation at any time in the post-operative period following partial hepatectomy or major liver surgery.
- Subjects with uncontrolled infection. Patients may be entered into the study provided antimicrobials have been administered for at least 48 hours with an appropriate response observed prior to randomization.
- Subjects with respiratory organ failure (as per CLIF-C OF scoring: $PaO_2/FiO_2 \leq$ 200 mmHg or 27 kPa or $SaO_2/FiO_2 \leq$ 214).
- Subjects with haemodynamic instability:
    - i) persistent hypotension (mean arterial pressure < 65 mmHg) with evidence of tissue hypoperfusion, not responsive to volume resuscitation and/or low dose vasopressor support;

[12] See appendix 2 for further definitions and justification.

[13] See appendix 2 for further definitions.

[14] A patient can be HIV +ve as long as they are clinically stable and **not presenting** with any AIDS defining illness i.e.
a CD4+ T-cell count below 200 cells/µL
a CD4+ T-cell percentage of total lymphocytes of less than 15%
or one of the defining illnesses such as PCP, Kaposi's sarcoma, CMV, Candidiasis etc

- ii) a norepinephrine dose of > 0.2 µg/kg/min, or a second pressor (terlipressin for variceal haemorrhage and/or hepato-renal syndrome does not count as pressor, unless it is specifically used to treat systemic hypotension) at screening or randomization. Patients can be reconsidered for study inclusion after at least a 24 hour period of norepinephrine requirement < 0.2 µg/kg/min.

- Subjects not considered appropriate for full active treatment including organ support or those with a Do Not Attempt Cardio-Pulmonary Resuscitation order (DNACPR).Subjects with active or history of non hepatic malignancy unless adequately treated or in complete remission for five or more years.
- Patients with HCC outside Milan criteria.
- Significant systemic or major illness other than liver disease, including coronary artery disease, cerebrovascular disease, pulmonary disease, renal failure, serious psychiatric disease, that, in the opinion of the Investigator would preclude the subject from participating in and completing the study.
- Any subject who has received any investigational drug or device within 30 days of dosing or who is scheduled to receive another investigational drug or device in the course of the study; concomitant observational studies are allowed.
- Evidence of uncontrolled seizures.
- Subjects diagnosed with Creutzfeldt-Jakob disease.
- In females: known pregnancy or lactating.
- Subjects weighing less than 30 kg (as per contra-indication of oXiris and septeX)
- Where subjects present with a known allergy to heparin or have type II thrombocytopaenia caused by heparin (HIT syndrome type II).
- In the opinion of the investigator, it is unsafe for the patient to be considered for the study.

5.3.3. Criteria and procedures for subject withdrawal or discontinuation

Other than those required for liver dialysis, no additional Investigation or interventions are expected over and above the routine SOC, which the patient will continue to receive. It is anticipated, therefore, that all subjects will continue to be highly compliant with the CIP study procedures. Non-compliance with the CIP study procedures will be documented by the investigator and reported to the Sponsor as agreed. Persistent non-compliance may lead to be withdrawn of the patient from the study.

The participant may, however, voluntarily choose to withdraw from the study at any time for any reason. This will not in any way affect their routine care. If the participant loses capacity during the study, the legal representative (if appointed) also has the right to withdraw the participant in this instance, acting on the presumed wishes of the participant.

The investigator also has the right to withdraw a patient at any time due to failure to follow CIP, administrative, safety or other reason. Stopping rules (for the study and for the DIALIVE treatment) have been developed as follows:

A) Rules for discontinuing DIALIVE therapy during a treatment session, based on safety grounds
   a. Persistent shock (MAP < 65mmHg or < 90% pre-treatment value, whichever is higher) for > 2 hrs with features of tissue hypoperfusion, unresposive to volume resuscitation and/or low dose noradrenaline (0.1µg/kg/min). Treatment can be restarted once haemodynamic stability is restored.
   b. Deteriorating respiratory failure as defined by worsening PaO_2/FiO_2 to < 200 mmHg (27 kPa) and SaO_2/FiO_2 to < 214. Treatment can be recommenced following a 12 hour recovery period.
   c. Disseminated Intravascular Coagulation (DIC). Thrombocytopenia alone (to below exclusion criterion threshold) without evidence of DIC is not a stopping rule. Platelet treatment should be considered in this case.
   d. Progression to more than 3 organ failures

e. In the opinion of the PI, the treatment is unsafe to continue

B) Study stopping rules for patients randomized to either the SOC or the DIALIVE arm
   a. Withdrawal of patient consent by the patient (or if applicable the appointed respresentative, where the patient were to lose capacity during this process).
   b. Following review of safety data by the DSMB if they wish to stop the study on safety grounds

The reasons for screen failure or withdrawal shall be documented in the eCRFs and in the patient notes. If a withdrawal is on the grounds of safety the Investigator will seek permission from the patient, or legal representative as appropriate, to follow up his/her status outside of the Investigation, but at least until resolution or full clarification of the adverse event.

5.3.4. Point of enrolment

Patients will be recruited through in-patient wards across multiple European centres. Approximately one (1) patient every four (4) months per centre will be recruited with an estimated three (3) patients per centre in total, although competitive recruitment will be utilised. This recruitment strategy is based upon the data accumulated during the performance of the CANONIC study [5].
Following consent and confirmation of eligibility, patients will be randomised electronically via the eCRF . The site will be issued with the subject ID number and informed of their treatment arm. Patients will be deemed enrolled at the point of randomization (Day 0).

5.3.5. Total expected duration of the clinical investigation.

The total duration of the study will be approximately 39 months.

5.3.6. Expected duration of each subject's participation.

After signing the Informed Consent, the patient can be screened for compliance with the in/exclusion criteria for up to 3 days. Patients from cohort 1, 2 and 3 are followed for 28 days, those from cohort 4 and 5 for 90 days.

Patients will be randomized on study day 0 followed by a maximum treatment window period of 10 days (DIALIVE arm). All patients will be intensively followed during the initial 28 days post-randomization and complete the study after completion of study day 28 (cohort 1, 2 and 3) or day 90 (cohort 4 and 5)..

5.3.7. Number of subjects included in the clinical investigation.

30 evaluable subjects will be randomised to receive either standard of care (SOC) or treatment with DIALIVE (1:1 randomisation).
As this is a first-in-man trial, and no hypothesis is to be tested, the sample size is arbitrarily set to obtain adequate information in a small patient population. This reduces the total number of patients exposed to potential study related risks while allowing the investigators to learn maximally from this potential treatment for ACLF-patients for which no alternative

treatment exists and the Data Safety Monitoring Board to make appropriate decisions on study (dis)continuation by comparing occurrence of adverse events between control and treatment arms in each of the 5 cohorts of patients.

5.3.8. Estimated time needed to select this number (i.e. enrolment/recruitment period).

There is an estimated 39 months recruitment period that has been assigned. Start was on February 2017 and patient recruitment is anticipated to run till December 2019.

5.4. Procedures

See Schedule of assessments below for Standard of Care and DIALIVE Patients:

General overview of study design

SOC Patients	Prior Screening	Screening		Study Days						
Study Procedures		0-72hrs	Day 0	Day 1	Day 3	Day 5	Day 10	Day 14 +/- 2 days	Day 28 +/- 5 days	Day 90 +/- 5 days
Informed consent[15]	X									
Inclusion/exclusion criteria		X	X[16]							
Demographics		X								
Medical history		X								
Complete physical examination		X		X	X	X	X	X	X	
Weight (kg) and height (cm)		X		X	X	X	X	X	X	
Vital signs		X		X	X	X	X	X	X	
12-lead ECG		X		X						
Viral screen (local laboratory)[17]		X								
Clinical laboratory (local laboratory)[18]		X		X	X	X	X	X	X	
Full infection screen (local laboratory)		X		X		X	X			
Pregnancy test (women only)		X								
Coagulation (local laboratory)[19]		X		X	X	X	X	X	X	
Randomisation			X							
AE assessments				X	X	X	X	X	X	X[20]
Concomitant medications				X	X	X	X	X	X	
CLIF-C OF Score, CLIF-C ACLF score, ACLF grade		X		X	X	X	X	X	X	

15 Informed consent must occur before ANY study specific procedures commence. Thorough clinical examination and assessment of FBC, U&Es, LFTs, Coagulation must be repeatedn if the test have been undertaken > 24 hours before randomization. Microbiology results obtained within the previous 72 hrs prior to screening may be used and samples not repeated. Viral serology results obtained within 8 weeks prior to screening maybe used and samples not repeated. Liver imaging results obtained 6 weeks prior to screening maybe used and tests not repeated unless there has been a significant change in clinical status

16 Confirmation only. Document in medical notes.

17 Tests performed: HBsAg, anti-HCV, and anti-HIV. Viral serology results obtained within 8 weeks prior to screening maybe used and samples not repeated.

18 Standard haematology and biochemistry as per local practice (Including CRP).

19 Tests performed: Platelets, PT, INR, PTT, Fibrinogen

20 Survival data only via a telephone call.

SOC Patients	Screening	Study Days							
Study Procedures	**0-72hrs**	**Day 0**	**Day 1**	**Day 3**	**Day 5**	**Day 10**	**Day 14 +/- 2 days**	**Day 28 +/- 5 days**	**Day 90 +/- 5 days**
MELD, MELD-Na	X		X	X	X	X	X	X	
Pugh and Lille Scores	X		X	X	X	X	X	X	
eGFR	X		X	X	X	X	X	X	
West Haven grade	X		X	X	X	X	X	X	
Blood sample for biomarkers including Albumin (HNA2) and Endotoxin Plasma Concentration (initial processing locally, samples are transferred to Biobank Royal Free Hospital for analysis)[21]			X	X	X	X			
Urine sample for biomarkers (initial processing locally, samples are transferred to Biobank Royal Free Hospital for analysis)[22]			X	X	X	X			
PBMCs (initial processing locally, samples are transferred to Biobank Royal Free Hospital for analysis)[23]			X	X	X	X			
Endotoxin Activity Assay (local processing and analysis)			X	X	X	X			

21 Blood Biomarker tests : cCK18/M30, flCK18/M65, Caspase 3, Caspase 7, IL-18, soluble CD163, cytokines (TNF-α, IL-6, IL-8, IL-10), chemokines (CX3CL1, CXCL3, CCL2, CCL5)
11 Includes blood for Human non-mercapt albumin -2 (HNA-2) and Endotoxin Plasma Concentration (LAL Assay).
22 Urine Biomarker tests: NGAL, TLR4, IL-18
23 Peripheral blood mononuclear cells (PBMC) Royal Free Hospital, London only.

DIALIVE Patients	Pre-screening	Screening	Treatment Period								
Study Procedures		**0-72hrs**	**Day 0**	**Day 1[24]**	**Day 2**	**Day 3**	**Day 5**	**Day 10**	**Day 14 +/- 2 days**	**Day 28 +/- 5 days**	**Day 90 +/- 5 days**
Informed consent[25]	X										
Inclusion/exclusion criteria		X	X[26]								
Demographics		X									
Medical history		X									
Complete physical examination		X		X	X	X	X	X	X	X	
Weight (kg) and height (cm)		X		X	X	X	X	X	X	X	
Vital signs[27]		X		X	X	X	X	X	X	X	
12-lead ECG		X		X							
Viral screen (local laboratory)[28]		X									
Clinical laboratory (local laboratory)[29]		X		X	X	X	X	X	X	X	
Full infection screen (local laboratory)		X		X			X	X			
Pregnancy test (women only)		X									
Coagulation (local laboratory)[30]		X		X	X	X	X	X	X	X	
Randomisation			X								

24 DIALIVE treatment must commence before end of day 1. DIALIVE treatment may commence on day 0 at the discretion of the investigator. If DIALIVE treatment commences on Day 0, then Day 1 tests must be perfomed before treatment commences on Day 0 and therefore does not need repeating again on Day 1.

25 Informed consent must occur before ANY study specific procedures commence. Thorough clinical examination and assessment of FBC, U&Es, LFTs, Coagulation must be repeated if the test have been undertaken > 24 hours before randomization. Microbiology results obtained within the previous 72 hrs prior to screening may be used and samples not repeated. Viral serology results obtained within 8 weeks prior to screening maybe used and samples not repeated. Liver imaging results obtained 6 weeks prior to screening maybe used and tests not repeated unless there has been a significant change in clinical status. s

26 Confirmation only. Record in medical notes.

27 Blood pressure (supine), respiratory rate, body temperature will be recorded throughout DIALIVE treatment (as per Standard of Care)

28 Tests performed: HBsAg, anti-HCV, and anti-HIV. Viral serology results obtained within 8 weeks prior to screening maybe used and samples not repeated

29 Standard haematology and biochemistry as per local practice (including CRP). Performed throughout DIALIVE treatment as per local practices.

30 Tests performed: Platelets, PT, INR, PTT, Fibrinogen. Performed throughout DIALIVE treatment as per local practices.

DIALIVE Patients	Screening	Treatment period								
Study Procedures	**0-72hrs**	**Day 0**	**Day 1[31]**	**Day 2**	**Day 3**	**Day 5**	**Day 10**	**Day 14 +/- 2 days**	**Day 28 +/- 5 days**	**Day 90 +/- 5 days**
AE assessments			X	X	X	X	X	X	X	X[32]
Concomitant medications			X	X	X	X	X	X	X	
CLIF-C OF Score, CLIF-C ACLF score, ACLF grade	X		X	X	X	X	X	X	X	
CLIF-AD Score (if relevant)	X		X	X	X	X	X	X	X	
MELD, MELD-Na	X		X	X	X	X	X	X	X	
Pugh and Lille Scores	X		X	X	X	X	X	X	X	
eGFR	X		X	X	X	X	X	X	X	
West Haven grade	X		X	X	X	X	X	X	X	

31 DIALIVE treatment must commence before end of day 1. DIALIVE treatment may commence at day 0 at the discretion of the investigator. If DIALIVE treatment commences on Day 0, then Day 1 tests must be perfomed before treatment commences on Day 0 and therefore does not need repeating on Day 1.

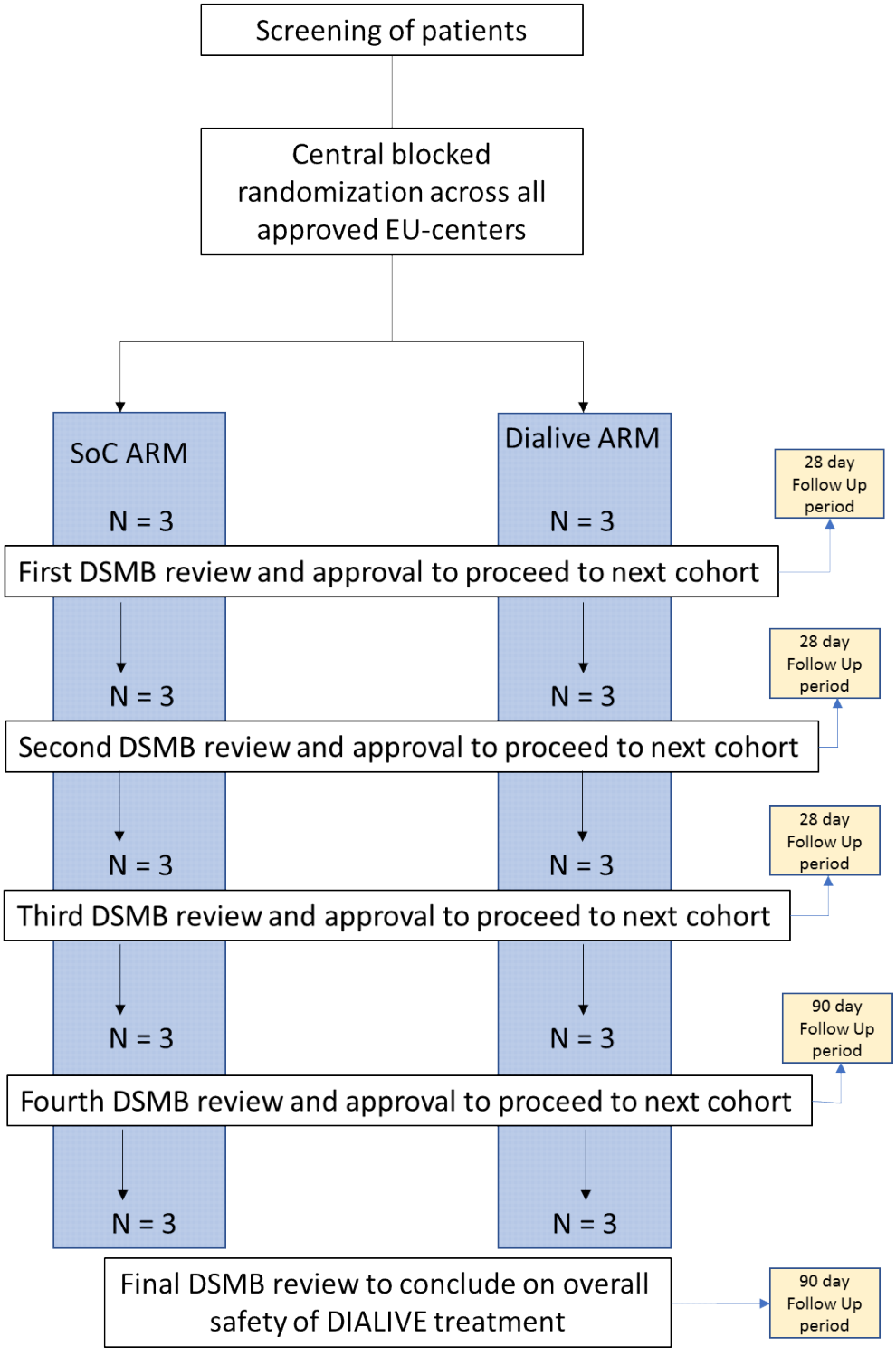
32 Survival only via telephone call

DIALIVE Patients-During Treatment

Study Procedures	0-72hrs Screening	Day 0[33] Randomisation	Day 1 – day 10 Treatment Period	Day 14 +/- 2 days	Day 28 +/- 2 days
PBMCs (initial processing locally, samples are transferred to Biobank Royal Free Hospital for analysis)[34]			Pre-treatment sample and post-treatment session sample		
Endotoxin blood sample (local processing for endotoxin activity analysis EAA)			Pre-treatment sample and post-treatment session sample. If patient not receiving DIALIVE treatment on study day 5 or study day 10 please collect a sample on these days and process		
Blood sample for biomarkers to include Albumin HNA2 and Endotoxin plasma concentration (initial processing locally, samples are transferred to Biobank Royal Free Hospital)[35]			Pre-treatment sample and post-treatment session sample[36] If patient not receiving DIALIVE treatment on day 5 or study day 10 please collect a sample on these days and process		
Urine sample for biomarkers (initial processing locally, samples are transferred to Biobank Royal Free Hospital)[37]			Pre-treatment sample and post-treatment sessione sample If patient not receiving DIALIVE treatment on study day 5 or study day 10 please collect a sample on these days and process		
Dialysate Fluid (initial processing locally, samples are transferred to Biobank Royal Free Hospital)			One sample to be collected prior to commencement of the first DIALIVE treatment session only, from the Dialysate bag		
Ultrafiltrate/Effluent fluid (initial processing locally, samples are transferred to Biobank Royal Free Hospital)[38]			On every DIALIVE treatment day: collect samples after 1hr, 4hrs, and 8-10hrs during treatment.		
Preparation and monitoring of patient per DSMB recomendations			Treatment on ICU, pre-loading fluid, appropriate arterial/venous lines, arterial blood gas analyses every 2 hrs, haemolysis screen[39], DIC screen if clinically indicated.		

33 DIALIVE treatment must commence before end of day 1. DIALIVE treatment may commence at day 0 at the discretion of the investigator. If DIALIVE treatment commences on Day 0, then pre treatment cycle bloods as indicated for Day 1 must be collected in line with the patients treatment cycle days.
34 Peripheral blood mononuclear cells (PBMC) Royal Free Hospital, London only.
35 Blood Biomarker tests : cCK18/M30, fICK18/M65, Caspase 3, Caspase 7, IL-18, soluble CD163, cytokines (TNF-α, IL-6, IL-8, IL-10), chemokines (CX3CL1, CXCL3, CCL2, CCL5)
24 Includes bloods for Human non-mercapt albumin -2 (HNA-2) and Endotoxin Plasma Concentration (LAL)
36 Pre-treatment is prior to initiating DIALIVE treatment. Post-treatment is within one hour after ending a DIALIVE treatment session)
37 Urine Biomarker tests: NGAL, TLR4, IL-18
38 Effluent is collected from the effluent line and from the waste collection bags.

39 Haemolysis screen: FBC including reticulocyte count, blood film, unconjugated and conjugated bilirubin, LDH, haptoglobin and urinary free haemoglobin only at end of DAY 1.


Screening of patients
Central blocked randomization across all approved EU-centers
SoC ARM
N = 3
Dialive ARM
N = 3
28 day Follow Up period
First DSMB review and approval to proceed to next cohort
N = 3
N = 3
28 day Follow Up period
Second DSMB review and approval to proceed to next cohort
N = 3
N = 3
28 day Follow Up period
Third DSMB review and approval to proceed to next cohort
N = 3
N = 3
90 day Follow Up period
Fourth DSMB review and approval to proceed to next cohort
N = 3
N = 3
Final DSMB review to conclude on overall safety of DIALIVE treatment
90 day Follow Up period

5.4.1. Additional sponsor representative(s) activities (excluding monitoring).

Sponsor representatives will be available to assist sites in the device set up.

5.5. Monitoring plan

A contract research organisation (CRO) will be utilised by the Sponsor, Yaqrit, to undertake the management and delivery of the clinical investigation. A project plan will be agreed and implemented to ensure effective conduct. Each site will be evaluated and the qualifications of the PIs verified and documented in a site selection report. There will be an agreement between the Sponsor, CRO and the sites to define responsibilities for study conduct between all parties.

Monitoring will verify that the rights and well-being of the subjects are protected and that the clinical investigation is conducted according to ethical and Good Clinical Practices (GCP), Declaration of Helsinki, Data Protection regulations and the overarching ISO 14155, as well as any regional or national regulations, as appropriate.

The monitor will conduct a Site Initiation Visit (SIV) at each study centre prior to patient recruitment activity. A delegation log will be completed identifying key personnel involved in the study, signatures, functions and designated authorisations.

The monitor will conduct on-site routine monitoring visits in accordance with the agreed monitoring plan during the study, to ensure adherence to the CIP, accurate data recording in the electronic Case Report Form (eCRF) and to monitor recruitment rates and adherence to follow up schedules. The Investigator(s) shall permit and assist the monitor to carry out verification of completed CRFs against data in the source documents.

The frequency of monitoring will be in accordance with the risk of the study. Whilst the study utilises two CE marked devices (filters), the study team has deemed the risk of the study to be **High Risk.** This is a first into man clinical investigation with a un-CE marked device (combination of two CE marked filters in new disease indication).

A SIV will be conducted at each site after all applicable approvals have been received. The monitor will ensure that each principal investigator has received and understood the requirements and content of the CIP, IB, the informed consent form, CRFs and Instructions for use of DIALIVE. Verification of site training on the set up and use of DIALIVE will also be undertaken.

At each visit the monitor shall ensure;

- Compliance with the CIP (and any amendments), ISO 14155 and other applicable regulatory requirements for medical devices
- The device is being used in accordance with the CIP and instructions for use
- Informed consent of patients has been obtained before any clinical investigation-related procedures are undertaken
- Traceability of the devices is appropriately maintained and device stored appropriately
- Source documents are accurate, complete, up-to-date and maintained appropriately.
- Verify that investigator repeatedly attempted to retrieve clinical information from patients considered lost-to-follow up.
- CRFs and queries are complete, recorded in a timely manner and consistent with source
- Adverse events are recorded and reported without unjustified delay
- Investigator study documentation (Investigator Site File) contains all required documents

A monitoring report will be generated and sent to the Sponsor after each visit. Additional site monitoring visits will be agreed between the Sponsor and CRO if any significant site issues are identified after a routine visit.

The level of Source Data Verification (SDV) will be agreed between the Sponsor and CRO. It is 100% for the patient informed consent forms, regulatory and ethical approvals and all study reported serious adverse events.

A close-out visit will be conducted at each site to ensure that the PI records are complete, all documents needed for the Sponsor are retrieved and all previously identified issues have been resolved. Arrangements for archiving will be made at this visit.

Additional detail regarding Monitoring aspects can be found in the associated Yaqrit risk based Monitoring Plan.

5.5.1. Confidentiality

All data will be handled in accordance with the EU Data Protection Directive (95/46/EC) and applicable national requirements. The CRFs will not contain the subject's name or other personal identifiable data. The subject's initials, date of birth and trial identification number, will be used for identification. Subjects will be assigned a trial identification number upon enrolment into the study. The study site will maintain a master subject identification Log. If national authorities require specific information to be included in the local/national Informed Consent, then this will be implemented, especially with reference to GDPR. To this end, also the information obtained from the medical staff of the investigational centers will be treated by sponsor and CRO in a confidential manner and only used for the purposes of the study – as is most often already outlined in the specific Investigator Agreements.

5.5.2. Record keeping and archiving

Archiving will be authorised by the Sponsor following submission of the end of study report. Investigators are responsible for the secure archiving of essential trial documents as per their institutional policy. All essential documents will be archived for 5 years after completion of trial. Destruction of essential documents will require authorisation from the Sponsor.

6. STATISTICAL CONSIDERATIONS

An independent statistician will be responsible for all statistical aspects of the investigation.

All statistical analyses will be carried out on the safety population, defined as the subset of randomised patients who receive at least one treatment. No imputation of missing values will be implemented. Only available data will be analysed. In addition, a sensitivity analysis will be carried out to check the main safety results using SAE-data of all evaluable patients, defined as all those patients who received standard of care or those who received the correct dialysis treatment cycle.

Due to the nature of the study all statistical analyses will be descriptive. The categorical variables will be summarised by means of the count and percentages corresponding to each variable category, while continuous variable will be summarised by the mean and Standard Deviation of each distribution or the median and min-max values in case of those variables non-normally distributed.

For each treatment arm, treatment-emergent AEs, SAEs, SADEs, ADEs, USADEs and DDs will be summarised by organ system and treatment causality. The number and percentage of patients discontinuing the study due to AEs, SAEs, SADEs or other stopping criteria confirmed by the DSMB (withdrawal of patient consent; development of septic shock; progression to ACLF Grade 3b or more; disseminated Intravascular coagulation; unanticipated serious adverse device effects, device deficiencies, or in the opinion of the PI, the treatment is unsafe to continue) will be summarised by treatment arm.

Secondary and exploratory parameters (changes from baseline at each study visit) will be descriptively analysed by treatment arm by means of the appropriate statistics.

Statistical analyses of safety data will be run when each study cohort is completed (minimally one week FU) and the corresponding results will be communicated to the DSMB.

Special interim data extraction required to comply with the EU ALIVER program.

Access to the ALIVER safety clinical trial data by IBM, one of the Partners of the ALIVER-Consortium, is required to determine if the ALIVER dataset has the same variable set of patient characteristics with those which were included in the CANONIC dataset [5]. This availability is critical to ensure compatibility of the predictive models which IBM is building for ALIVER with the safety clinical trial as input data. Also critical, is an assessment and subsequent potential adjustment of the model to allow for inconsistencies between the CANONIC and the safety clinical trial data e.g. specifications for names, types, and codes of patient characteristics. Access to the clinical data is required at regular interim time points to implement this in two main steps. Initially a process will be executed to filter out patient characteristics that are not considered critically useful in the prediction of mortality rates of ACLF patients. The filtering will be carried out applying statistical criteria using a multivariate feature selection analysis based on the Mutual Information. The filtering will also be carried out using criteria provided by clinicians to determine the usefulness of patient characteristics gathered using exploratory and non-exploratory based laboratory approaches. The second step will be to manage any missing values that could be absent from the safety clinical data provided by considering statistical information extracted from the variables available in the data. If the performance of the CANONIC predictive machine learning models decreases significantly when tested, using the safety clinical data provided, then alternative analytics approaches will be considered and determined.

The techniques which are envisaged for application tor the ALIVER safety data are:
- Standard descriptive statistics to determine the overall structure of the data.
- Data missing values replacement – statistical approach.
- Filtering/Selection of groups of patient characteristics according to previously stated usefulness analyses.
- Machine Learning models such as Logistic Regression, XGBoost and, for future work, Deep Neural Networks.

The results obtained from this research work will be made available in a report form not before the clinical trial is completed to guarantee confidentiality and to avoid any bias. No study end-points are assessed by this activity.

7. DATA MANAGEMENT

7.1.1. Procedures for data review, database cleaning, and issuing and resolving data queries.

All clinical investigators will have current Good Clinical Practice (GCP) training, specifically ISO 14155 standards for conducting a clinical Investigation.

Responsibilities

Data Management will be the responsibility of the Sponsor/designee. All documents and data shall be produced and maintained in a way that assures control and traceability. All documents, and subsequent versions, related to a clinical investigation shall be identifiable, traceable and appropriately stored to provide a complete history of the clinical investigation. Only authorised individuals will have access to the electronic system after appropriate training has been provided.

Data Collection

All collected patient data will be treated confidentially and identified only by a unique patient identification number. Medical records relating to clinical investigation, including those that are electronically maintained and those that may contain information that would identify an individual patient will remain confidential, but may be reviewed by, released, and/or transmitted to representatives of the hospital, regulatory authorities, or its agents when reasonable and appropriate for the conduct of the study.

An electronic Case Report Form (eCRF) will be used in the study and completed for every patient who is enrolled for participation. The eCRF will be developed and held by the data management centre of European Foundation for Chronic liver Failure (EF-CLIF), Barcelona, Spain. Data entered in the eCRF must be consistent with the source documents (medical and nursing notes, laboratory reports, ECGs, echocardiogram). Data must be entered by the site in a timely manner and be consistent with the source. The PI (or designee) will be responsible for the ensuring the eCRFs are signed. Any changes to the initial data recorded must be signed for to ensure an appropriate audit trail is maintained. The Trial Monitor(s) will carry out on-site monitoring during the study and at the end of the study to ensure accuracy and quality of data (Source Data Verification). Site investigators will ensure the Monitor's access to patients' medical records, laboratory reports, imaging reports, and other patients' records needed. Site Investigators will cooperate with the Trial Monitor to ensure that any problems detected in the course of these monitoring activities are resolved.

Avoidance of confusion.

The procol allows for a start of DIALIVE dialysis on the day of randomization (DAY 0). As this constitutes the first day of treatment in the DIALIVE-arm, it might be confused with "DAY 1" of the study scheme. The currently available electronic data base (eCRF) also does not provide data entry options for "DAY 0" data collection, other than directly related to the randomization assignment. If dialysis is performed on DAY 0, then clinical data are required to be entered on the eCRF indicated as "DAY 1". The date of treatment will allow, however, to retrospectively reconstruct the exact timing of dialysis sessions and to identify the 'eligible dialysis cycle' to be considered for the end-point analysis.

7.1.2. Procedures for verification, validation and securing of electronic clinical data systems

The study database will be located at the above data management centre. The database will be aligned with the case report forms so that the data captured are complete, accurate, reliable and consistent. Database design will be documented at each stage of development, including (but not limited to) test runs, training exercises, security checks, periodic reviews to confirm data integrity and compliance with operational specification. Once completed, the system will be validated to ensure that it is fit for purpose for the trial.

Appropriate software will be used in developing/designing a database to ensure:

- Ease of use with appropriately controlled access
- Prevention of duplicate entries
- Field validations, where appropriate

- Consistent data coding (e.g. MedDRA coding or other convention which is developed at the start of the study)
- Consistent and accurate downloads for analyses
- Full audit trail possible of data entered and any changes made.
- Regular back-ups

Prior to database lock, the entire database will be verified to ensure that there are no outstanding errors or unjustified missing data. When all queries have been resolved the database will be locked.

Statistical review of the Database

There will be a requirement to ensure that all the trial data have been received, verified, fully coded and cleaned for analysis with all queries resolved before locking the database for analysis. Unlocking of the database will only be undertaken in a strictly controlled environment and rationale for doing so will be fully documented.

The designated statistician will receive the full and dated download of the database with a complete and accurate dataset used for analysis. This will be retained separately from the live database to allow reproducible analyses (database-lock).

A final copy of the database will be provided to the study site. Where data is transferred electronically, this will be in accordance with applicable Data Protection regulations. There will be a documented record of data transfer and measures in place for the recovery of original information after transfer.

7.1.3. Data and biosample retention

Archiving will be authorised by the Sponsor following submission of the end of study report. Investigators are responsible for the secure archiving of essential trial documents and the trial database as per their trust policy. All essential documents will be archived for 5 years after completion of trial. Destruction of essential documents will require authorisation from the Sponsor.

Biosamples will be used for data analysis as specified and destroyed no later than 5 years after study closure, unless local national regulations specifically allow longer retention times (e.g. UK).

7.1.4. Clinical quality assurance

The trial management group (TMG), consisting of the coordinating investigator, site investigators and experts from relevant specialties (if required) will meet routinely in accordance with the TMG terms of reference, to discuss and address any concerns regarding the conduct of the study. The TMG will be consulted for appropriate review and adjudication of adverse events. Please refer to the TMG Charter for further details.

8. AMENDMENTS TO THE CIP

Amendments to this CIP may be necessary to protect the safety of the patients and integrity of the data. In collaboration with the Investigator(s), the CIP amendments will be documented and submitted for ethical and regulatory approval prior to implementation. All changes will be evaluated for impact per Sponsor SOPs. Amendments will be considered implemented after all ethical and regulatory approvals are received and all key sponsor and site staff has been trained. This process does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

The Prismaflex utilizes certain software to appropriately run the pumps according to specific settings. Per the user manual of oXiris and septeX a software version 4.0 or higher is to be used. For the DIALIVE study, in Austria a software version 8.1 only will be allowed.

9. DEVIATIONS FROM CLINICAL INVESTIGATION PLAN

A deviation is either a violation of the conditions and principles of GCP in connection with that trial; or the CIP relating to that trial, as amended from time to time.

The Investigator shall not deviate from this CIP except in situations that affect the subject's rights, safety and well-being, or the scientific integrity of the clinical investigation.

9.1. Procedures for recording, reporting and analysing CIP deviations.

Any deviations form the CIP that occur must be documented on the deviation log and maintained by the study site throughout the duration of the Investigation. Deviations will be submitted to Data Management so they maybe included in the analysis.

9.2. Notification requirements and time frames.

Requests for deviations by the investigator will be responded to by the Sponsor within 48 hours of receipt.

9.3. Corrective and preventive actions and principal investigator disqualification criteria.

Refer to the Yaqrit Risk-based Monitoring Plan, for corrective and preventative actions and principal investigator disqualification criteria.

10. DEVICE ACCOUNTABILITY

The DIALIVE system has been assessed against the Medical Devices Directive 93/42/EEC (MDDs) Essential Requirement (ERs) aspects as listed in Annex I of the IB. It is concluded that DIALIVE complies with the MDD ERs except for those aspects that are the subject of this investigation:

Essential Requirements 1 and 3 relating to patient safety and 6 relating to determination of side effects

The quality of the haemofilters used in DIALIVE is assured by design and production certified compliant with MDD Annex II (Full Quality Assurance System) and ISO 13485 Quality Management Systems for Medical Devices – System requirements for regulatory purposes.

The same applies for the inter-dialyser connection tubing.

The commercially available filters and connecting tube (Device) will be purchased directly from the suppliers and re-packaged by Yaqrit Ltd into a box labeled "exclusively for clinical investigation". The sterility of all component parts will not be altered in any way. Yaqrit Ltd will release and distribute the required number of devices to the sites ready for use. Each device will be assigned a unique device code to ensure full traceability once sent to sites. The sponsor will

maintain a master list of all devices. The device shall be stored according to the storage conditions listed in the approved IB.

Access to the DIALIVE device shall be controlled and the device shall only be used in the clinical investigation according to this CIP. The Sponsor will maintain records to document the physical location of all devices from shipment to sites to destruction. The instructions for use are detailed in the IB. DIALIVE treatment will be delivered by either (a) Trained study specific staff provided by Yaqrit Limited or (b) Site specific staff that have been specifically trained in the use of DIALIVE and signed training logs will be maintained in the Trial Master file.

Each site will maintain accountability records from receipt of the devices through to its use on a per patient basis and destruction following each treatment to ensure a full audit trail is maintained. This shall include the following information;

a) the date of receipt,

b) identification of each investigational device (batch number/serial number or unique code),

c) the expiry date, if applicable,

d) the date or dates of use,

e) subject identification,

f) date on which the investigational device was returned/explanted from subject, if applicable, and

g) the date of return of unused, expired or malfunctioning investigational devices, if applicable.

11. STATEMENTS OF COMPLIANCE

11.1. Declaration of Helsinki, International Standards and national regulations

The clinical investigation shall be conducted in accordance with the ethical principles of the Declaration of Helsinki, MDD 93/42/EEC, ISO 14155:2011 and all other applicable national regulations.

11.2. Approvals

The clinical investigation shall not commence at the site until ethics and regulatory approval and any institutional approval (as applicable) is received. All additional requirements imposed by the ethics committees and/or regulatory authority will be followed. No device will be release to site until all applicable approvals have been verified and the site initiation has been undertaken/scheduled.

11.3. Insurance

The Sponsor will maintain appropriate trial insurance for the clinical investigation as detailed in the Clinical Investigation Agreement(s).

12 INFORMED CONSENT PROCESS

Fully informed consent will be obtained at the initial screening visit. It will be the responsibility of the Investigator, or designee (on the delegation log) to obtain written informed consent from each subject prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study
The inclusion of patients with reduced capacity or who are unconscious (HE grade 3 or 4) is restricted to the approval based on local and / or national legislation. In different European countries, different rules apply. The applicable approach will be documented and applied according to the local or national Ethics Committee guidelines, recommendations or obligations.

Two approaches need to be considered.

A. Patient is conscious at start of enrollment process but might be at jeopardy to lose capacity as decided by the treating investigator. To provide additional protection of subjects, each subject will be offered the option – in line with local and national regulations – to nominate a consultee/representative at the point of consent who, should the patient loose capacity during the study, will act under their presumed wishes and ensure any study intervention conducted will have the consent of the consultee/representative. If a consultee/representative is appointed, he/she must have agreed to act in this capacity before the patient is randomized (i.e. during the screening phase) and this must be documented in the medical notes by site. Where verbal agreement has been initially sought, the site must ensure the Patient Informed Consent Form is co-signed and filed in the site file as soon as is practicable but not later than patient discharge from hospital. Where a patient does not have a consultee/representative (family member, legal representative) and in case he/she would prefer to have one appointed, a professional consultee/representative (independent of the study) will be offered to the patient. The consultee/representative will not undertake any decision making on behalf of the patient unless they are deemed to have lost capacity based on medical assessment.

B. Patient is unconscious at start of enrollment process. As this constitutes a 'vulnerable patient' per the definition of the ISO 14155, special care is to be provided that all local and national regulations are implemented. Inclusion of this kind of vulnerable patients might be prohibited, while in other countries or centers tolerated under specific conditions. These conditions might be linked, but are not restricted to the optimal treatment of the patient by the investigator and/or to the release of the clinical data which need to be approved at study end by either the patient's or the legal representative's consent, OR the upfront written consent by a legal representative, identified and approved per the local/national regulations

"Adequate time" will be given for consideration by the patient before taking part. The investigator, or designee, will record when the Informed Consent documents has been given to the patient. The Investigator or designee will explain the patients are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason. The patient will be given the opportunity to ask questions prior to signing the consent. No clinical trial procedures will be conducted prior to obtaining appropriate consent. A copy of the signed Informed Consent Form (ICF) will be given to the participant. The original signed form will be retained at the study site and a copy placed in the medical notes. If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated if necessary and subjects will be re-consented as appropriate within the time limits of the study.

As this is not an emergency treatment, no patients shall be enrolled into the study without full informed consent following the local/national legislation. Once a patient provided appropriate consent, this consent remains valid until study completion, withdrawal or exclusion.

Each Investigator will maintain a log of all subjects enrolled into the trial, to ensure assigned trial codes and names can be linked. This will be retained at each site.

12. ADVERSE EVENTS, ADVERSE DEVICE EFFECTS AND DEVICE DEFICIENCIES

12.1. The definitions are compliant with ISO 14155 and MEDDEV Guidance 2.7.1 rev 3

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device. **Note 1:** This definition includes events related to the investigational medical device or the comparator **Note 2:** This definition includes events related to the procedures involved **Note 3:** For users or other persons, this definition is restricted to events related to investigational medical devices
Adverse Device Effect (ADE)	Adverse Event related to the use of an investigational device. **Note 1:** This definition includes AEs resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device **Note 2:** This definition includes any event resulting from use error or from intentional misuse of the investigational medical device
Serious Adverse Event (SAE)	Any adverse event that: • Led to death, injury or permanent impairment to a body structure or a body function • Led to serious deterioration in the health of the subject, that either resulted in - a life-threatening illness or injury, or - a permanent impairment of a body structure or a body function, or - in-patient or prolonged hospitalization, or - in medical or surgical intervention to prevent life-threatening illness • Led to foetal distress, foetal death or a congenital anomaly or birth defect Planned hospitalization for a pre-existing condition or a procedure required by the CIP, without a serious deterioration in health is NOT considered a SAE.
Serious Adverse Device Effect (SADE)	An ADE that has resulted in any of the consequences characteristic of an SAE
Unanticipated Serious Adverse Device Effect (USADE)	An SADE, which by its nature, incidence, severity or outcome has <u>not been</u> identified in the current version of the risk analysis report.
Device Deficiency (DD)	Inadequately of a medical device with respect to its identity, quality, durability, reliability, safety or performance. **Note 1:** this includes malfunctions, use errors, and inadequate labeling

An adverse event does not include:

- Medical or surgical procedures; the condition that leads to the procedure is an adverse event.
- Pre-existing disease, conditions, or laboratory abnormalities present at the start of the study that do not worsen in frequency or intensity.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalizations for cosmetic or elective surgery or social/convenience admissions);
- The disease being studied or signs/symptoms associated with the disease unless more severe than expected for the subject's condition.
- Expected post-operative course (see section 3)

12.2. Reporting requirements and timelines

- The SAE reporting form in appendix 1 of the MEDDEV guidance will be used unless national/local requirements specify a different reporting requirement.

In line with MEDDEV guidance 2.7/3 rev 3 May 2015 and Annex X section 2.3.5 of the MDD[40]

Term	Reporter	Reported to	Reporting Timeline from awareness of the event
Adverse Event (AE)	Investigator	Sponsor/CRO	As soon as reasonably possible
Adverse Device Effect (ADE)	Investigator	Sponsor/CRO	As soon as reasonably possible
Device Deficiency (DD)	Investigator	Sponsor/CRO	Immediately, not later than 3 calendar days after site awareness of event
	Sponsor/CRO	CA/Ethics Committee	Immediately but no later than 2 calendar days if the event may have led to an SAE if; • suitable action had not taken • intervention had not been made • if circumstances had been less fortunate
Serious Adverse Event (SAE), Serious Adverse Device Effect (SADE), Unanticipated Serious Adverse Device Effect (USADE)	Investigator	Sponsor/CRO	Immediately, not later than 3 calendar days after site awareness of event
	Sponsor/CRO	CA/Ethics Committee	Immediately but not later than 2 calendar days of sponsor becoming aware of event if event indicates an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients, users or persons, or a new finding to it For all other events Immediately but not later than 7 calendar days of sponsor becoming aware of event
New findings/updates in relation to already reported events	Sponsor/CRO	CA/Ethics Committee	Immediately but not later than 7 calendar days of sponsor becoming aware of event

[40] Special national requirements might apply and will be implemented

12.3. Assessments of adverse events

Each adverse event will be assessed by the PI for the following criteria:

12.3.1. Severity

Category	Definition
Mild	The adverse event does not interfere with the volunteer's daily routine, and does not require intervention; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the volunteer's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health Note: A severity rating of severe does not necessarily categorise the event as an SAE.

12.3.2. Seriousness

Seriousness as defined for an SAE in section 13.1.

12.3.3. Causality

The relationship between the use of DIALIVE treatment and the occurrence of each adverse event shall be assessed and categorised. During causality assessment activity, clinical judgment shall be used and the relevant documents, (IB and the CIP) shall be consulted, as all the foreseeable serious adverse device effects (SADE) and the potential risks are listed and assessed there. The presence of confounding factors, such as concomitant medication/treatment, the natural history of the underlying disease, other concurrent illness or risk factors shall also be considered. For the purposes of this investigation the device related expected events will be outlined in the IB, The CIP will outline the disease expected events and cross refer to the IB.

The above considerations apply also to the serious adverse effects (SAEs) occurring in the comparison group.

For the purpose of harmonising reports, each SAE will be classified according to five different levels of causality. The Sponsor and the investigators will use the following criteria to assess the relationship of the serious adverse event to the investigational medical device or procedures.

Not related Relationship to the device or procedures can be excluded when:

- the event is not a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has no temporal relationship with the use of the investigational device or the procedures;
- the serious event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious event;
- the event involves a body-site or an organ not expected to be affected by the device or

procedure;
- the serious event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
- the event does not depend on a false result given by the investigational device used for diagnosis when applicable;
- harms to the subject are not clearly due to use error;
- In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious event.

Unlikely The relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.

Possible The relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.

Probable The relationship with the use of the investigational device seems relevant and/or the event cannot reasonably explained by another cause, but additional information may be obtained.

Causal relationship The serious event is associated with the investigational device or with procedures beyond reasonable doubt when:
- the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- the event has a temporal relationship with investigational device use/application or procedures;
- the event involves a body-site or organ that o the investigational device or procedures are applied to; o the investigational device or procedures have an effect on;
- the serious event follows a known response pattern to the medical device (if the response pattern is previously known);
- the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious event (when clinically feasible);
- other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- harm to the subject is due to error in use;
- the event depends on a false result given by the investigational device used for diagnosis when applicable;
- In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious event.

The sponsor and the investigators will distinguish between the Serious Adverse Device Effects (SADEs) related to DIALIVE treatment and those related to investigation procedures (any procedure specific to the clinical investigation). An adverse event can be related both to procedures (inherent in the study) and the investigational device. Complications of procedures are considered not related if the said procedures would have been applied to the patients also in the absence of investigational device use/application.

Particular attention shall be given to the causality evaluation of unanticipated serious adverse (device) events (USADEs). The occurrence of unanticipated events related to the use of DIALIVE could suggest that the clinical investigation places subjects at increased risk of harm than was to be expected beforehand. The DSMB will review all

safety data at specific time points throughout the study as outlined in the DSMB charter.

12.3.4. Expectedness

Category	Definition
Expected	An adverse event that is consistent with the information about DIALIVE listed in the Investigator Brochure (IB) and CIP
Unexpected	An adverse event that is not consistent with the information about DIALIVE listed in the Investigator Brochure (IB) and CIP

The reference document to be used to assess expectedness against the intervention is the IB.
The CIP will be used as the reference document to assess disease related and/or procedural expected events.

12.4. Procedures for recording and reporting Adverse Events and Device Deficiencies

12.4.1. . Investigator responsibilities:

The site Investigator shall report any SAE, SADE, USADE and DDs which occur in a subject to the Sponsor/CRO immediately but no later than 3 calendar days after site awareness of the event. The immediate report for all events may be made orally or in writing (using the Sponsor's/CRO SAE form or DD form, as applicable) and shall be followed by a detailed written report on the event.

The PI will respond to any queries raised by the Sponsor/CRO as soon as possible.

The Sponsor/CRO will report any SAE or DD to the REC and/or Competent Authority according to national guidelines.

All deaths of a patient during their participation in the study will be reported as a SAE irrespective of whether the death is related to trial participation or an unrelated event.

Where the event reported consists of, or results in the death of a subject, the investigator shall supply the Sponsor/CRO with any additional information requested by the Sponsor/CRO. Where the death has been reported to the relevant ethics committee, the investigator shall supply any additional information requested by that committee. All serious adverse events will be recorded in the hospital notes, the eCRF and a SAE form.

The investigator shall keep detailed records of all adverse events and device deficiencies relating to the clinical investigation, which are reported to them, by trial participants or users. The investigator shall document all relevant information on Sponsor/CRO provided AE logs, SAE forms and DD forms.

12.4.2. Reporting of all Adverse Events and Device Deficiencies: Investigator and Sponsor responsibilities

Investigator responsibilities shall be as per section 13.4. The sponsor shall keep detailed records of all adverse events and device deficiencies relating to the clinical trial, which are reported to them by the trial investigators. The Sponsor/CRO shall ensure that all relevant information about a reportable event, which occurs during the course of this clinical investigation, is reported as soon as possible to the competent authority and relevant ethics committees per their reporting requirements and according to the timelines in section 13.2. Any additional relevant information should be sent within the same time frame as the initial report. The Sponsor/CRO is responsible for informing the appropriate regulatory authorities, ethics committees and other investigators of any reportable events that have

occurred with the study device in any clinical investigation according to the guidelines set forth by either the ethics committees of record or regulatory authority in the country where the clinical investigation is taking place.

12.4.3. Safety Update Reports

These will be undertaken in accordance with national requirements.

12.4.4. Progress reports

Progress reports will be submitted to the ethics committees yearly as per national requirements. The Sponsor/CRO will prepare and submit the progress reports.

12.5. Foreseeable adverse events and anticipated adverse device effects

Listed below are known complications associated with liver disease

- Gastrointestinal bleeding
- Spontaneous Bacterial Peritonitis (SBP)
- Hepatic Encephalopathy (HE)
- Hypotension
- Septic shock
- Respiratory failure
- Cardiovascular failure
- Renal insufficiency
- Hepatorenal syndrome
- Death

Any existing condition will be recorded at baseline during screening. Further events relating to an existing condition will not be recorded as an AE unless in the opinion of the PI, the condition has worsened since baseline. All AEs will be recorded from the point of consent and reported to the Sponsor in accordance with section 13.2.

During initial human dialysis sessions, and subsequent DSMB reviews, the following (potential) device related SAEs were observed; these were incorporated in the latest version of the Investigator Brochure:

- Hypotension: this resulted in a series of recommendations to follow during the DIALIVE treatment (see §3.2 of CIP)
- Higher then expected albumin drop during dialysis led to additional in vitro tests supporting the need to administer 5 g albumin every hour of dialysis (see §3.4 of CIP and §8.1 of IB)
- Anemia was observed in one patient, requiring blood cell pack administration to restore the hemoglobin level. (see page §3.2 of CIP and §9.1, 11.2 and 12.3i of IB)

Reference is made to section 3.2 of this document, where also the anticipated events related to extracorporeal circulation and to kidney dialysis are summarized

12.6. Emergency contact details for reporting serious adverse events, serious adverse device effects and device deficiencies.

All SAEs and USADEs will be reported to the CRO via the eCRF. In the event access to the eCRF is not available, paper SAE forms will be completed by the site and emailed to:

DIALIVE.safetyandperformance@fakkel-bvba.com

or via fax (+32 1137 5327).

Please refer to Appendix 6 for Country Specific Safety Reporting.

13. VULNERABLE POPULATION

Many subjects able to consent for themselves will be enrolled in the investigation. To provide additional protection of subjects, each subject will nominate a consultee at the point of consent who, should the patient loose capacity during the study, can act under their presumed wishes of the subject and ensure any study intervention conducted will have the consent of the consultee. If the patient does not have a consultee then they may be offered a professional consultee (independent of the study). In all instanties, the treating physician is required to adhere to Good Clnical Practices, i.e. to offer the best care at all times to the patient, irrespective of his/her study participation and health condition.
Only if the national and/or local Ethics Committee does have legislation to allow the inclusion of unconscious patients, then these patients fulfilling all other in/exclusion criteria can be considered for study participation. All applicable regulations will need to be implemented prior study inclusion of this kind of vulnerable patient; if regulations cannot be fully complied with then the patient will not be considered for study inclusion. The possibility to include these vulnerable patients will be assessed and documented upfront prior first study patient enrollment in a given center.

14. SUSPENSION OR PREMATURE TERMINATION OF THE CLINICAL INVESTIGATION

The Sponsor may suspend or prematurely terminate either the clinical investigation in an individual investigation site or the entire clinical investigation for significant and documented reasons.

The PI, ethics committee, or regulatory authority may suspend or prematurely terminate participation in a clinical investigation at the investigation sites for which they are responsible.

If suspicion of an unacceptable risk to subjects arises during the clinical investigation, the Sponsor will suspend the clinical investigation while the risk is assessed. All information will be sent to the Data Safety Monitoring Board (DSMB) for review. The Sponsor shall;

a) Terminate the clinical investigation if an unacceptable risk is confirmed by the DSMB. All other Investigators will be informed or

b) If following review the DSMB concludes appropriate corrective actions have been undertaken and are acceptable and decides to lift the temporary suspension, the Sponsor will obtain all the necessary approvals for re-instating the trial and will inform all Principal Investigators.
The reports of the DSMB outcome will be forwarded to the national Competent Authority. The outcome will be implemented as a protocol amendment after the 1st and 2nd DSMB review. These DSMB outcome reports and

amendment(s) must be re-approved by the requiring National Competent Authority (Germany) prior to study continuation and patient enrollment in Germany if the next cohort is allowed.

If, for any reason, the sponsor suspends or prematurely terminates the investigation at an individual investigation site, the sponsor shall inform the responsible regulatory authority as appropriate and ensure that the ethics committee is notified,

If suspension or premature termination occurs, the Sponsor shall remain responsible for providing resources to fulfil the obligations from the CIP and existing agreements for following up the subjects enrolled in the clinical investigation, and the PI or authorized designee shall promptly inform the enrolled subjects at his/her investigation site, if appropriate. The method and the timing of this communication will depend on the circumstances and the perceived risks.

15. LEGAL REPRESENTATIVE

The clinical trial started in 2016 in UK and was broadened to include different European study centers as of 2017. In the event of UK leaving the European Community (Brexit), the Medical Device Regulation requires non-EU based manufacturers to be represented in a member state. YAQRIT Ltd, sponsor of the DIALIVE trial, has appointed the CRO FAKKEL as the legal representative for the duration of the study.

Contact details:

FAKKEL-bvba
Groenendael 43/001
3400 Landen, Belgium
Contact person : Jaak Minten
Telephone: +32-475-923-149
Fax: +32-11-37-53-27

16. PUBLICATION POLICY

After the study has been officially closed, a report on the evaluation will be completed. The clinical investigation report shall take into account the data from each investigation site and for all subjects. No subject shall be identifiable either from the clinical investigation report or the published results. The clinical investigation report will be made available to the all PIs for review and comment. The Sponsor will maintain records confirming that the clinical investigation report has been provided for review. The final, signed report will be submitted to the authorities in accordance with national requirements.

The results of this clinical investigation will be made available for publication according to sponsor SOPs and per the Clinical Investigation Agreement (CIA).

17. SIGNATURES

The Investigators agree to comply with the responsibilities of the Principal Investigator as defined in ISO 14155:2011 as well as EU GCP and other applicable national legislation, the EU Data Protection Directive (95/46/EC) and applicable national legislation, local Information Governance Policies, the Sponsor's SOPs, Study Specific SOPs and other regulatory requirements as amended.

The Sponsor substantiates that this CIP is in accordance with ISO 14155:2011 and the Medical Devices Directives 93/42/EEC (MDD). The Sponsor agrees to comply with the responsibilities of the Sponsor as defined in ISO 14155:2011 as well as EU GCP and other national Regulations, the EU Data Protection Directive (95/46/EC) and applicable national legislation, local Information Governance Policies, the Sponsor's SOPs, Study Specific SOPs and other regulatory requirements as amended.

SIGNATURES TO FOLLOW

CO-ORDINATING INVESTIGATOR AND SPONSOR SIGNATURES

I have read CIP number YAQ-002 V7.0.0 dated 10 October 2019. I agree to conduct the study as detailed in this CIP and in compliance with the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable regulatory requirements and guidelines

Coordinating Investigator

Dr Banwari Agarwal

Signature

Date
11 October 2019

Sponsor Representative

Daniel Green

Signature

Date

11 October 2019

Principal investigator

…… Signature Date

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Appendix 1a- Acute-on-Chronic Liver Failure Grading Scheme per CLIF-OF scoring system.

Grade	Subgroups	ACLF grade equivalent (for DIALIVE study)
No ACLF*	• Patients with no organ failure • Patients with a single hepatic, coagulation, circulation or respiratory failure, no AKI and no HE • serum creatinine <1.5mg/dL and no hepatic encephalopathy (HE) • Patients with single cerebral failure and serum creatinine <1.5mg/dL	
Grade 1	• Patients with renal failure (sCr > 2 mg/dL) • Patients with other single failure organ failure with serum creatinine ≥1.5 and <2 mg/dl and/or HE grade 1-2	• Patients with serum bilirubin > 20 mg/dL (364 µmol/L) • AKI stage 1b (sCr > 1.5 mg/dL or 134 µmol/L)
Grade 2	• Patients with 2 organ failures	
Grade 3 a	• Patients with 3 organ failures	
Grade 3 b	• Patisents with more than 3 organ failure	

From: Moreau, R., et al., *Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis.* Gastroenterology, 2013. **144**(7): p. 1426-37, 1437 e1-9.

Appendix 1b:Chronic Liver Failure Consortium (CLIF-C) Scoring Systems

CLIF-C Organ Failure (CLIF-C OF) Scoring System

Organ System	Score = 1	Score = 2	Score = 3
Liver (mg/dl)	Bilirubin < 6	6 ≤ Bilirubin ≤ 12	Bilirubin >12
Kidney (mg/dl)	Creatinine <2	Creatinine ≥2 <3.5	Creatinine ≥3.5 or renal replacement
Brain (West-Haven)	Grade 0	Grade 1-2	Grade 3-4
Coagulation	INR < 2.0	2.0 ≤ INR < 2.5	INR ≥ 2.5
Circulation	MAP ≥70 mm/Hg	MAP <70 mm/Hg	MAP < 65 mmHg; Vasopressors
Respiratory: PaO_2/FiO_2 or SpO_2/FiO_2	>300 >357	≤300 - > 200 >214- ≤357	≤200 ≤214

The shaded areas represent 'Organ Failures'

From: Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. Journal of Hepatology 2014; 61: 1038–1047

CLIF-C Acute Decompensation (CLIF-C AD) Formula

10*0.03*Age + 0.66*Ln Creatinine + 1.71*Ln INR + 0.88*Ln WBC +-0.05*Sodium + 8

From: Jalan R, Pavesi M, Saliba F, Amoros A, Fernandez J, Holland-Fischer P, et al. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-onchronic liver failure. Journal of Hepatology 2015; 62: 831–840

CLIF-C Acute-on-Chronic Liver Failure (CLIF-C ACLF) Formula

10*[0.33*CLIF-OFs + 0.04*Age + 0.63*Ln WCC – 2]

From: Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. Journal of Hepatology 2014: 61: 1038–1047

Appendix 2: a) AKI criteria for decompensated cirrhosis
b) Lille score.

a. AKI criteria

The definitions of Acute Kidney Injury (AKI) grading are:

- AKI stage 1A: acute rise in serum creatinine (sCr) of ≥ 0.3 g/dl (27 µmol/L) in ≤ 48 hrs, or
  sCr ≥ 1.5 – 2 times the baseline sCr in 7 days (but absolute sCr value ≤ 1.5 mg/dL
  AKI stage 1B: sCr > 1.5 mg/dL (134 µmol/L)
  .
- AKI stage 2: sCr ≥ 2.1 – 3 times the baseline sCr in 7 days

- AKI stage 3: 3.1 – 4 times baseline sCr in 7 days, or
  sCr ≥ 4 mg/L (354 µmol/L) with evidence of acute rise of ≥ 0.3 g/dl (27 µmol/L) in ≤ 48 hs

AKI stage 1B denotes significant kidney injury in the context of decompensated cirrhosis and equates to single renal organ failure or ACLF 1. AKI stages 2 and 3 should be used as per CLIF-OF score.

From: Huelin P, Piano S, Sola E, Stanco M, Sole C, Moreira R, et al. Validation of a staging system for acute kidney injury in patients with cirrhosis and association with acute-on-chronic liver failure. Clin Gastroenterol Hepatol 2017;15:438–445

b. Lille score.

Day 0 29 / 10 / 2018 (dd/mm/yyyy)

Date of Birth / / (dd/mm/yyyy)

Bilirubin µmol/L (at Day 0)

µmol/L **(at Day 7)**

Creatinine µmol/L (at Day 0)

Albumin* g/L (at Day 0)

Patient's prothrombin time sec (at Day 0)

** In patients who have been treated with albumin infusions, in order to avoid the use of an artificial value, it is recommended to use the available albumin value before infusion of albumin*

Lille Model Score = (exp(-R))/(1 + exp(-R))

Where R = 3.19 - (0.101 X age) + (0.147 X baseline albumin) + (0.0165 X change in bilirubin level) - (0.206 X creatinine) - (0.0065 X baseline bilirubin) - (0.0096 X prothrombin time)

From: Louvet A., Naveau S., Abdelnour M., et al. The Lille Model: a new tool for Therapeutic Strategy in patients with severe alcoholic hepatitis treated with steroids. HEPATOLOGY, Vol. 45, No. 6, 2007: 1348-1354.

Appendix 3: Model for End-Stage Liver Disease (MELD) Scoring Systems

MELD Formula
3.8*ln Bilirubin (mg/dl) +11.2* in INR + 9.6* ln Creatinine (mg/dl) + 6.4

From: Kamath P., Wiesner R.H., Malinchoc M. et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001; 33: 464-470

MELD-Sodium Formula
3.8*ln Bilirubin (mg/dl) +11.2* in INR + 9.6* ln Creatinine (mg/dl) + 1.59 (135-Na (meq/l) + 6.4

From: Biggins S.W., Kim W.R., Terrault NA. et al. Evidence-based incorporation of serum sodium concentration into MELD. Gastroenterology 2006; 130: 1652–1660

Appendix 4: West Haven Criteria for Semi-quantitative Grading of Mental State

Grade 1	Trivial lack of awareness
	Euphoria or anxiety
	Shortened attention span
	Impaired performance of addition
Grade 2	Lethargy or apathy
	Minimal disorientation for time or place
	Subtle personality change
	Inappropriate behavior
	Impaired performance of subtraction
Grade 3	Somnolence to semistupor, but responsive to verbal stimuli
	Confusion
	Gross disorientation
Grade 4	Coma (unresponsive to verbal or noxious stimuli)

From: Ferenci P., Lockwood A., Mullen K. et al. Hepatic encephalopathy – definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002; 35: 716 –721

Appendix 5: Definitions of acute decompensation:

Acute development of large ascites was defined by the development of grade 2 to 3 ascites, according to the International Ascites Club Classification, [1] within less than 2 weeks; it could be a first episode of ascites or a new episode. Patients with chronic refractory ascites who were admitted to the hospital frequently for therapeutic paracentesis due to rapid reaccumulation of large ascites were not included in this definition.

Acute hepatic encephalopathy was defined by the acute development of a change in mental status in a patient with previous normal consciousness and no evidence of an acute neurologic disease.[2] It could be the first episode of hepatic encephalopathy or a new episode. Patients with chronic hepatic encephalopathy were not included in this definition.

Acute gastrointestinal hemorrhage was defined by the development of an upper and/or lower gastrointestinal bleeding of any etiology.[3]

Although bacterial infections are not specific complications of cirrhosis, they were considered as such because of their high prevalence and association to abnormalities related to cirrhosis, including bacterial translocation and impaired leukocyte functions.[4, 5] Spontaneous bacterial peritonitis, spontaneous bacteremia, urinary tract infection, pneumonia, and cellulitis, the most frequent infections in cirrhosis,[5] as well as any other type of acute bacterial infection were included in this definition.

1. *Moore et al. Hepatology*. 2003; 38: 258–266
2. *Blei et al. Am J Gastroenterol*. 2001; 96: 1968–1976
3. *Garcia*-Tsao et al. *N Engl J Med*. 2010; 362: 823–832
4. *Arviniti et al. Gastroenterology*. 2010; 139: 1246–1256
5. *Gustot et al*. *Hepatology*. 2009; 50: 2022–2033

Appendix 6: Country Specific Safety reporting requirements

Member State	Ethics Committee Requirements	Competent Authority Requirements	Comments
United Kingdom	SAEs: Only reports of **related and unexpected within 15 days** of the chief investigator becoming aware of the event. Use REC specific template for reporting non-clinical trial of investigational medicinal product events. There is no requirement for annual safety reports in addition to the information provided through the annual progress report.	**MHRA** When reporting SAEs please include the total number of patients treated in the UK at the time of reporting. This includes events occurring outside the UK, where the investigation is also being conducted in the UK. When submitting the MEDDEV *2.7/3* reporting Excel spreadsheet please email this to aic@mhra.gsi.gov.uk quoting CI/2016/0032 (MHRA's reference number) **Summary reports** Reports of **all serious adverse events** must be made on a **3 monthly basis** in tabular format broken down by event type. To include; • number of serious adverse events • number of participants affected by those events • percentage of the total number of enrolled participants affected by those events Include a summary analysis of the serious events together with the manufacturer's/sponsor conclusions	UK utilizes MEDDEV 2.7/3 requirements for CA submissions. Health Research Authority requirements included in REC submissions.

Member State	Ethics Committee Requirements	Competent Authority Requirements	Comments
Germany	As per MEDDEV requirements	**BfARM** SAEs: A causal relationship between the SAE and the investigational medical device, a comparator device, diagnostic or therapeutic procedures performed as part of the clinical trial or other conditions of the trial conduct **cannot be excluded-RELATED** Immediately but not later than 2 calendar days SAEs: A causal relationship between the SAE and the investigational medical device, a comparator device, diagnostic or therapeutic procedures performed as part of the clinical trial or other conditions of the trial conduct **can be excluded-UNRELATED** Immediately but not later than 7 calendar days SAE summary evaluation	Use German SAE report form for single cases in Germany. If event occurs outside Germany use MEDDEV 2.7.3 On quarterly basis using MEDDEV excel file. Sheets to be separated into two sheets for those events occurring inside Germany and those occurring outside of Germany. On quarterly basis using the SAE summary evaluation report.
France	SAEs: No SAE reporting to CPP required. Annual report to be submitted.	As per MEDDEV requirements. Other safety data requiring immediate notification within 15 days of sponsor awareness: any new element that could significantly modify the evaluation of the risk-benefit ratio of the investigational medical device An annual safety report required.	Use ANSM template for reporting to CA.

Spain	SAE: only if country related: immediately but not later than 15 days after awareness. Unexpected SAE that led or could have led to death: only if site related: immediately but not later than 7 days after awareness. At least once a year the annual safety report.	As per MEDDEV 2 At least once a year the annual safety report.	
Denmark	An annual safety report is required. SUSAR: suspected unexpected serious adverse reactions should be reported immediately. Annually and throughout the year the sponsor or investigator must submit reports on SA(D)E and USA(D)Es. In addition, a safety assessment on trial subject's safety should be presented. Reports must be forwarded in pdf format.	An annual safety report is required. SUSAR: suspected unexpected serious adverse reactions should be reported immediately. The investigator and/or sponsor must indicate if the adverse event is associated with the investigational device (SADE), that the event is serious and that the event is unexpected (USA(D)E).	An e-form to be completed on the DKMA webpage.
Italy	According to timelines specified in MEDDEV 2.7/3 December 2010	According to MEDDEV An annual report must be submitted	No Specific template to be used
Austria		BASG: According to MEDDEV 2.7 rev 3 All SAEs if related immediately and -SAES as strong breach of the public safety: not later than 2 days -Death and unexpected SAEs: not later than 10 calendar days -Other SAES (not Death _Non unexpected SAE): : not later than 30 calendar days	MEDDEV 2.7/3 SAE Report Table v2 (XLS) And Notification form (Word)
Romania	According to timelines specified in MEDDEV 2.12/1 rev 8 from January 2013	According to timelines specified in MEDDEV 2.7 rev 3	Ro utilizes MEDDEV 2.7/3 requirements for CA submissions.

Belgium	Annual report required	FAGG - Each SAE - Each Device deficiency - Each AE that could have been a SAE, but was prevented by correct handling of the responsible persons SAEs occurring in patients treated in Belgium and outside of Belgium. Notification as fast as possible, SAEs immediately Annual Safety Report required.	Safety reporting to AFMPS/FAGG by using MEDDEV 2.7/3 reporting Excel spreadsheet

Appendix 7: Summary report of the DSMB outcome

A) upon review of the safety aspects observed in cohort 1 of the study.

Upon completion of patient enrollment in cohort 1of the study, the 7 SAEs observed were reviewed by the DSMB on the 19th April 2018. The following recommendations were formulated by the DSMB:

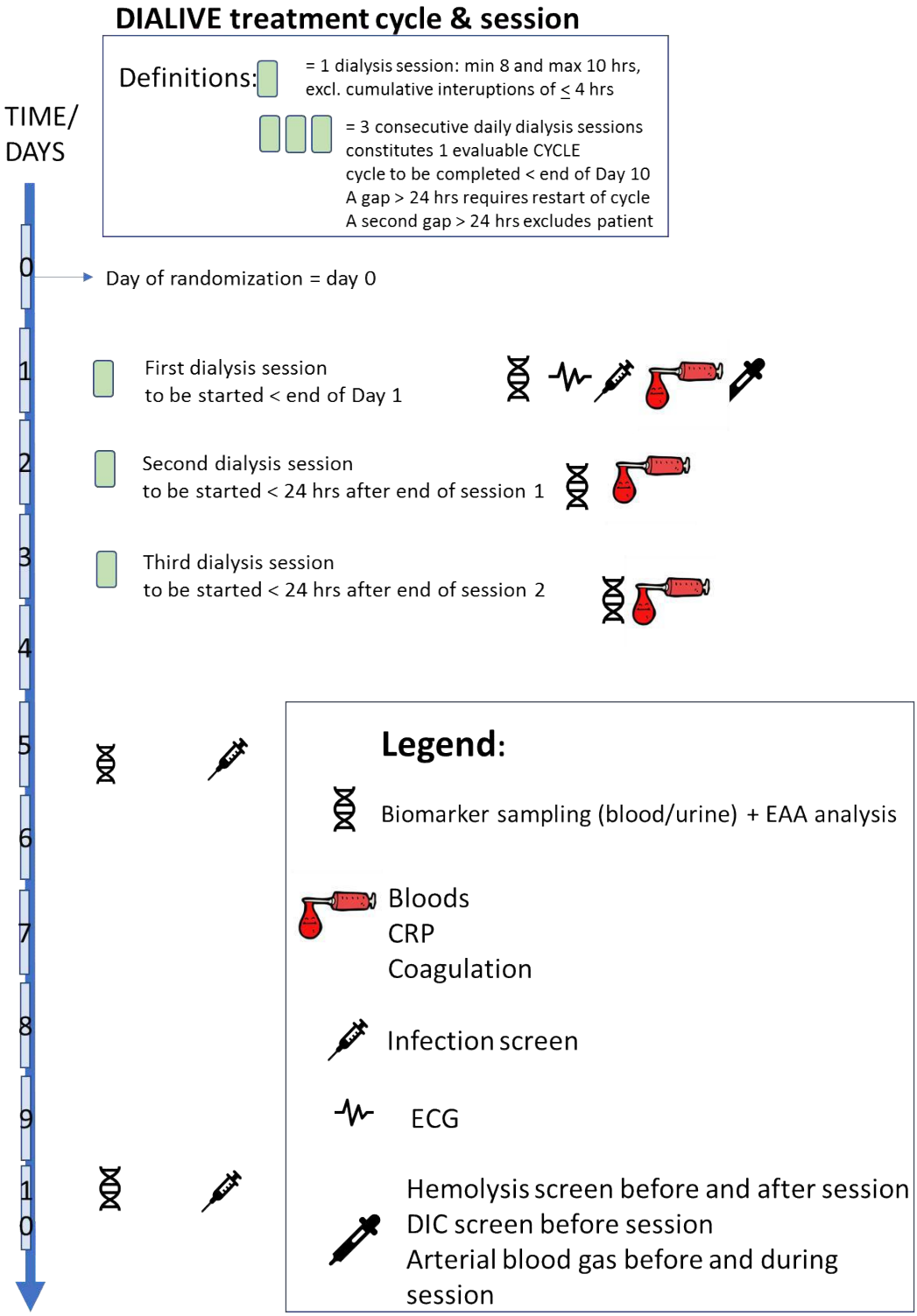
1. DSMB unanimously recommended the continuation of the DIALIVE study and approved enrollment of another 6 patients (i.e. the completion of enrollment in the second cohort of the study).
2. A question was raised on the possibility of standardising anticoagulation regime across centres. It was acknowledged by all that this is a complex area and that there is no straightforward answer to this. Our current knowledge of what should be an optimal anticoagulation strategy for this device is limited. More information is required before a definitive standardized anticoagulation regime can be recommended. The current study protocol addresses this issue by incorporating detailed assessment of coagulation and haemostasis as part of the study. This would then hopefully provide better insight into this matter to then help formulate an informed anticoagulation regime. But until then we would continue with local policies used for standard CRRT therapy.
3. It was recommended to provide adequate guidance within the protocol to avoid inclusion of severely ill patients. The specificity of the ACLF grading in determining the expected mortality in an individual patient can sometimes be challenging. It is possible that an ACLF grade 2 is clinically sicker than a stable ACLF grade 3. Efforts will be made to address this issue in the next iteration of the study protocol, possibly by introducing more stringent exclusion criteria. It was also discussed that Point-of-Care measurements may be suboptimal as compared to central laboratory blood chemistry values. The co-ordinating PI, Banwari Agarwal, confirmed that it is the current recommendation to all investigators to rely only on central laboratory results for medical judgements.

There is no need to adjust the protocol prior to proceeding with the study. It is important that additional information is accrued on more study patients to better understand the optimal treatment of the ACLF-patient. The number of currently enrolled patients is too small to make appropriate conclusions. Therefore, additional study patients are required.

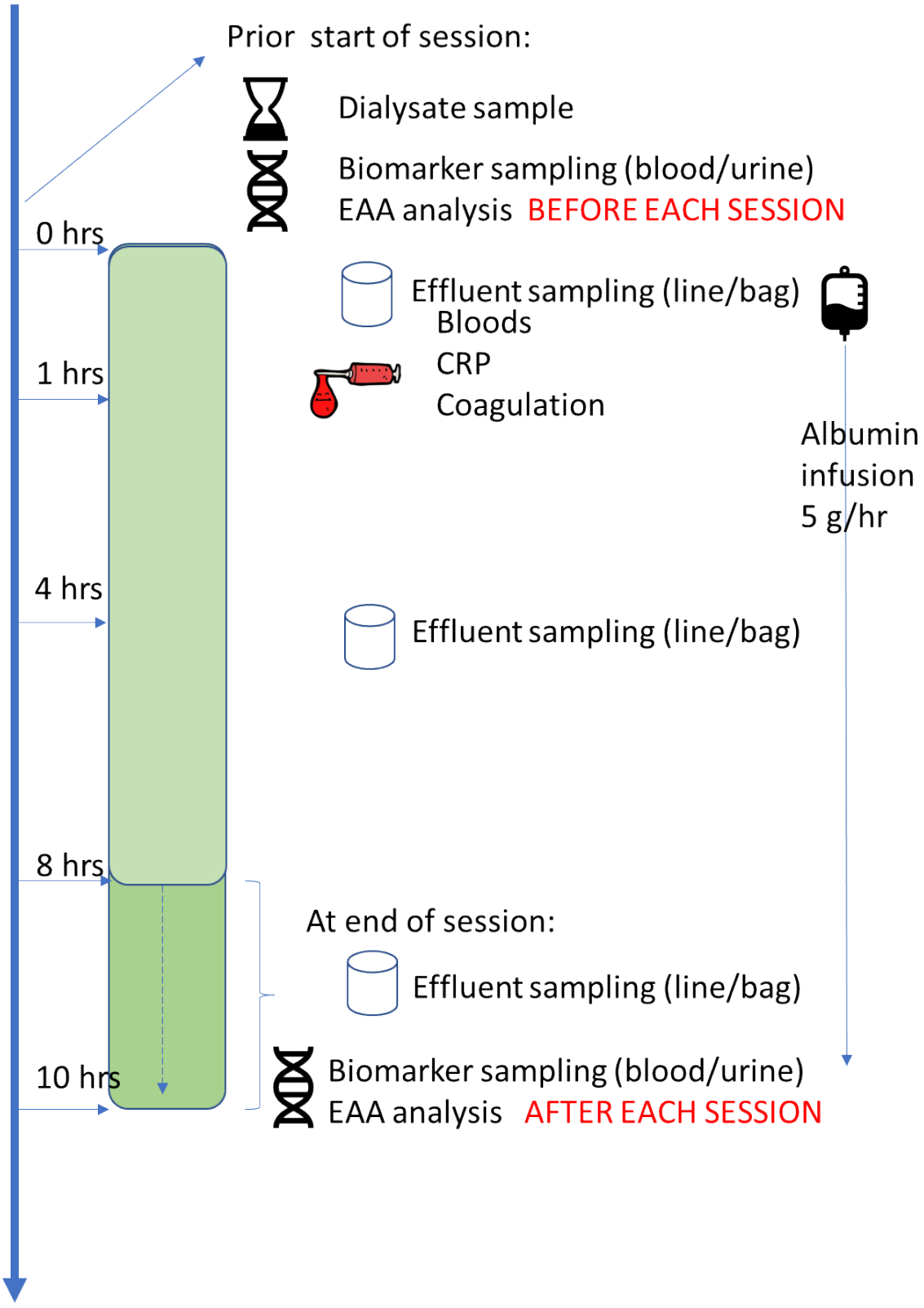
B) upon review of the safety aspects observed in cohort 2 of the study.

14 subjects have been enrolled to the study so far; 8 DIALIVE and 6 SOC, 2 of 8 DIALIVE patients were non-evaluable as per the study protocol since they failed to complete the minimum treatment required for analysis. Overall, with the exception of the 2 non-evaluable DIALIVE patients, the intervention seems to be safe and is well tolerated with some expected adverse events such as a degree of hypotension, mild platelet consumption and potential complications related to the lines being used to deliver the treatment. The two deaths in the DIALIVE arm, both occuring within 24-36 hours of treatment having been commenced, appear to be multifactorial including progression of the disease itself and the learning curve of the sites using such treatment for the first time thus sometime not realising the nuances of such treatment. Following the recommendations made at the interim DSMB meeting whereby a standard document was created to capture these nuances for ready reference to the site teams, the three DIALIVE patients treated since then had a very stable treatment course.
We therefore, based on the evidence provided to us by the central study team and the sponsor, feel reasonably confident that the study should continue to the next cohort as and when practical.

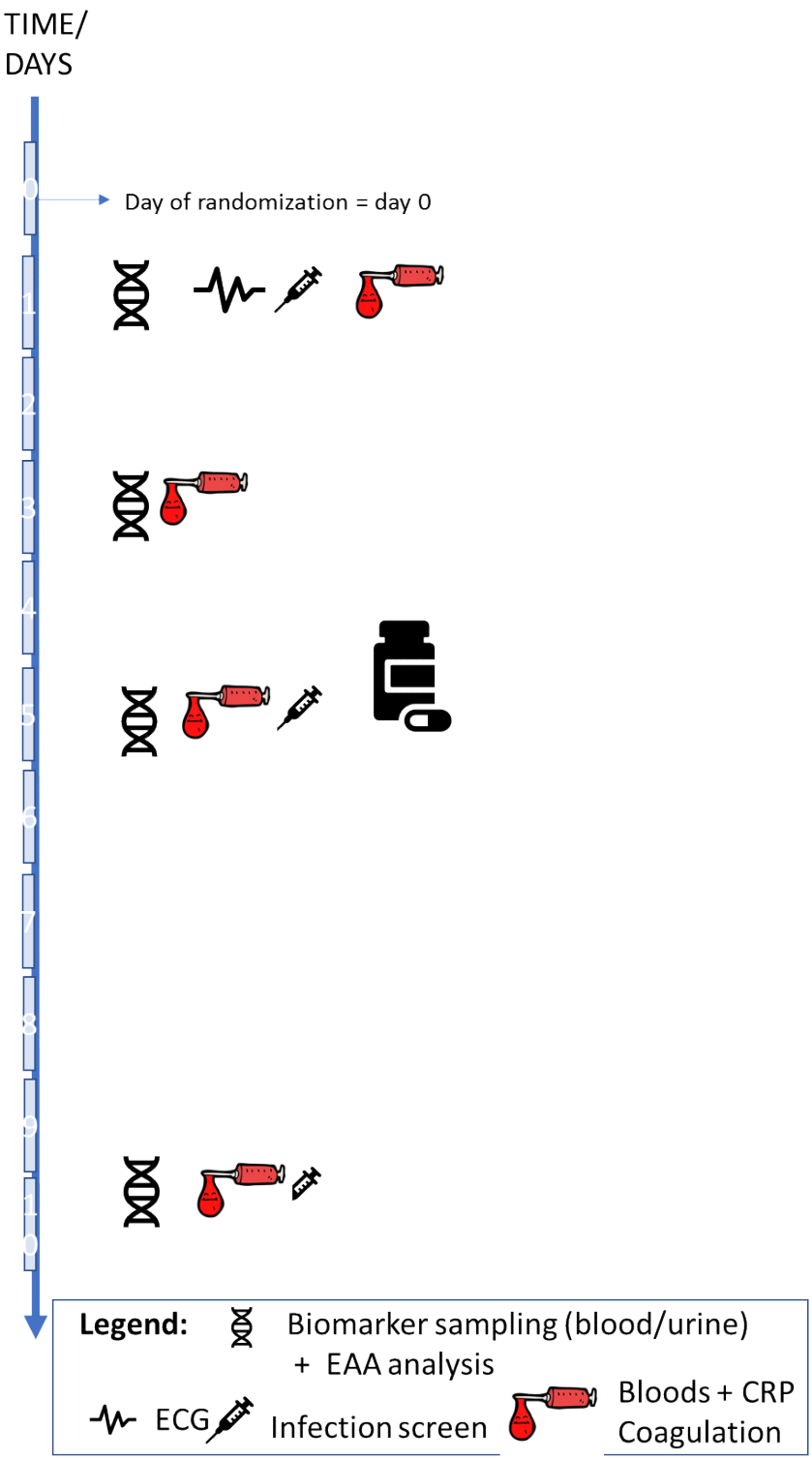
Appendix 8: Illustrations of clinical procedures.



One dialysis session (same for ALL sessions):



Clinical Procedure for SoC



Appendix 9 Amendment History- Country Specific

Amendment No.	CIP version no.	Author(s) of changes	Details of changes made
1 (Substantial) UK Only	4.0 dated 8th December 2016	Yvanne Enever	P1-Company logo amended, addition of DIALIVE in title and addition of EUDAMED number. P2- Principal Investigator amended to Coordinating Investigator P3-Addition of additional EU Sites (Germany, France, Spain) P8- Overall Synopsis amended; • addition of DIALIVE • Primary End Point outcome measure amended from Days 1-7 to Days 1-10 • Amendment to % of subjects discontinuing due to "SADE between days 1-10 in DIALIVE arm only" P9-Amended secondary objective to "DIALIVE arm only" P10-Exploratory end points now include additional wording • "compared with SOC group" • "serum" creatinine • "plasma induced" neutrophils and additional test IL1RA P11-Addition of SAE and number of visits altered to clarify the following; • Day 0=Randomisation • Day 5 assessments now replaces day 7 (both arms) • Day 10 assessment for safety (both arms) • Day 21 removed Removal of wording "after last treatment" Trial dates amended to reflect additional approval requirements P15-Additiona of IB as reference for DIALIVE set up P24-Addition of "compared with SOC" added to Primary objective Addition of wording "to evaluate improvement in clinical parameters between patients receiving DILAIV treatment vs SOC" P25-Addition of European requirements in section 5.1 • EU data protection Directive (5.1 section c) • Addition of footnote re supply of Prismaflex platform (section 5.1 d) • Clarification that 24 evaluable patients are required and drop outs will be replaced (section 5.1 e) P26-Additional comment re drop outs (section 5.2) and additional wording "by the patient or their appointed consultee…. during this process" (section 5.1 c) P27-Increase in number of sites from 4 to 7, clarification of 3 patients/site, clarification re randomization=day 0 and criteria for screening failures (section 5.2 d), clarification of screening phase, treatment phase (DIALIVE) and that all patients will complete study after completion of day 28 visit (section 5.1 f), the word evaluable added (section 5.2 g), recruitment period extended to 18 months (section 5.2 h)

Amendment No.	CIP version no.	Author(s) of changes	Details of changes made
1 (Substantial) cont…	4.0 dated 8th December 2016	Yvanne Enever	P29-30 Schedule of assessment amended to align both groups; • Screening period day-3 to day 0 • Day 1 (first day of treatment DIALIVE arm) • Day 5 assessments • Day 10 assessments (safety) • Day 14 window period added • Day 21 removed • Day 28 window period added • Clarification of samples taken locally vs centrally • Clarification of timings for Biomarkers, endotoxin and albumin samples P31-Addition of Fig 1 Schedule and Amendment of Fig 2 to alter timings of assessments P33-insertion of word monitor and electronic, removal of monitoring schedule specifics as will be added to monitoring plan P34-EU data protection provision added (section 5.5 a), SAE added (section 6 P36-Clinicians amended to Site investigators P37-Clarification regarding release of device and addition of unique device code for accountability purposes (section 10), addition of Ethics approval and removal of HRA approval and addition of wording "no device will be released to site…undertaken/scheduled" (section 11 b) P38-EU data protection provisions added P45 addition of "safety update reports will be in accordance with national provisions" (section 13.5 a), amendment of safety reporting numbers for SAEs and USADEs (section 13.6) P47-signatures amended to account for additional investigators P48 addition of PI declaration of compliance P49-Additonal PI signatures P56-58 Addition of Appendix 5 –EU country specific safety reporting requirements P59-60-Addtion of Appendix 6-Amendment History
2 (Non Substantial) UK Only	CIP v4.1 dated 27th January 2017	Yvanne Enever	P1 Addition of wording "Project is supported in part by a grant of the European Community (ALIVER EU-grant No 733057)" as required by the European commission grant conditions. P 12 and 14-Clarification of filter name to be used during albumin replacement in the DIALIVE arm P 12-to correct the serum albumin level to be achieved as this stated in error that a level of of >30 g/L should be achieved rather than establishing the level of baseline for the patient. P45-corrected the safety email address of the CRO P48 Amended version of CIP in compliance statement.

Amendment No.	CIP version no.	Author(s) of changes	Details of changes made
3. Substantial Amendment UK only Substantial Amendment Number 1 France, Germany and Spain	CIP v5.0 dated 16th October 2017	Banwari Agarwal/Jaak Minten/Yvanne Enever	To align all versions of CIP across EU member states following EC and CA approvals in Germany, France and Spain. P1 Version and date change. P2-3 All site details removed and a separate master list will be maintained by the CRO. Only the Coordinating Investigator is now identified. Removal of Edinburgh as a site and replacement of Basildon Hospital (UK). P6-7 Updates to abbreviations. P8 Additions of definitions of SOC and DIALIVE treatment to ensure it is explicit for site staff. This does not change change the original study concept. P10 To clarify in the secondary end points DIALIVE + SOC vs SOC alone. The secondary outcomes have not changed. To add in the requirements for plasma endotoxin and albumin measurements to ensure explicit requirements outlined across both arms. To provide greater clarity of when the measurements will be taken from. P11 To outline that some exploratory end points will be centre specific according to local capabilities. The addition of assessment of coagulation and haemostasis as a result of the 2 device related SAEs seen in subject 001. P12-Greater clarity provided to ensure sites understand the DIALIVE treatment session vs DIALIVE treatment cycle requirements. This does not alter the original study concept. Additional wording to outline the statistical analysis requirements ensuring that **all** patients will be evaluated for SAEs in line with the primary end point for the study. P13 Clarity regarding screening assessment requirements and commencement of DIALIVE treatment prior to end of Day 1. This allows DIALIVE patients who meet the Day 1 requirements at randomization (Day 0) to commence treatment. Further clarification has been provided regarding the use of test results obtained prior to screening to ensure tests are not unnecessarily repeated. P14 Further clarification has been provided to outline the rationale and use of the Hepalbin filter for replacement albumin and to amend the levels of albumin replacement for DIALIVE patients based on the exposure of patient 001 to the DIALIVE and additional in vitro testing conducted. P17 Clarification provided to ensure patients requiring CRRT will undergo this after their DIALIVE a treatment. P20 Additional pre clinical results (invitro data) has been added. No study adaptations are required as a result of these data. The IB has been updated accordingly. P 23/24 Risks-hypoalbuminemia and potential mechanical haemolysis added and aligned with update of the IB P28 The full primary and secondary end points for the study have been added

			based on feedback from the German Competent Authority. Assessment of coagulation and haemostasis added to further explore the findings seen in patient 001. P31 Additional wording has been included to clarify the use of corticosteroids and to outline the determination of HE assessment. P32 The additional exclusion to include known allergies to heparin has been included based on the recommendation of the German Competent Authority. Additional wording included to reflect the requirement of a patient loosing capacity as per the original approval for the study. Clarification of patient withdrawal criteria included to outline the re-inclusion of patients following response to antibiotic therapy after 24-48hrs. P35 Additional wording to again clarify the commencement of DIALIVE treatment before the end of Day 1 and that screening failure patients (due to the inability to commence DIALIVE treatment before end of Day 1) maybe re-consented and re-screened. Additional wording to further describe the statistical requirements of the study. P 34-P39 Separation of Assessment Schedules per arm. Additional clarification on test requirements and parameters for taking samples which are in line with the newly developed Laboratory Manual. The test requirements have not changed from the original study concept. P42 Additional wording to clarify the statistical considerations. P45 Reference made to the TMG Charter. P46 Update to ensure compliance with national regulations rather than UK only regulations P47 Additional wording to further clarify the requirements of the consultee. P53 The DIALIVE safety email address corrected P54 Requirement to send the outcome reports of the DSMB to the Competent Authority as required by the German Competent Authority and the requirement to implement the outcome as an amendment requiring approval. P56 Removal of PI signatures to have one signature page per site to allow ease of signature collection. P63-65 Update to Appendix 6 to be in line with National requirements P66-69 Inclusion of Amendment history per Member State
4. Substantial amendment for AUSTRIA	CIP v 5.1 dated 31 May 2018 with Amendment 1 dated 10th December 2018	Jaak Minten	§2.1 Model number of Prismaflex (Model Ref: 60 23014700) and applied software version (version 8.1) is added. §2.3 A footnote is added to refer to the absence of model numbers for both the septeX and oXiris filters as this is the brandname given by Gambro/Baxter. § 3.3. The “first use syndrome”, which is not specifically related to the DIALIVE but rather a risk associated with any type of extracorporeal circulation exposure, is added in the section. § 3.4, Risk associated with the administration of albumin have been added to the bullet “albumin” and the use of telepressin is indicated.

			§ 6.3.2. In/exclusion criteria. Per the user manual of oXiris and setpeX the body weight of the treated patient should be above 30 kg. Patient lighter than 30 kg will not be allowed to be enrolled in the study §9 Amendments to the CIP. The Prismaflex utilizes certain software to appropriately run the pumps according to specific settings. Per the user manual of oXiris and septeX a software vesion 4.0 or higher is to be used. For the DIALIVE study, in Austria a software version 8.1 only will be allowed.
5. Substantial amendment for Denmark	CIP v 5.1 dated 31 May 2018 with Amendment 1 dated November 2018	Jaak Minten	§6.1.7. Expected dropout rate is specified and 28 days of FU for patients dropping out early is added. § 6.1.7.. The follow up procedure for patients that have not completed a treatment cycle is specified to be 28 days. §6.3.3. It is specified that patients who experience a serious adverse device or procedure related event and subsequently withdraw from the study will be followed until the reported issue is solved/clarified. §6.5. In case a subject is lost to follow-up, it is specified that the monitor will check the appropriateness of the investigational site's attempts to contact the subject and/or his contact person. § 6.5. It is specified that the Informed Cosnent Forms, letters of authority (EC and CA) and all serious adverse events are for 100% monitored. §8.1.3. Biomarker sample retention time specified (limited to 5 yrs).
6. Substantial amendment for Belgium	CIP v 5.1 dated 31 May 2018 with amendment v 5.2 BE dated December 2018	Jaak Minten	Title: the title was adjusted to include the underlying disease of ACLF, i.e. "alcohol related cirrhosis".
7Substantial amendment.	CIP v 6.0 All countries	Jaak Minten	Version 6.0 attempts to make the CIP uniform for all countries where the study is executed. It includes the modifications that are addressed in version 5.1 (for countries Romania, Austria, Belgium and Denmark – listed above) and in addition: - Implementation of the DIALIVE treatment recommendations as specified by the DSMB following their safety review of 3rd July 2018 - Implementation of more clarification and specification for the appropriate selection criteria of ACLF patients based on 1, 2 or 3 organs failing (grade 1, 2 and grade 3a) - Implementation of the need to obtain more clinical evidence of the treatment aspects of the DIALIVE dialisys as recommended by the investigators / DSMB and by increasing the number of observations, but without jeopardizing the safety review as established by the DSMB charter

			These changes resulted in the specific modifications of following elements: Title: includes reference to “alcoholic cirrhosis”. Summary: - Target patient population is adjusted to include AKI 1b, ACLF 3a and patients with sole high bilirubine levels - includes in/exclusion criteria, omitted section of Definitions. - Timing and frequency of biomarker sampling adjusted to increase accuracy of parameter changes induced by treatment - Added 3 month follow up for patients of cohort 4-5 to assess survival - Added clnical benefit endpoints on clinical and economical aspects. - Adjusted statistical analysis section towards ‘unblinded’ analysis and interim data extractions to comply with ALIVER European research program - Added footnote ‘6’ to guide investigators during screening - Referenced the national legislation on GDPR and vulnerable patient population. Table of contents is corrected. List of abbreviations: list completed with additional abbreviations. §2.2. Included section on Definitions (from previous Summary): screen failures and use of corticosteroids is clarified; ‘nuts and bolts’ of DIALIVE treatment section added; adjusted treatment cycle to 3 sessions of 8-10 hrs. §2.4. Corrected Figure 1 to specify that DIALIVE is sub-part of total extracorporeal circulation circuit. § 2.6. Moved description to §2.2. Definition. § 2.7. and §2.8. Literature section is updated to include info on CANONIC study, on ACLF treatment and referring to recent literature, providing additional rationale for EAA analysis and endotoxin removal. §3.2.Risk of hypotension added; completed mitigation action in case of misuse. §3.5. Specification added that outcome of review by TMG & DSMB is implemented to mitigate additional observed risks. § 4.1 Adjusted primary objective towards the appropriate target population. Reworded the secondary objective. § 4.2. Elaborated on the underlying reason for conducting the study, keeping the hypothesis as specified. §5. Specified that DIALIVE treatment is to be done in ICU or HDU environment with adequate monitoring capabilities. §6.1 and 6.1.7 Sample size adjusted from 24 to 30. §6.1.2 & 6.1.3. Formulated end-points more precisely. §6.1.3. Adjusted biomarker sampling to include D2 (DIA) and D3 (SoC), and start and end of each dialysis session; added D90 follow up; added clnical and economical outcome endpoints.

			§ 6.1.6. Added 'luminometer' as sponsor's property. §6.1.7. Adjusted sample size from 24 to 30. Added dropout rate of 17% and 28 days FU for patients dropping out early. §6.2. specified use of IWRS and 5 instead of 4 cohorts of patients to reflect new sample size. §6.3.1. Inclusion criteria. Inclusion of ACLF3a, AKI 1b and sole severe liver failure patients. Specified "consultee" or "legal representative" as defined by national/local legislation. Defined better patients under corticosteroids and omitted 'uncontrolled infected' patients (as is exclusion criterion). §6.3.2. Exclusion criteria. Specified better "haemodynamical instability", "respiratory organ failure", added "ACLF 3b", and "INR > 3 as exclusion criteria. §6.3.3. Added/modified rules of therapy discontinuation and study stop. §6.3.4. Removed definition on start time of DIALIVE treatment. §6.3.5. Adjusted study duration. §6.3.6. Adjusted duration of patient participation in study. §6.3.7. Adjusted sample size of 24 to 30. §6.3.8. Adjusted study duration. §6.4. Procedures.Implemented schemes to illustrate the study design, adjusted scheme on different study cohorts, adjusted tables to reflect actions on D3 and D90 for SoC and D2/3 and D90 for DIALIVE patients, and added action to be implemented in DIALIVE patients on D1-10 per recommendations of DSMB. Removed irrelevant measures. §6.5.a. Added that modifications requested by EC/CA will be implemented per local/national regulations, and that GDPR is adhered too. §7. Specfied the unblinded nature of statistical safety analysis and the need for interim data extractions per the ALIVER program requirements from the EU. §13. Adjusted the Informed Consent Procedure to comply with multi-national and local regulatory/ethical considerations to allow or disapprove inclusion of patients who have reduced capacity or are unconscious, with or without additional consent from a consultee, legal representative, next of kin or other appointed person. Consent must be obtained per local/national regulations and consent remains valid until study completion, withdrawal or exclusion. §14.6 Added information on risks observed during dialysis in patients from the first 2 cohorts: hypotension, albumin depletion and anemia. Added reference to §3.2 with known risks associated with extracorporeal circulations. §15. Added paragraph on vulnerable unconscious patients: local and national regulations must be complied with prior allowing inclusion of an unconscious patient. §18. Adjusted version and date of signature page. §19. Adjusted bibliography to reflect current references.

			Appendix 1: Adjusted table with ACLF definitions, provided more complete reference information. Appendix 2: Incerted definitions of AKI criteria and Lille score. Appendix 3-4: provided more complete references for MELD score and West Haven criteria. Appendix 6: added specific AE-reporting requirements for the countries AU, RO, BE, DK. Appendix 7: Added summary of DSMB outcome review meeting after 1st and 2nd cohort of patients. Appendix 8: added illustrations of clinical procedures to apply treatlment and perform blood/uring/biomarker sampling correctly. Appendix 9: updated table with current changes.
8.Substantial amendment (all countries)	Version 7.0	10 Oct 2019	§2.2. Definition of first dialysis session on DAY 0 is further clarified. §2.2. Timing of effluent sampling is specified to be between 8-10 hrs instead of 8-12 hrs. §5.4. Added weight/height measurements on Day 2 and Day 3 of study schedule of the DIALIVe-arm §5.4. Removed the note under graph of study schedule as redundant. § 7. 1.1. Added paragraph on how to avoide confusion when 'day 1 of treatment' falls on DAY 0 of the study schedule. §12. Made lay-out changes to have correct numbering of paragraphs. §15. Added section on the assignment of legal representative.