CONFIDENTIAL

VTL-308 PROTOCOL (AMENDMENT 1)

Title A Randomized, Open-label, Multicenter, Controlled, Pivotal Study to Assess Safety and Efficacy of ELAD® in Subjects with Alcohol-Induced Liver Decompensation (AILD)

IND 005479

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Sponsor Vital Therapies, Incorporated
15010 Avenue of Science, Suite 200
San Diego, CA 92128

Amendment 1 20 November 2015

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PROTOCOL SYNOPSIS

Sponsor: Vital Therapies, Inc., San Diego, CA

Product Name: ELAD®

Protocol Number and Title: VTL-308, A Randomized, Open-Label, Multicenter, Controlled, Pivotal Study to Assess Safety and Efficacy of ELAD in Subjects with Alcohol-Induced Liver Decompensation (AILD)

Planned Study Centers: Approximately 40 study centers in the United States and Europe

Phase of Development: Phase 3

Study Objectives:

The primary objective of the study is to evaluate safety and efficacy of ELAD® with respect to overall survival (OS) of subjects with a clinical diagnosis of alcohol-induced liver decompensation (AILD) through at least Study Day 91, with follow-up Protocol VTL-308E providing additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308 study termination (after the last surviving enrolled subject completes Study Day 91). The primary objective will be assessed using a Kaplan-Meier survival analysis of the Intent-to-Treat (ITT) population utilizing a log-rank test.

The secondary objective is to evaluate the proportion of survivors at Study Day 91 using a chi-squared test.

Study Design:

VTL-308 is a randomized, open-label, multicenter, controlled, pivotal study of subjects with AILD. A minimum of 150 subjects meeting the eligibility requirements of the study will be randomly assigned in a 1:1 ratio to receive either standard of care treatment for AILD (as defined in the protocol) plus treatment with the ELAD System (ELAD group) or standard of care treatment for AILD alone (Control group).

Central randomization will be performed to ensure that neither Sponsor, site, nor study personnel will have any foreknowledge of subject treatment assignment.

Because ELAD treatment must take place in an Intensive Care Unit (ICU), or a Step-Down Unit (SDU) with an equivalent standard of care if written pre-approval has been given by VTL, all subjects considered for enrollment must be considered eligible for ICU or SDU placement. Control subjects need not be placed in an ICU or SDU setting unless deemed necessary by the Investigator due to the severity of their illness.

Screening evaluations and assessments will be completed for both ELAD and Control subjects and reviewed against inclusion/exclusion criteria prior to Randomization. For both study groups, ELAD-treated and Control, the time of Randomization will define the time of study entry (Hour 0, Study Day 1, study baseline) and inclusion in the ITT population. Laboratory draws for the first post-randomization blood tests required by the protocol will begin with the next standard laboratory draw (typically the following morning).

In addition to the Screening evaluations, because there will be a delay between Randomization and initiation of ELAD treatment, a further assessment to confirm the subject's safety eligibility will be undertaken. This assessment will be conducted in the same manner for both ELAD and Control subjects. Either the principal investigator or sub-investigator trained on the study must have evaluated the safety eligibility results and be immediately available when ELAD treatment begins in order to assess changes in the subject's condition since Randomization. This is especially important when evaluation changes during the 24 hours preceding initiation of ELAD treatment in order to determine whether the subject remains eligible for extracorporeal treatment.

For all subjects randomized, the following key safety factors must be evaluated within 6 hours prior to ELAD treatment initiation, or at End of Study Day 2 (±6 hours) for Control subjects: platelet count, international normalized ratio (INR), serum creatinine, evidence of infection unresponsive to antibiotics, hemodynamic instability, bleeding status, and status with respect to ventilation, intubation, or need for hemodialysis. These factors will be assessed in accord with the pertinent exclusion criteria (2, 3, 4, 7, 9, 10, 15, and 16, relative to the safety evaluation time point), and subjects must be assessed within 6 hours prior to ELAD treatment initiation, or at End of Study Day 2 (±6 hours) for Control subjects, and subjects must continue to be eligible based on these exclusion criteria to remain in the modified intent-to-treat (mITT) population.

In addition, vital signs will be taken, total bilirubin measured and a MELD score will be calculated.

The standard of care labs may fulfill this requirement provided they are drawn and evaluated within 6 hours prior to ELAD treatment initiation.

- If an ELAD subject fails to meet these criteria, ELAD treatment will not be initiated, the subject will continue to receive standard of care, and the subject will be exluded from the modified intent-to-treat (mITT) population.
- If a Control subject fails to meet these criteria, the subject will continue to receive standard of care, and the subject will be excluded from the mITT population.

Subjects randomized to the ELAD group will be treated with ELAD for a minimum of 3 days (72 hours).

ELAD treatment may be interrupted for a period of *less* than 6 hours then restarted with the same set of cartridges provided that, in the opinion of the Investigator, the subject remains eligible for treatment. ELAD treatment may be interrupted for a period of *more* than 6 hours but less than 72 hours, if, for example, to allow for the conduct of a diagnostic or therapeutic procedure or for management of an adverse event, or should there be a need to interrupt the use of anticoagulants. Should this circumstance arise, a new set of ELAD cartridges must be used if treatment of the subject will continue, and the subject must meet the same safety eligibility criteria as are required prior to the initiation of treatment (See Section 5.4.2.1.1).

ELAD treatment will be discontinued and will not be restarted if any of the following discontinuation criteria are met. If ELAD treatment is discontinued, subjects will receive standard of care therapy alone and be followed through Study Day 91. Discontinuation criteria include the following:

- Continued ELAD treatment is judged to be futile, defined as an increase of more than 25% in total bilirubin at 72 (+6) hours (includes clinical consideration of impact of artifactual hyperbilirubinemia) compared to the most temporal measurement of total bilirubin taken within 6 hours prior to ELAD initiation. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin (e.g., administration of packed red blood cells, plasma exchange);
- Subject has been off treatment for more than 72 hours;
- Subject has suffered an adverse event that, in the Investigator's opinion, requires ELAD treatment to be discontinued, and/or which cannot be resolved within a period of 72 hours;
- Subject has suffered a disseminated intravascular coagulopathy (DIC) event;
- Subject develops an indication for hemodialysis;
- Subject requires an extracorporeal procedure that takes precedence over ELAD treatment;
- Subject has been treated for a maximum of 120 hours within five (5) 24-hour periods regardless of treatment interruption;
- Subject undergoes orthotopic liver transplantation;
- Subject is discharged from the hospital;
- The first set of ELAD cartridges has been replaced for any reason and the second set also requires replacement, or the subject fails safety eligibility checks for the second set;
- Subject or legally-authorized representative withdraws consent for further ELAD treatment;
- Investigator decides to stop ELAD treatment;
- ELAD System performance issues arise that cannot be resolved within a period of 72 hours;
- Subject experiences catheter-related issues that cannot be resolved within a period of 72 hours.

Subjects in both groups will be evaluated throughout the 91-day study period.

If standard of care therapy as defined by the institution is consistent with discharging the subject home, then the subject should be discharged. Prior to discharge, the subject will be advised to attend all protocol-required visits. Instructions for home visits will also be reviewed.

An extension of this study, VTL-308E, will provide extended follow-up of subjects enrolled in VTL-308 over a period of 5 years to determine incidence of survival, cancer, liver transplant, and quality of life. Data obtained

from the extension study will provide additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308 study termination (after the last surviving enrolled subject completes Study Day 91).

Efficacy Analyses

Primary EfficacyAnalysis:

Overall survival (OS) of AILD subjects through at least Study Day 91

OS will be assessed using a Kaplan-Meier survival analysis of the intent-to-treat (ITT) population utilizing a log-rank test, with follow-up Protocol VTL-308E providing additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308 study termination. Model-based estimates and confidence limits will be calculated for median survival by treatment group and hazard rates along with the hazard ratio and its confidence limits. This analysis will also be carried out on the mITT, Per-Protocol (PP) and Safety populations as sensitivity analyses. Two-tailed alpha for the log-rank test will be set at 0.05. The statistical analysis plan will outline the methods used to account for missing data in this and all other analyses.

Secondary Efficacy Analyses:

Proportion of Survivors at Study Day 91

A chi-square test will be used to evaluate the proportion of subjects who survived at End of Study Day 91 based on the ITT population. Two-tailed alpha will be set at 0.05. This analysis will also be carried out on the mITT, PP and Safety populations as sensitivity analyses.

Safety Assessment

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and incidences will be presented by treatment group using MedDRA Preferred Terms within System Organ Classes for the Safety Population. The following will also be summarized by treatment group:

- Adverse Events
- Mean Arterial Pressure (MAP)
- Clinical laboratory values
- The use of concomitant procedures including concomitant blood product administration (e.g., whole blood, packed red blood cells, platelets, fresh frozen plasma, albumin, cryoprecipitate)
- Concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial line)

Inclusion Criteria
Subjects must meet ALL inclusion criteria to be eligible for the study:

1. Age ≥18;
2. Total bilirubin ≥16 mg/dL (≥273.6 µmol/L);
3. A clinical diagnosis of alcohol-induced liver decompensation (AILD), based upon lab test or medical history or family interview with a causal relationship and temporal association (6 weeks or less) of alcohol use and hospital admission for this episode of AILD;
4. Maddrey score ≥32;
5. Subjects must have AILD that is severe acute alcoholic hepatitis (sAAH) diagnosed with either:
    a. A confirmatory liver biopsy, OR
    b. Two or more of the following:
        i. Hepatomegaly,
        ii. AST > ALT,
        iii. Ascites,
        iv. Leukocytosis (WBC count above lab normal at site);

    Note: Subjects will be classified as either:
        a. AILD that is sAAH with **no** underlying liver disease other than alcoholic liver disease, OR
        b. AILD that is sAAH **with** evidence of underlying liver disease other than alcoholic liver disease which must be documented by:
            i. Liver biopsy, AND/OR
            ii. Laboratory findings, AND/OR
            iii. Medical history;
6. Not eligible for liver transplant during this hospitalization;
7. Subject or legally-authorized representative must provide Informed Consent;
8. Subject must be eligible for Standard of Care treatment as defined in the protocol.

Exclusion Criteria
Subjects must NOT have any of the exclusion criteria to be eligible for the study:

1. Age ≥50;
2. Platelet count <40,000/mm^3;
3. International Normalization Ratio (INR) >2.5;
4. Serum Creatinine ≥1.3 mg/dL (≥115.04 µmol/L);
5. MELD score ≥30;
6. AST >500 IU/L;
7. Evidence of infection unresponsive to antibiotics (e.g. increased tissue involvement relative to initial diagnosis, clinical worsening of symptoms, etc.) indicated by any of the following:
    a. Presence of sepsis or septic shock; OR
    b. Positive blood cultures (bacteremia, fungemia) within 72 hours prior to Randomization; OR
    c. Presence of spontaneous bacterial peritonitis during the 2 days prior to Randomization; OR
    d. Clinical and radiological signs of pneumonia;
8. Evidence of reduction in total bilirubin of 20% or more in the previous 72 hours. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin (e.g., administration of packed red blood cells, plasma exchange);
9. Evidence of hemodynamic instability as defined by the following:

a. Systolic blood pressure <90 mmHg with evidence of diminished perfusion unresponsive to fluid resuscitation and/or low-dose pressor support; OR
b. Mean arterial pressure (MAP) <60 mmHg with evidence of diminished perfusion unresponsive to fluid resuscitation and/or low-dose pressor support; OR
c. Requirement for escalating doses of vasopressor support prior to Screening; OR
d. Subject on vasopressors, including but not limited to those listed below, at doses above the following at Screening or Randomization:
   - Dobutamine: 5.0 µg/kg/min
   - Dopamine: 2.0 µg/kg/min
   - Norepinephrine: 0.02 µg/kg/min
   - Phenylephrine: 1.0 µg/kg/min
   - Vasopressin: 0.02 U/min

10. Evidence of active bleeding, major hemorrhage defined as requiring ≥2 units packed red blood cells to maintain a stable hemoglobin occurring within 48 hours prior to Randomization, or with banding of gastroesophageal varices during the 7 days immediately preceding screening;
11. Clinical evidence of liver size reduction due to cirrhosis [liver size of the craniocaudal diameter (sagittal view) <10 cm when measured on the mid clavicular line (or equivalent measurement) by ultrasound, or liver volume <1200 cc as determined by CT or MRI], unless Investigator interpretation of the clinical evidenceindicates liver size of <10 cm or volume <1200 cc is not considered reduced for the individual subject, and Sponsor agrees;
12. Occlusive portal vein thrombosis impairing hepatopetal flow, or evidence of bile duct obstruction;
13. Evidence by physical exam, history, or laboratory evaluation, of significant concomitant disease with a life expectancy of less than 3 months, including, but not limited to:
    a. Severe acute or chronic cardiovascular, central nervous system, or pulmonary disease;
    b. Cancer that has metastasized or has not yet been treated;
    c. Severe metabolic abnormalities that have not been corrected (See Section 5.1.3);
14. Subject has chronic end-stage renal disease requiring chronic hemodialysis for more than 8 weeks (not classified as hepatorenal syndrome);
15. Subject ventilated or intubated;
16. Subject on hemodialysis;
17. Subject has liver disease related to homozygous hemachromotosis, Wilson's disease, has non-alcoholic fatty liver disease, or Budd-Chiari Syndrome;
18. Serological evidence (including viral titers) of active viral hepatitis A, B or C infection. If the investigator suspects that the subject may be at risk for viral hepatitis A, B or C, and no serology is available, then serologies must be obtained prior to Randomization, as a positive serology would be exclusionary;
19. Pregnancy as determined by serum β-human chorionic gonadotropin (HCG) results, or subjects of child-bearing potential not willing to use effective means of contraception, without history of medical or surgical sterilization;
20. Participation in another investigational drug, biologic, or device study within one month of enrollment, except for observational studies (the observational study setting should not affect the safety and/or efficacy of the VTL-308 clinical trial);
21. Previous liver transplant;
22. Previous enrollment in the treatment phase of another ELAD trial;
23. Have a Do Not Resuscitate or a Do Not Intubate (DNR/DNI) directive (or such local equivalent) or any other Advanced Directive limiting Standard of Care in place (the DNR/DNI criterion is not applicable in Europe);
24. Refusal to participate in the VTL-308E follow-up study;
25. Inability to provide an address for home visits.

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ABBREVIATIONS

AAH	Acute Alcoholic Hepatitis
AASLD	American Association for the Study of Liver Diseases
ACLF	Acute on Chronic Liver Failure
AE	Adverse Event
AILD	Alcohol-induced liver decompensation
ALT	Alanine Aminotransferase
AOCH	Acute on Chronic Hepatitis
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
C&S	Culture and Sensitivity
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CMH	Cochran-Mantel-Haenszel
CO_2	Carbon Dioxide
CRF	Case Report Form
CT	Computed Tomography
CVVH	Continuous Venovenous Hemofiltration
CVVHD	Continuous Venovenous Hemodialysis
DNR/DNI	Do Not Resuscitate/Do Not Intubate
DSMB	Data and Safety Monitoring Board
EASL	European Association for the Study of the Liver
EC	Ethics Committee
eCRF	Electronic Case Report Form
ECS	Extracapillary Space
EDC	Electronic Data Capture
EVL	Endoscopic Variceal Ligation
FDA	Food and Drug Administration
FiO_2	Fraction of Inspired Oxygen
HAV	Hepatitis A Virus
HCG	β-Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HE	Hepatic Encephalopathy

HIT	Heparin-Induced Thrombocytopenia
HIV	Human Immunodeficiency Virus
HRS	Hepatorenal Syndrome
ICH	International Conference on Harmonization
ICS	Intracapillary Space
ICU	Intensive Care Unit
INR	International Normalization Ratio
IRB	Institutional Review Board
ITT	Intent-to-Treat
IWRS	Interactive Web Response System
LAR	Legally-authorized Representative
LDH	Lactate Dehydrogenase
LTFU	Lost to follow-up
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model of End-stage Liver Disease
mITT	Modified Intent-to-Treat
mTSR	Modified Treatment Services Review Questionnaire
NAC	N-acetylcysteine
NH_3	Ammonia
NSAID	Nonsteroidal Anti-Inflammatory Drug
O_2	Oxygen
OS	Overall Survival
P_{BR}	Blood return pressure
P_{BW}	Blood withdrawal pressure
P_{EC}	ELAD cartridge pressure
PegIFN	Peginterferon
Peth	Phosphatidylethanol
P_F	Filter pressure
PFS	Progression Free Survivors
P_{PUG}	Pre ultrafiltrate generator pressure
P_{UF}	Ultrafiltrate withdrawal pressure
PP	Per Protocol (analysis)
PT	Prothrombin Time
PTT	Partial Thromboplastin Time

PTx	Pentoxifylline
RBV	Ribavirin
SAAG	Serum-Ascites Albumin Gradient
sAAH	Severe Acute Alcoholic Hepatitis
SAE	Serious Adverse Event
SBP	Spontaneous Bacterial Peritonitis
SD	Study Day
SDU	Step-Down Unit
SOC	Standard of Care
SUSAR	Suspected unexpected serious adverse reaction
TIPS	Transjugular intrahepatic portosystemic shunt
TLFB	Alcohol Timeline Followback Questionnaire
TTP	Time to Progression
UNOS	United Network for Organ Sharing
US	Ultrasound
USA	United States of America
VTL	Vital Therapies, Inc.

1 INTRODUCTION

The liver is one of the largest and most metabolically-complex organs in the body, but has a remarkable capacity for regeneration in response to injury. Vital Therapies, Inc. (VTL) has developed ELAD®, an extracorporeal human hepatic cell-based liver treatment (hereafter referred to as the ELAD® System or ELAD®) to improve survival in patients with liver failure secondary to acute hepatocellular insult with a temporal and causal relationship to alcohol use, and to provide liver support continuously to a subject with compromised liver function. It is postulated that this allows time for the subject's native liver to recover from the acute decompensation, stabilize, potentially regenerate and be restored to a functional state, or to maintain the subject until a suitable donor organ can be found for transplantation.

The current configuration of the ELAD System draws blood from the subject via a dual-lumen catheter placed in a large vein using an extracorporeal pumping unit, and then separates the plasma fluid (ultrafiltrate) from cellular components using a specifically-designed ultrafiltrate generator cartridge. While the cellular components are returned to the subject via the venous access, the ultrafiltrate is circulated at a high flow rate through the four metabolically-active ELAD cartridges. The active ingredients within the ELAD C3A cell cartridges are cloned, immortalized human hepatoblastoma cells (VTL C3A cells) derived from a subclone of the human hepatoblastoma cell line HepG2. The metabolic activity of ELAD cartridges containing VTL C3A cells can be monitored by measuring oxygen and glucose consumption, as well as by the production of hepatocyte-specific proteins.

After circulation through the cartridges, the ultrafiltrate passes through a 0.2-µm pore size filter, is recombined with the cellular components of the subject's blood, and is returned to the subject through the dual-lumen catheter. The toxins metabolized by the VTL C3A cells are thereby returned to the subject to be excreted by the renal or gastrointestinal system. This circulation is maintained continuously for the duration of ELAD treatment, typically between three and five days.

Hepatocellular damage, secondary to a variety of insults (infectious agents, alcohol, exogenous drugs, autoimmunity, fatty liver disease, etc.), can result in chronic liver disease if the underlying etiology is not effectively treated. This condition is characterized histopathologically by increasing degrees of fibrosis and cirrhosis, and frequently remains subclinical and/or undiagnosed. Often as a result of a secondary insult, the chronic hepatitis can decompensate, leading to a life-threatening disorder known as Acute on Chronic Liver Failure (ACLF) (Jalan 2012).

There are a number of types of ACLF, including acute flare of Chronic Hepatitis B and Alcohol-induced Liver Decompensation (AILD). AILD is defined as a progressive inflammatory liver disease leading to an acute form of alcohol-induced liver injury which arises when the proximate cause of the acute decompensation is the consumption of alcohol. A specific, well-defined subset of AILD is Severe Acute Alcoholic Hepatitis (sAAH), generally defined as progressive inflammatory liver disease leading to an acute form of alcohol-induced liver injury that occurs with the consumption of large amounts of alcohol in patients with relatively mild, underlying chronic alcoholic liver disease. These sAAH patients typically present with a characteristic pattern of jaundice, ascites, hepatomegaly, AST > ALT, and leukocytosis. Liver biopsies in sAAH subjects reveal the following histological characteristics: hepatocellular damage/ballooning, Mallory bodies, polymorphonuclear infiltration, steatosis, lobular fibrosis,

megamitochondria, and bilirubinostasis (Altamirano 2014). However, liver biopsy is deemed to be too invasive for routine use in US patients (O'Shea 2010). Various degrees of fibrosis and hepatitis are present in AILD patients (Altamirano 2014 and Orrego 1987).

The damage to the liver from continuing insults causes the gradual development of fibrosis in the liver over time, which results in a decrease of both liver function and the ability to regenerate after decompensation (Schuppan and Afdhal 2008). The fibrosis progresses to cirrhosis when this process has been underway for many years. The progression of fibrosis to cirrhosis results in a shrunken liver, distortion of hepatic lobules, and continued loss of hepatocytes (due to replacement with fibrotic tissue) that leads to progressive and recurrent episodes of decompensation. This progressive loss of hepatocyte mass impairs the liver's inherent ability to regenerate following decompensation. In AILD patients with hepatomegaly, there appears to be sufficient hepatocyte mass that may allow hepatic regeneration and reversal of the decompensation. They can be discriminated from patients with end-stage liver disease by measurements of liver size using imaging techniques such as ultrasound, CT scan, or MRI.

Treatment options for patients with AILD are limited. In particular, patients with severe sAAH (defined as a Maddrey Discriminant Function of >32) have a poor prognosis, with 90-day survival of around 50% (Maddrey 1978). Regimens that have been used for the past 40 years, including corticosteroids (Morris 2005; Thursz 2015), theophylline (Kendrick 2010) with corticosteroids, pentoxifylline (Parker 2013; Lebrec 2010; and Thursz 2015), and infliximab (Naveau 2004), have had no significant effect on the long-term survival of patients with AILD. Other contraindications to steroid use in patients with AILD include active gastrointestinal bleeding, renal failure, acute pancreatitis, active tuberculosis, uncontrolled diabetes, and psychosis (Singal 2011; Singh 2015). Subjects who do not respond to seven days of steroid therapy (as defined by the Lille score) have a particularly dismal prognosis, with 6-month survival rates of less than 25% (Louvet 2007). A recent study of >1100 subjects with a clinical diagnosis of sAAH (the STOPAH study) demonstrated a reduction in 28-day mortality in subjects administered steroids that did not reach statistical significance, with no improvement in survival at 90 days or 1 year. This study also revealed no survival benefit at any time point for pentoxifylline relative to placebo (Thursz 2015).

It has been hypothesized that the common characteristic of subjects that have benefited from ELAD treatment is that they have more extensive hepatic reserve capacity than those with end-stage liver disease accompanied by cirrhosis, thereby having increased potential for liver regeneration. However, there is no accepted method to measure liver reserve capacity or to quantify liver regeneration. The extent of chronic liver damage must be estimated, for example by assessing liver size, medical history and subject condition. Using these parameters, the current ELAD clinical trial program has been designed to focus on those subjects without evidence of end-stage liver disease, in whom a single course of ELAD treatment of 3-5 days may induce stabilization, possibly associated with increasing hepatic reserve secondary to liver regeneration, thereby leading to improved survival in the absence of liver transplantation. In addition, data from the VTI-208 study have indicated that subjects with secondary organ complications, such as acute kidney injury and moderate to severe coagulopathy have an increased mortality risk if administered ELAD treatment compared with standard of care therapy and these subjects will be excluded from future ELAD clinical trials (Gustot 2015).

In a Phase 2 study (VTI-206) of ELAD, AILD was defined based on clinical judgment and substantiated by evidence from laboratory tests, medical history, or family interview of a temporal association (6 weeks or less) and causal relationship between the use of alcohol and the onset of symptoms. Analysis of this predefined subset of subjects in VTI-206 provided preliminary evidence that those treated with ELAD along with standard of care may have prolonged survival versus standard of care alone.

VTI-208 was designed to provide data on the safety and clinical utility of the ELAD System in the treatment of subjects with AILD. The primary endpoint evaluated overall survival of subjects with a clinical diagnosis of alcohol-induced liver decompensation (AILD) up to at least Study Day 91, with follow-up Protocol VTI-208E providing additional survival data up to a maximum of 5 years that was included, as available, through VTI-208 study termination (after the last surviving enrolled subject completed Study Day 91). This was assessed using a Kaplan-Meier survival analysis of the ITT population utilizing a log-rank test. The study was conducted at 40 centers in the US, Australia, and Europe.

In addition to collecting long-term survival data, VTI-208E will help inform the incidence of cancer, liver transplant, and an assessment of quality of life over the extended 5-year follow-up period.

The VTI-208 study included 203 AILD subjects in the ITT population, 96 and 107 subjects randomized to ELAD treatment and Control (standard of care only) groups, respectively. The Kaplan-Meier analysis of the overall survival (OS) in the ITT population was not statistically different. However, in a predefined subset of 120 subjects with MELD scores <28 that consisted of 51 and 69 subjects in the ELAD-treated and Control groups, respectively, the Kaplan-Meier analysis of the OS did approach statistical significance. In another pre-specified exploratory analysis of subjects with less than the median age of 46.9 years, the Kaplan-Meier analysis of the OS favored the ELAD treated AILD subjects. These analyses provide the rationale to conduct this VTL-308 study in AILD subjects younger than 50 years old who have MELD scores <30. Other subset analyses suggest that subjects with acute renal dysfunction, defined as serum creatinine ≥1.3 mg/dL and severe coagulopathy (INR >2.5) (Moreau 2013), should be excluded from this study.

The safety profile of ELAD observed in the VTI-208 study was similar to that observed in prior ELAD studies.

2 STUDY OBJECTIVES

The primary objective of the study is to evaluate safety and efficacy of ELAD® with respect to overall survival (OS) of subjects with a clinical diagnosis of alcohol-induced liver decompensation (AILD) through at least Study Day 91, with follow-up Protocol VTL-308E providing additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308 study termination (after the last surviving enrolled subject completes Study Day 91). The primary objective will be assessed using a Kaplan-Meier survival analysis of the Intent-to-Treat (ITT) population utilizing a log-rank test.

The secondary objective is to evaluate the proportion of survivors at Study Day 91 using a chi-squared test.

The exploratory objectives will identify potential differences between treatment groups in factors related to standard demographics, selected baseline characteristics, medical history, and standard of care. These factors will be prespecified in the SAP, and are based on previously-identified and medically-pertinent influences on treatment outcomes in alcoholic liver disease, as well as on factors shown to be important in prior studies of ELAD. In addition, the relationship between outcomes and ELAD System performance, therapeutic interventions of interest and administration of concomitant pharmacotherapies of interest will be assessed in order to help inform product labeling and use. Furthermore, changes in certain biomarkers of interest will also be evaluated.

3 STUDY DESIGN

VTL-308 is a randomized, open-label, multicenter, controlled, pivotal study of subjects with AILD. A minimum of 150 subjects meeting the eligibility requirements of the study will be randomly assigned in a 1:1 ratio to receive either standard of care treatment for AILD (as defined in the protocol) plus treatment with the ELAD System (ELAD group) or standard of care treatment for AILD alone (Control group).

Central randomization will be performed to ensure that neither Sponsor, site, nor study personnel have any foreknowledge of subject treatment assignment.

Because ELAD treatment must take place in an Intensive Care Unit (ICU), or a Step-Down Unit (SDU) with an equivalent standard of care if written pre-approval has been given by VTL, all subjects considered for enrollment must be considered eligible for ICU or SDU placement. Control subjects need not be placed in an ICU or SDU setting unless deemed necessary by the Investigator due to the severity of their illness.

Screening evaluations and assessments will be completed for both ELAD and Control subjects and reviewed against inclusion/exclusion criteria prior to Randomization. For both study groups, ELAD-treated and Control, the time of Randomization will define the time of study entry (Hour 0, Study Day 1, study baseline) and inclusion in the ITT population. Laboratory draws for the first post-randomization blood tests required by the protocol will begin with the next standard laboratory draw (typically the following morning).

In addition to the Screening evaluations, because there will be a delay between Randomization and initiation of ELAD treatment, a further assessment to confirm the subject's safety eligibility will be undertaken. This assessment will be conducted in the same manner for both ELAD and Control subjects. Either the principal investigator or sub-investigator trained on the study must have evaluated the safety eligibility results and be immediately available when ELAD treatment begins in order to assess changes in the subject's condition since Randomization. This is especially important when evaluation changes during the 24 hours preceding initiation of ELAD treatment in order to determine whether the subject remains eligible for extracorporeal treatment.

For all subjects randomized, certain key safety factors must be evaluated within 6 hours prior to ELAD treatment initiation, or at End of Study Day 2 (±6 hours) for Control subjects: platelet count, international normalized ratio (INR), serum creatinine, evidence of infection unresponsive to antibiotics, hemodynamic instability, bleeding status, and status with respect to ventilation, intubation, or need for hemodialysis. These factors will be assessed in accord with the pertinent exclusion criteria (2, 3, 4, 7, 9, 10, 15, and 16 relative to the safety evaluation time point), and subjects must continue to be eligible based on these exclusion criteria to remain in the modified intent-to-treat (mITT) population.

In addition vital signs will be taken, total bilirubin measured, and a MELD score will be calculated.

The standard of care labs may fulfill this requirement provided they are drawn within 6 hours prior to ELAD treatment initiation.

- If an ELAD subject fails to meet these criteria, ELAD treatment will not be initiated, the subject will continue to receive standard of care, and the subject will be exluded from the modified intent-to-treat (mITT) population.

- If a Control subject fails to meet these criteria, the subject will continue to receive standard of care, and the subject will be excluded from the mITT population.

Subjects randomized to the ELAD group will be treated with ELAD for a minimum of 3 days (72 hours).

ELAD treatment may be interrupted for a period of *less* than 6 hours then restarted with the same set of cartridges provided that, in the opinion of the Investigator, the subject remains eligible for treatment. ELAD treatment may be interrupted for a period of *more* than 6 hours but less than 72 hours, if, for example, to allow for the conduct of a diagnostic or therapeutic procedure or for management of an adverse event, or should there be a need to interrupt the use of anticoagulants. Should this circumstance arise, a new set of ELAD cartridges must be used if treatment of the subject will continue, and the subject must meet the same safety eligibility criteria as are required prior to the initiation of treatment (See Section 5.4.2.1.1).

ELAD treatment will be discontinued and will not be restarted if any of the following discontinuation criteria are met. If ELAD treatment is discontinued, subjects will receive standard of care therapy alone and be followed through Study Day 91. Discontinuation criteria include the following:

- Continued ELAD treatment is judged to be futile, defined as an increase of more than 25% in total bilirubin at 72 (+6) hours (includes clinical consideration of impact of artifactual hyperbilirubinemia) compared to the most temporal measurement of total bilirubin taken within 6 hours prior to ELAD initiation. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin (e.g., administration of packed red blood cells, plasma exchange);
- Subject has been off treatment for more than 72 hours;
- Subject has suffered an adverse event that, in the Investigator's opinion, requires ELAD treatment to be discontinued, and/or which cannot be resolved within a period of 72 hours;
- Subject has suffered a disseminated intravascular coagulopathy (DIC) event;
- Subject develops an indication for hemodialysis;
- Subject requires an extracorporeal procedure that takes precedence over ELAD treatment;
- Subject has been treated for a maximum of 120 hours within five (5) 24-hour periods regardless of treatment interruption;
- Subject undergoes orthotopic liver transplantation;
- Subject is discharged from the hospital;
- The first set of ELAD cartridges has been replaced for any reason and the second set also requires replacement, or the subject fails safety eligibility checks for the second set;
- Subject or legally-authorized representative withdraws consent for further ELAD treatment;
- Investigator decides to stop ELAD treatment;

- ELAD System performance issues arise that cannot be resolved within a period of 72 hours;
- Subject experiences catheter-related issues that cannot be resolved within a period of 72 hours.

Note: Regarding the judgment of futility of treatment based upon increase of bilirubin by 25%, Investigators are asked to use clinical judgment in assessing artifactual hyperbilirubinemia (e.g., resulting from transfusion of packed red blood cells) before discontinuing ELAD treatment if they believe ELAD to still be capable of providing clinical benefit. A call to the Medical Monitor should be made to discuss this, should it occur.

Subjects in both groups will be evaluated throughout the 91-day study period.

Subjects undergoing orthotopic liver transplantation during ELAD treatment will be evaluated exactly the same as any other subject who completed treatment early. The evaluation mandated for the first day after treatment will be carried out 24 hours post-transplantation. All subjects (ELAD and Control) receiving a liver transplant will be followed through Study Day 91.

If the subject (ELAD or Control) withdraws consent from the study at any time during the Study, the subject will no longer be monitored by the site through Study Day 91. All efforts should be made to ensure subject continues to consent for safety and follow up.

If standard of care treatment as defined by the institution is consistent with discharging the subject home, then the subject should be discharged. Prior to discharge, the subject will be advised to attend all protocol-required visits. Instructions for home visits will also be reviewed.

An extension of this study, VTL-308E, will provide extended follow-up of subjects enrolled in VTL-308 over a period of 5 years to determine incidence of survival, cancer, liver transplant, and quality of life. Data obtained from the extension study will provide additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308 study termination (after the last surviving enrolled subject completes Study Day 91).

A diagram of the study design and timeline is provided in Figure 2.

4 STUDY POPULATION

4.1 GENERAL CHARACTERISTICS OF THE PROPOSED STUDY POPULATION

Subjects under 50 years of age with a clinical diagnosis of AILD, associated with recent alcohol consumption, with elevated bilirubin, and without evidence of secondary organ failure, as characterized by acute kidney injury, severe coagulopathy, infection unresponsive to antibiotics, hemodynamic instability, recent bleeding and/or the need for intubation.

4.2 ANTICIPATED NUMBER OF RESEARCH SUBJECTS

Approximately 150 subjects meeting the defined eligibility requirements.

4.3 INCLUSION CRITERIA

Subjects must meet ALL inclusion criteria to be eligible for the study:

1. Age ≥18;
2. Total bilirubin ≥16 mg/dL (≥273.6 μmol/L);
3. A clinical diagnosis of alcohol-induced liver decompensation (AILD), based upon lab test or medical history or family interview with a causal relationship and temporal association (6 weeks or less) of alcohol use and hospital admission for this episode of AILD;
4. Maddrey score ≥32;
5. Subjects must have AILD that is severe acute alcoholic hepatitis (sAAH) diagnosed with either:
    a. A confirmatory liver biopsy, OR
    b. Two or more of the following:
        i. Hepatomegaly,
        ii. AST > ALT,
        iii. Ascites,
        iv. Leukocytosis (WBC count above lab normal at site);

    Note: Subjects will be classified as either:

    a. AILD that is sAAH with **no** underlying liver disease other than alcoholic liver disease, OR
    b. AILD that is sAAH **with** evidence of underlying liver disease other than alcoholic liver disease which must be documented by:
        i. Liver biopsy, AND/OR
        ii. Laboratory findings, AND/OR
        iii. Medical history;
6. Not eligible for liver transplant during this hospitalization;
7. Subject or legally-authorized representative must provide Informed Consent;
8. Subject must be eligible for Standard of Care treatment as defined in the protocol.

4.4 EXCLUSION CRITERIA

Subjects must NOT have any of the following exclusion criteria:

1. Age ≥50;
2. Platelet count <40,000/mm^3;
3. International Normalization Ratio (INR) >2.5;
4. Serum Creatinine ≥1.3 mg/dL (≥115.04 µmol/L);
5. MELD score ≥30;
6. AST >500 IU/L;
7. Evidence of infection unresponsive to antibiotics (e.g. increased tissue involvement relative to initial diagnosis, clinical worsening of symptoms, etc.) indicated by any of the following:
    a. Presence of sepsis or septic shock; OR
    b. Positive blood cultures (bacteremia, fungemia) within 72 hours prior to Randomization; OR
    c. Presence of spontaneous bacterial peritonitis during the 2 days prior to Randomization; OR
    d. Clinical and radiological signs of pneumonia;
8. Evidence of reduction in total bilirubin of 20% or more in the previous 72 hours. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin (e.g., administration of packed red blood cells, plasma exchange);
9. Evidence of hemodynamic instability as defined by the following:
    a. Systolic blood pressure <90 mmHg with evidence of diminished perfusion unresponsive to fluid resuscitation and/or low-dose pressor support; OR
    b. Mean arterial pressure (MAP) <60 mmHg with evidence of diminished perfusion unresponsive to fluid resuscitation and/or low-dose pressor support; OR
    c. Requirement for escalating doses of vasopressor support prior to Screening; OR
    d. Subject on vasopressors, including but not limited to those listed below, at doses above the following at Screening or Randomization:
        - Dobutamine: 5.0 µg/kg/min
        - Dopamine: 2.0 µg/kg/min
        - Norepinephrine: 0.02 µg/kg/min
        - Phenylephrine: 1.0 µg/kg/min
        - Vasopressin: 0.02 U/min
10. Evidence of active bleeding, major hemorrhage defined as requiring ≥2 units packed red blood cells to maintain a stable hemoglobin occurring within 48 hours prior to Randomization, or with banding of gastroesophageal varices during the 7 days immediately preceding screening;

11. Clinical evidence of liver size reduction due to cirrhosis [liver size of the craniocaudal diameter (sagittal view) <10 cm when measured on the mid clavicular line (or equivalent measurement) by ultrasound, or liver volume <1200 cc as determined by CT or MRI], unless Investigator interpretation of the clinical evidence indicates liver size of <10 cm or volume <1200 cc is not considered reduced for the individual subject, and Sponsor agrees;
12. Occlusive portal vein thrombosis impairing hepatopetal flow, or evidence of bile duct obstruction;
13. Evidence by physical exam, history, or laboratory evaluation, of significant concomitant disease with a life expectancy of less than 3 months, including, but not limited to:
    a. Severe acute or chronic cardiovascular, central nervous system, or pulmonary disease;
    b. Cancer that has metastasized or has not yet been treated;
    c. Severe metabolic abnormalities that have not been corrected (See Section 5.1.3);
14. Subject has chronic end-stage renal disease requiring chronic hemodialysis for more than 8 weeks (not classified as hepatorenal syndrome);
15. Subject ventilated or intubated;
16. Subject on hemodialysis;
17. Subject has liver disease related to homozygous hemachromotosis, Wilson's disease, has non-alcoholic fatty liver disease, or Budd-Chiari Syndrome.
18. Serological evidence (including viral titers) of active viral hepatitis A, B or C infection. If the investigator suspects that the subject may be at risk for viral hepatitis A, B or C, and no serology is available, then serologies MUST be obtained prior to Randomization, as a positive serology would be exclusionary;
19. Pregnancy as determined by serum β-human chorionic gonadotropin (HCG) results, or subjects of child-bearing potential not willing to use effective means of contraception, without history of medical or surgical sterilization;
20. Participation in another investigational drug, biologic, or device study within one month of enrollment, except for observational studies (the observational study setting should not affect the safety and/or efficacy of the VTL-308 clinical trial);
21. Previous liver transplant;
22. Previous enrollment in the treatment phase of another ELAD trial;
23. Have a Do Not Resuscitate or a Do Not Intubate (DNR/DNI) directive (or such local equivalent) or any other Advanced Directive limiting Standard of Care in place (the DNR/DNI criterion is not applicable in Europe);
24. Refusal to participate in the VTL-308E follow-up study;
25. Inability to provide an address for home visits.

5 STUDY PROCEDURES

Schedules of evaluations are presented by study period in Table 1, Table 2, Table 3, and Table 4.

The pre-screening, screening (inclusion/exclusion criteria assessment), and randomization process is a collaboration between the Investigator and the Sponsor Representative to confirm subject eligibility and ensure subject safety. The process involves pre-screening through the conduct of routine standard medical procedures, subject informed consent (enrollment) followed by conduct of the screening assessment (inclusions/exclusion criteria assessment), and randomization. The Investigator may contact the Medical Monitor or Chief Medical Officer at any time during this process.

5.1 PRE-SCREENING

Pre-Screening is the collection of information to determine subject eligibility *before* obtaining Informed Consent. It includes the review of data obtained from routine standard medical procedures to determine if a patient is potentially an eligible subject for enrollment in this study.

A record of all pre-screened subjects will be maintained by each site on the Pre-Screening Log in the Electronic Data Capture (EDC) system. If a subject is found ineligible for study participation prior to Screening, the reason(s) for ineligibility will be documented on the Pre-Screening Log electronic Case Report Form (eCRF).

It is possible that a subject who does not initially qualify for the study may have a change in status at a later date. If the Investigator believes that the eligibility of the subject has changed, the Sponsor Representative should be notified and the subject may be considered again.

5.1.1 Sponsor Notification

Investigational Review Board/Ethics Committee (IRB/EC) or other governing regulatory body policy dictates the manner in which medical information may be shared with the Sponsor.

1. The local policy allows de-identified medical information to be shared with the Sponsor prior to obtaining Informed Consent.

   OR

2. The local policy requires Informed Consent to be obtained prior to sharing any de-identified medical information with the Sponsor.

When a potential study subject is identified during the pre-screening process, the Investigator or designee must notify the Sponsor Representative by calling the Subject Enrollment Mobile Phone. Medical information may be shared at this time with the Sponsor according to local policy.

Results of medical information from tests or procedures that have been performed as part of routine standard medical care of the patient (and that may be shared with the Sponsor according to the local policy during the pre-screening process) must be provided to the Sponsor Representative. **All personal identifiers (patient names, patient numbers, hospital names and locations, etc.) must be removed/redacted from the data prior to sending them to VTL. Signed Informed Consent Forms must not be sent**.

5.1.2 Pre-Screening Procedures

The results of the tests and assessments listed below, if performed as Standard of Care (SOC) within the previous 24 hours, unless otherwise noted (see Section 5.2.3.2 regarding imaging requirements), should be reviewed. The below tests and assessments, if unavailable as SOC, should NOT be obtained specifically for study review until AFTER a signed Informed Consent Form is obtained. An overview of these procedures is presented in Table 1:

- Demographics (date of birth or age, race, ethnicity, height, weight, gender)
- Relevant medical history
- Documented evidence of temporal association (6 weeks or less) of alcohol use and hospital admission for this episode of AILD. Documented evidence includes patient or other credible person reporting alcohol use, and/or positive blood test
- Physical exam (performed by a Physician, Physician Assistant or Nurse Practitioner if within the scope of practice for institution)
- Vital signs (blood pressure, heart rate, respiration rate, temperature)
- Encephalopathy stage [West Haven Criteria, performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated
- Liver imaging to determine liver size and confirm the absence of occlusive portal vein thrombosis or bile duct obstruction (see Section 5.2.3.2 regarding imaging performed no more than six weeks prior to current hospitalization)
- Routine laboratory evaluations: Creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-fetoprotein
- Complete Blood Count (CBC): White blood cell count (WBC), hematocrit. hemoglobin and platelets
- Prothrombin time (PT), international normalized ratio (INR)
- Serum pregnancy test (if applicable)
- MELD and Maddrey scores will be calculated in accord with Section 5.2.4.

5.1.3 Pre-Screening Laboratory Abnormalities

If during pre-screening, severe metabolic abnormalities are noted, screening and randomization should not be considered until these abnormalities have been corrected. Examples of severe metabolic abnormalities (See Exclusion Criterion 13) include, but are not limited to:

- hypoglycemia (serum glucose <50 mg/dL or 2.8 mmol/L)
- hypocalcemia (serum calcium ≤3.52 mg/dL, ≤0.88 mmol/L, or ≤1.76 mEq/L)
- hypokalemia (serum potassium <7.8 mg/dL, <2 mmol/L, or <2.0 mEq/L)
- hypomagnesemia (serum magnesium <1.2 mg/dL, <0.5 mmol/L, or <1.0 mEq/L)
- hyponatremia (serum sodium <276 mg/dL, <120 mmol/L, or <120 mEq/L)
- hypophosphatemia (serum phosphate <0.93 mg/dL, <0.3 mmol/L, or <0.6 mEq/L)

5.2 SCREENING

The Screening process includes evaluation of the potential subject based on the inclusion/exclusion criteria provided in Section 4. It is the collection of information to determine subject eligibility *after* obtaining Informed Consent. It includes specific procedures to determine if a patient is an eligible subject for randomization into this study. Additional laboratory tests not required for study eligibility, but that are part of determining baseline laboratory values, must also be collected as part of this Screening process.

If a subject fails the Screening process, the subject's Screening information is collected in the EDC system and the subject will be considered a screen failure and not randomized into the study.

It is possible that a subject who does not initially qualify for the study may have a change in status at a later date. If the Investigator believes that the eligibility of the subject has changed, the Sponsor Representative should be notified and the subject may be considered again.

5.2.1 Informed Consent

A subject is enrolled in the study when the subject or their legally-authorized representative (LAR) has signed the Informed Consent Form. This marks the beginning of the Screening process during which study-specific screening procedures may be performed (Section 5.2.3). Screening eCRFs in the EDC system will be completed for all enrolled subjects regardless of the final eligibility determination.

5.2.1.1 *Informed Consent Process*

The Informed Consent Form (ICF) must be approved by the local IRB/EC or governing regulatory body. The ICF must be signed and dated by the subject or LAR and by the person obtaining consent. The original signed ICF will be retained in the subject's study records and a copy will be provided to the subject or LAR. The informed consent process must be documented in the subject's medical record.

If informed consent is obtained from a subject with altered mental status, capacity for consent must be clearly documented in the medical record as assessed by the Investigator or designee. An assessment tool for determining capacity for consent is available in Appendix B. If at any time during the study the subject regains capacity for consent, this assessment must be documented by the investigator and the subject must complete the consent process. If the subject does not agree to consent, they will be discontinued from participation in the study.

During the informed consent process, subjects will also be informed about participation in the follow-up registry extension of this study (VTL-308E). Subjects who decline participation in VTL-308E are not considered candidates for participation in VTL-308 (Exclusion Criterion 24).

5.2.2 Sponsor Notification

The Investigator or designee must notify the Sponsor Representative by calling the Subject Enrollment Mobile Phone *after* signed Informed Consent is obtained. Medical information may be shared at this time with the Sponsor according to local policy.

Results of medical information from tests or procedures that have been performed as part of the Screening Process (inclusion/exclusion criteria assessment) must be provided to the Sponsor

Representative. **All personal identifiers (patient names, patient numbers, hospital names and locations, etc.) must be removed/redacted from the data prior to sending them to VTL. Signed Informed Consent Forms must not be sent.**

5.2.3 Screening Procedures

The tests and assessments listed below must be performed prior to Randomization, unless otherwise noted (see Section 5.2.3.2 regarding imaging requirements). An overview of these procedures is presented in Table 1 of this protocol.

Procedures that are performed one time as part of the Screening process are as follows:

- Demographics (date of birth or age, race, ethnicity, height, weight, gender)
- Relevant medical history
- Documented evidence of temporal association (6 weeks or less) of alcohol use and hospital admission for this episode of AILD. Documented evidence includes patient or other credible person reporting alcohol use, and/or positive blood test
- Liver imaging to determine liver size and confirm the absence of occlusive portal vein thrombosis or bile duct obstruction (see Section 5.2.3.2 regarding imaging performed no more than six weeks prior to current hospitalization)
- Serologies (if not previously collected during this hospitalization, and investigator suspects subject may be at risk) for active viral hepatitis A, B or C infection or human immunodeficiency virus (HIV); a positive serology for active hepatitis would be exclusionary
- Serum pregnancy test^{++} (if applicable)

$^{++}$ Note: Subjects who are sexually active, without history of medical or surgical sterilization, must agree to practice a form of effective contraception. Instruction should be given that if a subject becomes pregnant or impregnates another during participation in the study, they must inform the Principal Investigator immediately. Female subjects that become pregnant and report this to the Principal Investigator will be followed through to the completion of the pregnancy, including delivery and status of the neonate. The partner of a male ELAD-treated subject who is impregnated during the time between Randomization to Study Day 91 will not be followed.

Procedures that are performed as part of the Screening process and which are used as Baseline values required for subsequent assessments of change in key parameters are as follows (Note: these tests and assessments must be performed within 24 hours prior to randomization):

- Physical exam (performed by a Physician, Physician Assistant or Nurse Practitioner if within the scope of practice for institution)
- Vital signs (blood pressure, heart rate, respiration rate, temperature)
- Encephalopathy stage [West Haven Criteria, performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated
- Routine laboratory evaluations: BUN, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, sodium,

potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, albumin, total protein, alkaline phosphatase

- Baseline laboratory evaluations (not used for study eligibility): partial thromboplastin time (PTT), ammonia, lactate, albumin, α-fetoprotein, lactate dehydrogenase (LDH)
- Complete Blood Count (CBC): White blood cell count (WBC), hematocrit, hemoglobin, and platelets
- Prothrombin time (PT), control PT, international normalized ratio (INR)
- Pulse oximetry and FiO_2 (with amount of oxygen and delivery method noted, if applicable)
- PEth Test

MELD and Maddrey scores will be autocalculated in accord with Section 5.2.4.

5.2.3.1 *PEth Test*

The PEth test blood sample (finger stick) will be collected to determine whether the subject has been drinking alcohol (Center for Substance Abuse Treatment, 2006; Allen 2003; Cluver 2007; Miller 2005). This specimen (identifiable by subject number only) will be sent to a central laboratory and the results will go directly to the outside data management group that handles the Sponsor's data to prevent any caregivers from knowing the results. Because subjects may want this information kept confidential from any caregivers, subjects should be made aware that it is for research only and results will not be entered into their medical records or given to any of the clinical trial team.

The kit to carry out the PEth test to determine alcohol use will be provided to the sites by VTL or designee and performed by an outside lab:

United States Drug Testing Laboratories
1700 South Mount Prospect Road
Des Plaines, Illinois 60018

Instructions for the test and for sending the samples to United States Drug Testing Laboratories will be included with the actual test materials delivered to the investigational site.

5.2.3.2 *Imaging Requirements*

Liver imaging will be used to confirm that there is no evidence of occlusive portal vein thrombosis or bile duct obstruction and to assess liver size, either by diameter or by liver volume (Exclusion Criteria 11 and 12). To determine subject eligibility for enrollment, the liver must be assessed by ultrasound in all subjects, to determine if there is occlusive portal vein thrombosis or bile duct obstruction, whether or not the ultrasound measurement is used to assess liver size. Liver size may be assessed by ultrasound, CT scan, or MRI. All imaging must be indicative of and have a temporal relationship to the subject's current clinical condition.

If the imaging is not performed during the current hospitalization, the Investigator or designee must document that it is representative of the subject's current clinical condition. Imaging must be performed no more than six weeks prior to the current hospitalization. The Sponsor will

request that imaging be repeated if the imaging studies that have been carried out are inadequate to ascertain patient safety and/or eligibility.

5.2.3.2.1 Measurement of Liver Diameter

Liver diameter can be assessed using ultrasound, CT or MRI to ensure adequate liver size per Exclusion Criterion 11. Imaging must be performed no more than six weeks prior to the current hospitalization. The craniocaudal diameter (sagittal view) of the liver must be at least 10 cm when measured on the mid-clavicular line. If the mid-clavicular (sagittal) measurement is not available, other craniocaudal planes may also be used [e.g., mid-axillary (coronal plane) or anterior-posterior (transverse plane)] provided that, in the opinion of the Investigator, the measurement is consistent with a mid-clavicular measurement (right lobe of the liver) of at least 10 cm. If liver size is not routinely measured at the institution, then it is recommended that liver measurement and the technique to measure the liver size be added to ultrasound orders for potential subjects.

5.2.3.2.2 Measurement of Liver Volume

If the ultrasound is unable to adequately assess liver size, a CT or MRI scan may be performed, and the liver volume may be calculated to ensure adequate liver size per Exclusion Criterion 11. The CT or MRI scan must be performed no more than six weeks prior to the current hospitalization. The liver volume must be at least 1200 cc.

5.2.4 Assessment of MELD and Maddrey Scores

The MELD scoring system accounts for the effects of dialysis, whether it be intermittent hemodialysis or continuous hemofiltration. For the purposes of this protocol, the MELD score will be autocalculated when the individual parameters (bilirubin, creatinine, INR, age, dialysis) are entered into the eCRFs. MELD and Maddrey scores should not be calculated manually outside of the eCRF/EDC system.

The parameters used to calculate the MELD score **must** be obtained using lab values from blood draws taken within a period of 6 hours of each other. If one of the three parameters for the MELD score needs to be reassessed, and the blood draw for this parameter falls outside of a period of 6 hours from one or both of the other parameters, then all three parameters have to be reassessed using the guidelines above.

Likewise, calculation of the Maddrey score must be done using laboratory values for the PT and total bilirubin collected from blood draws taken within 6 hours of each other. For the purposes of this protocol, the Maddrey is autocalculated when entered into the eCRFs and will autopopulate once calculation values are entered. These calculations must be done in the eCRF and should not be calculated manually.

5.3 RANDOMIZATION PROCESS

After initial subject eligibility has been confirmed by the Sponsor, the Investigator is notified. The investigator is now able to complete the randomization process using the EDC system. Once randomization occurs, study subjects randomized to the ELAD treatment group should be transferred to the ICU (or equivalent) prior to End of Study Day 2. Written preapproval must be obtained from VTL prior to ELAD treatment in a Step Down Unit (SDU).

The randomization to the ELAD treatment group or Control group will be 1:1.

Every effort should be made to notify the subject's personal physician about his/her inclusion in this study. If the subject cannot provide the physician's contact information, attempts to obtain it from the family or LAR should be made.

5.3.1 Interactive Web Response System (IWRS)/EDC Core Details

Subject enrollment will occur via the vendor-supplied EDC eClinical Suite. The system is an integrated platform containing the EDC, Adjudication, and Randomization (IWRS) modules.

The EDC eClinical Suite Randomization module provides secure, confidential, real-time access to a centralized randomization list. The potential for bias is reduced by ensuring that neither Sponsor, site, nor study personnel will have any foreknowledge of subject treatment assignment.

Prior to any entry into the system, each user will complete the training required to perform the applicable tasks specific to their role and function.

Controls are in place to limit access to key personnel for the following:

- Randomization List
- Randomization Controls
- Randomization Configuration
- Randomization

5.3.2 Subject Enrollment and Randomization Procedure

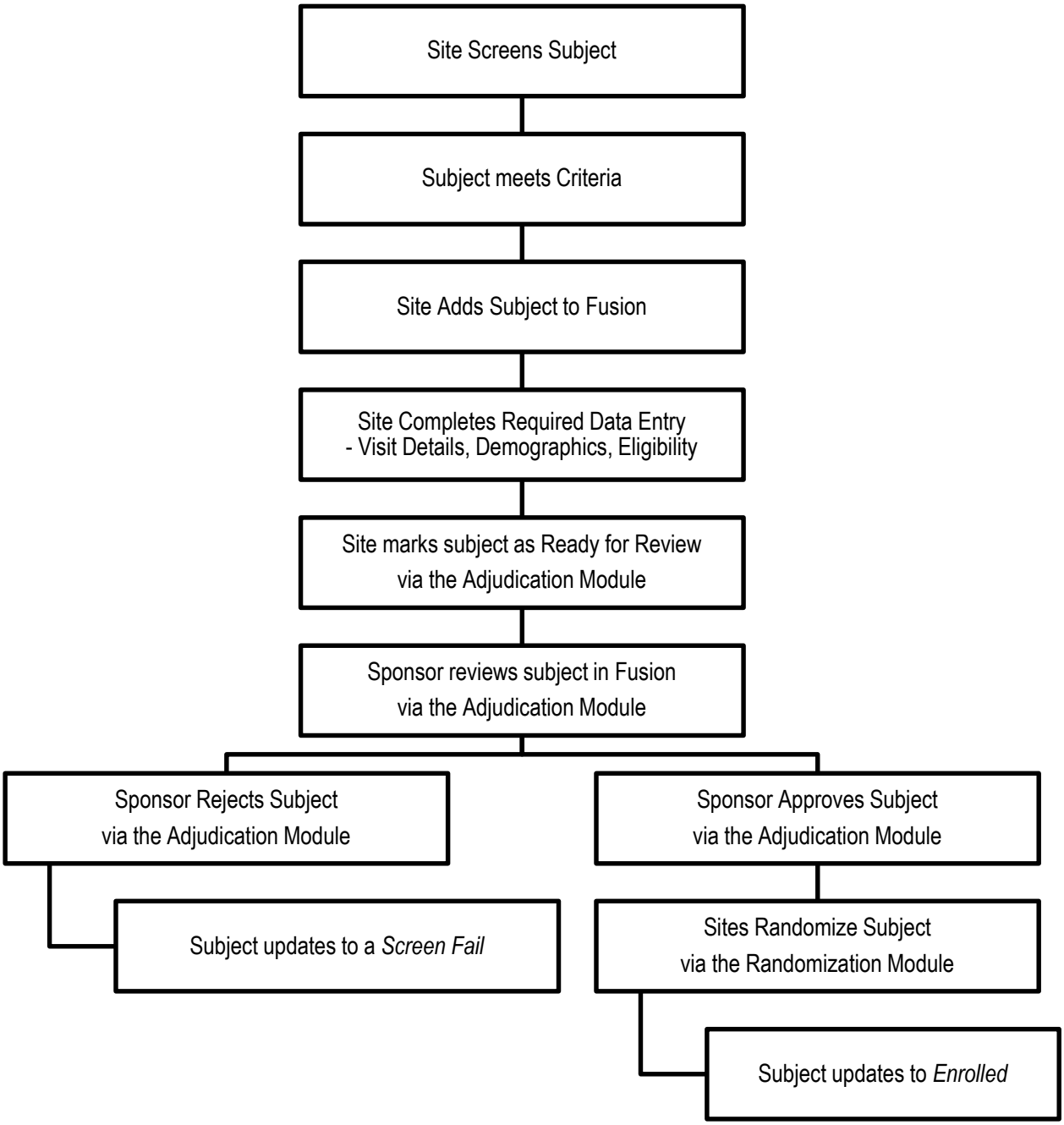
Site personnel will evaluate patients for eligibility. Patients who are not eligible will be recorded in the EDC system's Pre-Screening Failure module. If site evaluation indicates that the patient is eligible, the site will add the subject into the EDC system. The site will enter basic information, including Informed Consent and Demographic data, and then upload supporting documentation of the subject's eligibility into the EDC system. Once these steps are completed, the EDC system automatically notifies the designated Sponsor representative that the subject's data is available for adjudication.

The Sponsor representative reviews the submitted data and documentation and will either approve or reject the subject in the EDC system. The EDC system will automatically notify the site of this status update. For approved subjects, the site can proceed to randomization using the EDC system's Randomization module. Rejected subjects are designated as Screen Failures in the EDC system.

The subject will be randomized based on the randomization scheme defined and loaded into the system. The subject will be randomized to either the ELAD treatment or control group in a 1:1 ratio. This information is automatically populated into the EDC system on screen and within the audit log, and is available to the site and the sponsor. This information is also provided to the designated site and Sponsor team via an automatic system email notification once a subject has been randomized.

See Figure 1 below for a flow diagram of the subject enrollment process.

Figure 1. Subject Enrollment Process



5.3.3 Other Procedures

The following samples or procedures should be collected or performed at any time prior to starting ELAD treatment for subjects assigned to the ELAD group, and before End of Study Day 2 for subjects assigned to the Control group. Results are not required prior to initiation of treatment, however, if results are known prior to initiating treatment, and there is a positive culture result, appropriate treatment per medical judgment should be provided prior to initiating

treatment. If results become available after initiation of treatment, appropriate treatment per medical judgment should be provided.

- Blood culture samples (×2)** (See Section 5.6.1)
- Urine sample for culture and sensitivity (C&S) testing**
- Sputum sample for C&S testing**, if clinically able to obtain and process according to standard Microbiology Department procedures at Institution
- Peritoneal fluid sample for C&S testing, if ascites present and if one has not been done within prior 7 days or is contraindicated
- Blood collection for outside analysis (consented subjects at participating centers only) (See Section 5.6.3)
- Subject location

**Note: If repeat cultures are not allowed within 48 hours, then those closest to randomization will be used.

5.4 STUDY TREATMENT

Study Day 1 begins at the time of Randomization when protocol-specified standard of care (SOC) guidelines must be followed and concludes following the next morning's SOC lab draw.

Note: The SOC lab draw may be adjusted in order to initiate ELAD treatment within a 6-hour window to limit blood draws. Study Day 1, therefore, will be less than 24 hours. Study Day 91 is the final study day. All subjects are enrolled into the study with the expectation of completing all 91 days of the study.

5.4.1 Study Treatment Standards of Care

To eliminate potential bias, subjects entered into this study will receive the pertinent standard of care as defined by this protocol, independent of treatment group (ELAD/Control) assignment. Standard of Care treatment for *all* subjects, in both the ELAD and Control groups, is to be the same.

For anticoagulation guidelines for the VTL-308 Study please refer to Appendix E. There is also a summary table of the standard of care guidelines in Table 4, which were derived from those written and endorsed by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL).

There will be treatments that do not fall under the SOC listed in this protocol. For those treatments, it is imperative that Control and ELAD subjects are managed the same. For example, institutional standard of care for severe acute alcoholic hepatitis may involve the administration of concomitant medications such as pentoxifylline (PTx) and/or N-acetylcysteine (NAC). If this is the case the same SOC must be applied to both treatment groups unless contraindicated. In this case the administration of PTx or NAC would be captured in the concomitant medication section of the eCRF.

Any significant deviations from the standards defined below are to be noted in the eCRFs. These SOC cover the following conditions/events:

- Nutritional support
- Steroids
- Ascites
- Gastroesophageal varices
- Hepatic encephalopathy
- Hepatorenal syndrome (HRS)
- Hyponatremia
- Spontaneous bacterial peritonitis (SBP)
- Thrombocytopenia
- ICU discharge
- Hepatic Hydrothorax

5.4.1.1 *Standard of Care - Nutrition*

All subjects will receive Dietary consultations within 72 hours of Randomization if this has not already occurred. All Dietary consults include weight measurement. Should specialized Dietary personnel (Nutritionists) not be routinely available at the investigative site, Investigators will provide dietary oversight following the AASLD/EASL guidelines or hospital guidelines for both Control and ELAD-treated subjects. Nutritional guidelines that are standard for the hospital will be followed while subjects are in-house with particular attention given to vitamin, trace metal, and nutritional deficiencies (protein-calorie malnutrion) and overall general nutritional supplementation. Dietary follow-up will be given throughout the hospital stay including at hospital discharge. Upon discharge, standardized dietary follow-up, including measurement of weight, will be provided at each investigational site/clinic visit.

5.4.1.2 *Standard of Care - Steroid Administration*

In accord with AASLD guidelines, all subjects treated on the protocol (ELAD-treated and Control subjects), will be administered prednisolone/prednisone (40 mg daily) for 7 days unless specifically contraindicated. If steroids are not administered, the rationale for not administering steroids (e.g the specific contraindication) must be documented on the eCRF. After 7 days of prednisolone/prednisone therapy, subjects will be evaluated to assess whether steroid treatment should be continued. Based on clinical judgment and objective measures of clinical response, subjects not responding will be discontinued from steroid treatment and the method used to evaluate the clinical response, and the reason for termination recorded. Care should be exercised in the interpretation of measures such as the Lille score in subjects treated with ELAD due to the impact of extracorporeal therapy on serum bilirubin and prothrombin time. To the extent possible, the same criteria must be applied to both the ELAD-treated and Control subjects to determine continued steroid treatment. If continued use of steroids is judged to be beneficial to the subject, steroid use can be continued for up to 28 days (total). All subjects will stop prednisolone/prednisone 40 mg daily after 28 days of administration unless the Investigator believes that continued use of steroids is warranted. The clinical rationale for extending or stopping steroid dosing must be documented on the eCRFs. If steroid treatment is stopped, a tapering schema may be followed. The recommended tapering schema would take place over 7 days. If an alternative tapering schema is adopted, the rationale for adopting the tapering schema

must be documented. The treatment period of prednisolone/prednisone at 40 mg/day must take into account any prednisolone/prednisone treatment received prior to this hospital admission (e.g., treatment from a transferring hospital).

5.4.1.3 *Standard of Care - Ascites*

5.4.1.3.1 Abdominal Paracentesis

All subjects with ascites who are entered into the VTL-308 study will undergo an abdominal paracentesis during Screening, if clinically indicated (e.g., grades 2 [moderate] or 3 [large] ascites, worsening ascites, spontaneous bacterial peritonitis [SBP], etc.) and if one has not been done within 7 days prior to Screening. Subjects who had an abdominal paracentesis more than 7 days before the first day of Screening are to undergo a repeat paracentesis so that it is confirmed that patients do not haveSBP prior to Randomization. The purpose of this requirement is to assure that subjects are free of SBP since it is a co-morbidity that is associated with an increased risk of hepatorenal syndrome (HRS) and gastroesophageal bleeding, as well as a higher mortality. If subjects are on ELAD treatment, paracentesis may only be done if heparin anticoagulation has been discontinued for a minimum of 12 hours and should not be done if the subject has been diagnosed to have hyperfibrinolysis or disseminated intravascular coagulation.

5.4.1.3.2 Ascitic Fluid Analysis

All subjects with ascites treated with abdominal paracentesis entered into the VTL-308 study will have an ascitic fluid cell count and differential, ascitic fluid total protein, and serum-ascites albumin gradient (SAAG) conducted in the ascitic fluid sample obtained during Screening. The SAAG) is calculated by subtracting the ascites albumin from the serum albumin. A high gradient (SAAG >1.1 g/dL indicates portal hypertension). If the subject is suspected at Screening to have an ascitic bacterial infection due to associated co-morbidities, signs, or symptoms, ascitic fluid should be collected at the bedside in aerobic and anaerobic blood culture bottles prior to initiation of antibiotics. Post-paracentesis albumin infusion may not be necessary for a single paracentesis of <5 liters.

5.4.1.3.3 Treatment

All subjects entered into the VTL-308 study with ascites will be treated with a salt-restricted diet (approximately 2000 mg sodium) and an oral dual diuretic regimen consisting of spironolactone and furosemide (if clinically indicated) as soon as the subjects are stabilized and are able to eat and tolerate oral medications.

Long-term norfloxacin (or equivalent) is to be administered to subjects with no gastrointestinal bleeding or prior history of SBP if the ascitic fluid protein is <1.5 g/dL and at least one of the following is present: serum creatinine ≥1.2 mg/dL; BUN ≥25 mg/dL; serum sodium ≤130 mEq/L; and Child-Pugh ≥9 points with bilirubin ≥3 mg/dL.

Cellulitis can explain pain and fever in subjects with cirrhosis and ascites and should be treated with diuretics and antibiotics.

5.4.1.3.4 Management of Tense Ascites

Subjects entered into the VTL-308 study who are considered to have tense ascites that should be treated with a paracentesis will have this procedure conducted during Screening before

Randomization and the paracentesis should be followed with dietary sodium restriction and oral diuretics. Subjects who have less than 5 liters of ascitic fluid removed do not need a colloid infusion whereas subjects who had 5 or more liters of ascitic fluid removed are to have post-paracentesis colloid infusion (8 g of serum albumin for every liter of ascitic fluid removed) to prevent post-paracentesis circulatory dysfunction. If tense ascites recurs, paracentesis can be repeated at any time it is clinically required.

5.4.1.3.5 Refractory Ascites

Refractory ascites is defined as fluid overload that is unresponsive to a sodium-restricted diet and high-dose diuretic treatment (400 mg/day spironolactone and 160 mg/day furosemide) or rapid recurrence of ascites after a therapeutic paracentesis. Subjects with refractory ascites entered into the VTL-308 study are to have the therapeutic paracentesis conducted during Screening (before Randomization). In addition, if the volume of ascitic fluid exceeds 5 liters, subjects will be required to have a colloid infusion (8 g of serum albumin for every liter of ascitic fluid removed) to minimize electrolyte abnormalities and prevent post-paracentesis circulatory dysfunction.

5.4.1.3.6 Drugs contraindicated in Patients with Ascites

The following drugs are contraindicated in patients with ascites: nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin; angiotensin converting enzyme inhibitors and angiotensin receptor blockers; α1-adrenergic blockers; dipyridamole; and aminoglycosides alone or in combination with ampicillin, cephalothin, or mezlocillin.

5.4.1.4 *Standard of Care – Gastroesophageal Varices*

Note: Subjects with banding of gatroesophageal varices during the 7 days immediately preceding screening will not be eligible for enrollment in the study (see Exclusion Criterion 10).

All subjects with medium/large varices that have not bled but are Child-Pugh A, B, or C will be placed on non-selective β-blockers at the maximum tolerated dose sometime during Screening but before Randomization, unless there are clinical issues that contraindicate their use. These same subjects may be treated with endoscopic variceal ligation but this procedure and the follow-up endoscopies should be conducted after the subject completes Study Day 7 (Control) or completes ELAD treatment.

Subjects who have had active bleeding and have received blood products prior to Screening must be stable with no bleeding and no further need for blood products for 2 days prior to Randomization. Subjects who had active bleeding within 2 weeks prior to Randomization should be given oral norfloxacin 400 mg twice daily for 7 days (or equivalent); if the antibiotics cannot be administered orally, intravenous ceftriaxone 1 g/day for 7 days (or equivalent) is to be administered.

Subjects who have a variceal bleed will be treated according to the AASLD guidelines: blood transfusion (maintain hemoglobin level of 8 g/dL); short course of prophylactic antibiotics (e.g., oral norfloxacin 400 mg twice daily for 7 days; if the antibiotics cannot be administered orally, intravenous ceftriaxone 1 g/day for 7 days - or equivalents); pharmacologic therapy (e.g., vasopressin, terlipressin); endoscopy using endoscopic variceal ligation (EVL)/sclerotherapy (if EVL is not technically feasible), balloon tamponade (not to exceed 24 hours), or transjugular intrahepatic portosystemic shunt (TIPS); bleeding cannot be controlled or recurs despite

pharmacologic and endoscopic therapy, if needed. Subjects who are treated for recurrent variceal bleeding while in the study will be treated with non-selective β-blockers and EVL to prevent recurrence of bleeding. TIPS may be considered for appropriate subjects.

Treatment of type 1 gastric varices (extension of esophageal varices along the lesser curvature of the stomach) should follow the management guidelines for esophageal varices. Acute gastric fundal variceal hemorrhage should be treated with endoscopic obturation with tissue adhesives or EVL, and TIPS if bleeding cannot be controlled or recurs,

5.4.1.5 *Standard of Care – Hepatic Encephalopathy*

Subjects whose hepatic encephalopathy (HE) is stabilized at Screening, during the hospitalization, or the outpatient visits to Study Day 91 will be treated with prophylactic measures to prevent precipitating factors (e.g., variceal bleeding, SBP, HRS, infections), will be fed a nutritious diet (protein intake between 1.0 and 1.5 g/kg/day), and will be maintained on an adequate daily dose of lactulose to maintain two to three soft bowel movements per day. Selective digestive decontamination, for example rifaximin, may also be given.

5.4.1.6 *Standard of Care – Hepatorenal Syndrome (HRS)*

Hepatorenal syndrome (HRS) is to be diagnosed when ascites is present, the serum creatinine is >1.5 mg/dL, there is no reduction in serum creatinine after at least 2 days of diuretic withdrawal and volume expansion with albumin, there was no shock, no current or recent treatment with nephrotoxic drugs, and there is absence of parenchymal kidney disease.

Type I is characterized by a rapidly progressive reduction in renal function as defined by a doubling of the initial serum creatinine to a level >2.5 mg/dL or a 50% reduction of the initial 24-hour creatinine clearance to a level <20 mL/minute in less than two weeks. Type II does not have a rapidly progressive course.

Subjects who develop Type I HRS after randomization should be placed on pharmacotherapeutic SOC in accord with local practice, for example albumin/octreotide/midodrine (MOA) regimen in the US or terlipressin/albumin regimen (TA) ex-US. The treatment of Type II HRS that occurs after randomization will be driven by the clinical needs of the subject.

Renal replacement therapy may be useful in subjects who do not respond to MOA or TA, and who fulfill criteria for renal replacement therapy.

VTL requires that subjects who develop Type I HRS after randomization but prior to the start of ELAD treatment as well as while on ELAD treatment must be discontinued from ELAD treatment and are to be continued on SOC. VTL also requires that subjects on ELAD treatment who require renal replacement therapy/hemodialysis are to be discontinued from ELAD treatment before renal replacement therapy/hemodialysis is started.

5.4.1.7 *Standard of Care – Hyponatremia*

Even though there is no good evidence as to what level of serum sodium should prompt treatment, subjects in this study will be diagnosed to have hyponatremia when the serum sodium level is lower than 130 mmol/L per the EASL practice guidelines. Investigators need to determine whether the hyponatremia is secondary to hypovolemic hyponatremia (usually secondary to excessive diuretic administration) or hypervolemic hyponatremia (related to the

expansion of extracellular fluid volume associated with ascites and edema). Hypovolemic hyponatremia should be treated (e.g., stopping diuretics, fluid administration as needed per the clinical needs) whenever it occurs during the study. Hypervolemic hyponatremia should be treated with fluid restriction if it is practical; fluid restriction is necessary unless the serum sodium level is <125 mmol/L.

5.4.1.8 *Standard of Care – Spontaneous Bacterial Peritonitis*

All subjects entered into the VTL-308 study are to undergo an abdominal paracentesis for ascitic fluid testing, inclusive of culture, if clinical signs or symptoms suggestive of SBP are present (e.g., abdominal pain or tenderness, fever, encephalopathy, renal failure, acidosis, or peripheral leukocytosis) during the study. Ascitic fluid should be collected at the bedside in aerobic and anaerobic blood culture bottles prior to initiation of antibiotics.

SBP is diagnosed when the ascitic fluid polymorponuclear (PMN) count is ≥250 cell/mm^3 and the bacterial cultures are positive. Subjects who have an ascetic fluid PMN count ≥250 cell/mm^3 but the bacterial cultures are negative should be diagnosed to have culture-negative neutrocytic ascites. Symptomatic subjects with ascitic fluid PMN counts <250 cells/mm^3 are considered to have monomicrobial nonneutrocytic bacterascites.

Empiric therapy with intravenous cefotaxime (or equivalent) is to be started as soon as the cell counts are available and continued for 5 days; appropriate subjects can be treated with ofloxacin (or equivalent) for 8 days. Subjects eligible for empiric therapy include those with culture-positive neutrocytic ascites, culture-negative neutrocytic ascites, and monomicrobial non-neutrocytic bacterascites. Empiric therapy in subjects with culture-positive neutrocytic ascites is modified based on the culture and sensitivities. Subjects who develop a recurrence of SBP at any time after the resolution of the current episode of SBP are to have a repeat paracentesis and if the polymorphonuclear (PMN) cell counts are ≥ 250 cells/mm^{3}and/or clinical signs of SBP are present, the subject is to have empiric therapy, which is to be modified as needed when the culture and sensitivities are available. All episodes of SBP up to Study Day 91 are to be treated using this algorithm.

- Intravenous ceftriaxone (or other 3rd generation cephalosporin) for 7 days or norfloxacin twice daily for 7 days (or equivalent) is to be given to prevent bacterial infections in subjects who have gastrointestinal bleeding (variceal bleeding) between Study Day 1 and Study Day 91.
- Subjects who survived a prior episode of SBP prior to entering the VTL-308 study or the episode of SBP was treated while the subject was in the VTL-308 study will be started on long-term prophylaxis with daily norfloxacin (or trimethoprim-sulfamethoxazole or equivalent); if indicated, long-term antibiotic prophylaxis can be started at Randomization or following an episode of SBP that occurred between Study Day 1 to Study Day 91.
- Subjects with cirrhosis and ascites but no gastrointestinal bleeding are to be started on long-term prophylaxis with daily norfloxacin (or trimethoprim-sulfamethoxazole or equivalent) if the ascitic fluid protein is <1.5 g/dL and at least one of the following is present: serum creatinine ≥1.2 mg/dL, BUN ≥25 mg/dL, serum sodium ≤130 mEq/L, and Child-Pugh ≥9 points with bilirubin ≥3 mg/dL.

5.4.1.9 *Standard of Care - Thrombocytopenia*

Subjects with a platelet count <20,000/mm^3 without any clinical manifestations of bleeding during study treatment (ELAD and Control) will receive platelet transfusions based on the judgment of the clinical team. If the platelet count is <20,000/mm^3 and there are clinical manifestations of only localized contained bleeding, the subjects (ELAD and Control) must receive platelet transfusions. Subjects with a platelet count of <20,000/mm^3 and generalized bleeding are to be promptly assessed and, if on ELAD, considered for treatment discontinuation.

5.4.1.10 *Standard of Care – ICU Discharge*

Subjects may be admitted to the ICU (or SDU where hospital standards allow and VTL approves) before or during the VTL-308 study. Therefore, the subjects' status should be continuously reviewed to identify subjects who no longer require ICU care. It is recommended that subjects be discharged from the ICU when their physiologic status has stabilized or when their physiological status has deteriorated and further interventions are not warranted. It is recommended that subjects experiencing alcohol withdrawal receive ICU care until their detoxification stage has stabilized (i.e., absence of seizures and delirium requiring intense monitoring). In addition, the subject experiencing alcohol withdrawal should be hemodynamically stable prior to discharge (i.e., not requiring intense monitoring for vasopressor administration or mechanical respiratory support). Continuous and frequent follow-up with the subject is recommended on the SDU or medical/surgical unit post-ICU discharge. For discharge from the ICU, a general guideline is that the subject is no longer in need of vasopressors, mechanical ventilation, or continuous extracorporeal renal treatment.

5.4.1.11 *Standard of Care – Hepatic Hydrothorax*

Hepatic hydrothorax is defined as a large pleural effusion (usually unilateral and right-sided) that occurs in a subject with cirrhosis and ascites. The protein concentration in the pleural fluid is usually higher than in the ascitic fluid due to differences in the hydrostatic forces in the abdomen versus the chest. First-line therapy of hepatic hydrothorax consists of dietary sodium restriction and diuretics while TIPS is considered to be second-line treatment when it becomes refractory. Chest tube insertion is contraindicated in a subject with hepatic hydrothorax.

5.4.2 Pre-Treatment

Assessments will be carried out for both ELAD and Control subjects. For ELAD subjects, these assessments will be conducted in accord with Section 5.4.2.1.1 to determine eligibility for extracorporeal safety. For Control subjects, these assessments will be conducted as part of Study Day 2 evaluations in accord with Section 5.4.2.2.1.

5.4.2.1 *Pre-ELAD Treatment*

Prior to initiation of the extracorporeal treatment, ELAD subjects will be evaluated in accord with Section 5.4.2.1.1.

5.4.2.1.1 Pre-ELAD Safety Assessments

Either the principal investigator or sub-investigator trained on the study must have evaluated the safety eligibility results and be immediately available when ELAD treatment begins in order to assess changes in the subject's condition since Randomization. This is especially important when evaluating changes during the 24 hours preceeding initiation of ELAD treatment in order to

determine whether the subject remains eligible for extracorporeal treatment. When the ELAD Specialist confirms that ELAD treatment can begin in 6 hours or less, certain key safety factors must be evaluated. The following procedures will be completed (Table 3), and assessed in accord with the pertinent Exclusion Criteria 2, 3, 4, 7, 9, 10, 15, and 16 relative to the safety evaluation time point:

- Platelet count
- International Normalization Ratio (INR)
- Serum creatinine
- Evidence of infection unresponsive to antibiotics
- Hemodynamic instability assessment (blood pressure and MAP)
- Bleeding status
- Status with respect to ventilation, intubation, or need for hemodialysis

In addition the following will be done:

- Vital signs (blood pressure, heart rate, respiration rate, and temperature)
- Blood samples will be obtained for culture (×2) (See Section 5.6.1)
- Total bilirubin will be obtained and MELD scores will be calculated in accord with Section 5.2.4
- Partial thromboplastin time (PTT) will be re-evaluated to guide anti-coagulation protocols (See Section 5.5.2.1)

The ELAD Specialist and the Investigator or designee, prior to the initiation of ELAD treatment, will review the safety eligibility criteria listed above to ensure that the criteria are met, and that it is safe to initiate treatment. In addition, the Investigator or designee must confirm that the subject remains eligible for extracorporeal treatment. If these criteria are not met, the Sponsor Representative must be notified and ELAD treatment may not be initiated. The Medical Monitor or the Chief Medical Officer must be contacted by the Investigator or designee prior to initiation of ELAD treatment. All such procedures and evaluations must be recorded in the appropriate section(s) of the eCRFs.

Every effort must be made to initiate ELAD treatment as soon as possible after confirmation of the safety procedures. However, if treatment is delayed for any reason such that the time from the conduct of safety procedures is greater than 6 hours, the subject must be reassessed prior to the initiation of ELAD treatment in accord with the procedures above. In that case, the latest data would be recorded on the eCRF.

If the subject is no longer considered eligible for ELAD treatment, the ELAD cartridges will either be destroyed according to the institution's practices, or may be returned to VTL or designee. The reason the subject was deemed a Pretreatment Failure will be noted on the appropriate eCRFs. Pretreatment failure in the ELAD group will result in exclusion from the mITT population.

5.4.2.2 *Pre-Control Treatment*

Control subjects undergo the same evaluation as ELAD subjects in order to standardize data collection.

5.4.2.2.1 Pre-Control Safety Assessments

Either the principal investigator or sub-investigator trained on the study must have evaluated the safety eligibility results and be available at the safety eligibility evaluation timepoint for Control subjects (End of Study Day 2) (±6 hours) in order to assess changes in the subject's condition since Randomization. The following procedures will be completed (Table 3), and assessed in accord with the pertinent Exclusion Criteria 2, 3, 4, 7, 9, 10, 15, and 16 relative to the safety evaluation time point:

- Platelet count
- International Normalization Ratio (INR)
- Serum creatinine
- Evidence of infection unresponsive to antibiotics
- Hemodynamic instability assessment (blood pressure and MAP)
- Bleeding status
- Status with respect to ventilation, intubation, or need for hemodialysis

In addition the following will be done:

- Vital signs (blood pressure, heart rate, respiration rate, and temperature)
- Blood samples will be obtained for culture (×2) (See Section 5.6.1)
- Total bilirubin will be obtained and MELD scores will be calculated in accord with Section 5.2.4

All such procedures and evaluations must be recorded in the appropriate section(s) of the eCRFs.

If the subject is no longer considered eligible, the reason the subject was deemed a Pretreatment Failure will be noted on the appropriate eCRFs. Pretreatment failure in the Control group will result in exclusion from the mITT population.

5.4.3 Treatment Start

5.4.3.1 *ELAD Treatment Start*

Prior to ELAD treatment initiation, subjects will be given 100 mg hydrocortisone (or institutional equivalent) and 50 mg diphenhydramine (or institutional equivalent). The timing for premedication should be in accord with the institution's policy for premedication; if ELAD cartridges need to be replaced premedication does not need to be repeated. Anticoagulation therapy should be considered prior to initiation of ELAD treatment (See Section 5.5.2.1).

The clinical, laboratory, and hemodynamic assessments will be performed at specified intervals during the study treatment period for both the ELAD and Control groups. An overview of evaluations during study treatment is presented in Table 2 for Control subjects and Table 3 for ELAD subjects.

The Investigator or designee should assess the subject, per their Institutions SOC guidelines for placing a patient on an extracorporeal system, immediately prior to starting the ELAD System blood pump.

All ELAD System management and cartridge placement will be done by ELAD Specialists. Site staff will not be responsible for the management of the ELAD System.

As ELAD may provide some degree of liver function, the dosage of drugs known to be subject to liver metabolism should be carefully monitored by the Investigator or designee in accord with institutional standard of care.

5.4.3.1.1 Catheter Placement

Prior to initiation of ELAD treatment, a dedicated, dual-lumen, kink-resistent dialysis catheter must be placed by an experienced physician, preferably ultrasound-guided, into the right internal jugular vein (preferred site), left internal jugular, femoral, or subclavian veins. If available and accessible, the interventional radiology team should be involved in complicated cases. Catheters should be designed to deliver a minimum of 300 mL/min blood flow to the ELAD System. For insertion and care of the catheter, the Centers for Disease Control and Prevention (CDC) or the European CDC guidelines (or local equivalent) should be followed.

5.4.3.1.2 ELAD Treatment Duration

Subjects randomized to receive ELAD will be treated with ELAD for a minimum of 3 days (72 hours), unless discontinuation criteria are met (See Section 3). At the end of 3 days (72 hours), a total bilirubin measurement must be taken. If the total bilirubin has increased by 25% or more from the measurement within 6 hours prior to initiation of ELAD treatment, ELAD treatment must be discontinued. If the bilirubin has not increased by 25% then treatment should continue to a maximum of 5 days (120 hours) regardless of treatment interruption (see Section 6.6). Bilirubin should be measured at least 12 hours after any procedure known to artificially alter serum bilirubin (e.g. administration of PRBCs, plasma exchange).

Subjects randomized to receive ELAD treatment will also receive standard of care therapy, and will receive medical treatment in accordance with the SOC guidelines as delineated in Section 5.4.1 of this protocol and those of the institution. For overall ranges of the durations of ELAD treatments from past trials (presented in hours) see the Investigator's Brochure.

5.4.3.2 ***Control Treatment Start***

Subjects randomized to Control Treatment will initiate treatment in accord with the protocol-defined standards of care immediately following randomization.

The clinical, laboratory, and hemodynamic assessments will be performed at specified intervals during the study treatment period for both the ELAD and Control groups. An overview of evaluations during study treatment is presented in Table 2 for Control subjects and Table 3 for ELAD subjects.

5.4.3.2.1 Control Treatment Duration

Subjects randomized to the Control group receive standard of care therapy alone, and will receive medical treatment in accordance with the SOC guidelines as delineated in Section 5.4.1 of this protocol and those of the institution.

5.5 EVALUATIONS WHILE ON TREATMENT

All evaluations in this section are for both ELAD and Control subjects, unless specified otherwise.

5.5.1 Daily Evaluations through End of Study Day 7

The following procedures will be performed daily through End of Study Day 7, based on 24-hour periods following the End of Study Day 1, and must be performed a minimum of 12 hours apart and, if specified, either within the defined time window or during the Study Day. Any subject discharged from the hospital prior to End of Study Day 7 must return to the study site for the End of Study Day 7 evaluation only (+2 days). It is not necessary to have outpatients return for daily assessments. There may be instances where the ELAD treatment period is delayed; therefore daily evaluations may be extended beyond End of Study Day 7. Additional eCRFs may be added in this situation. See Table 2 for evaluations schedules for Control subjects and Table 3 for ELAD subjects:

- Physical examination (performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution)
- Vital signs (blood pressure, heart rate, respiration rate, temperature)
- Encephalopathy stage [West Haven Criteria, performed by a study physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated
- Routine laboratory evaluations: BUN, creatinine, glucose, sodium, potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, ALT, AST, bilirubin (total/direct), albumin, total protein, alkaline phosphatase
- Additional laboratory evaluations: ammonia, lactate, , α-fetoprotein, lactate dehydrogenase (LDH)
- Complete Blood Count (CBC): white blood cell count (WBC), hematocrit, hemoglobin, and platelets
- Blood collection for outside analysis (consented subjects at participating centers only - at End of Study Days 3, 5, and 7) (See Section 5.6.3)
- Blood culture samples ×2 – at End of Study Day 2 and End of Study Day 4 for Control subjects, or within 6 hours prior to ELAD treatment start and Hours 48 and 96 of treatment for ELAD subjects
- Ultrafiltrate samples must be drawn as described in Section 5.6.2 and Section 5.6.4.
- PT, PTT, and INR (If heparin is administered, PTT will be assayed in accord with the standard anticoagulation therapy protocol at the site.)
- Pulse oximetry and FiO_2 and method of delivery (for inpatients only)
- MELD score will be autocalculated in accord with Section 5.2.4
- Adverse events will be monitored continuously from Randomization and recorded
- Concomitant procedures including concomitant blood product administration (e.g., whole blood, packed red blood cells, platelets, fresh frozen plasma,

albumin, cryoprecipitate) will be monitored continuously from Randomization and recorded

- Concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial line) will be monitored continuously from Randomization and recorded
- Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented
- For outpatients Study Day 7 only: Follow-up Dietary consult (including weight)

5.5.2 Safety Evaluations While on Extracorporeal Treatment (ELAD only)

The following study procedures will be completed during the first 24 hours of ELAD treatment (see Table 3):

- Vital signs (blood pressure, heart rate, respiration rate, and temperature) collected between hours 1-3, 6-10, and 14-18
- PT, PTT, INR, and platelet count collected between hours 6-10, and 14-18
- If anticoagulants are administered, they will be managed using the standard anticoagulation therapy protocol at the site (See Section 5.5.2.1)

These 24-hour safety evaluations will all be repeated if the ELAD cartridges are replaced during treatment (See Section 6.5).

ELAD Specialists are responsible for all ELAD System management and monitoring. ELAD System performance will be monitored through pump speeds and system pressures (P_{BW}, P_{PUG}, P_{UF}, P_{EC}, P_F, P_{BR}). ELAD cartridge performance will be monitored by measuring oxygen, pH, glucose, and temperature.

The ELAD System Operator's Manual provides instructions for the management and monitoring of the ELAD System during treatment. The ELAD Specialists will document ELAD System performance in the ELAD System Monitoring Forms.

5.5.2.1 *Anticoagulation Therapy during ELAD Treatment*

Extracorporeal systems often require anticoagulation therapy. As an example, in subjects with a PTT of less than 50 seconds, extracorporeal circuits may clot more frequently than in subjects with a spontaneous PTT of more than 50 seconds if no anticoagulant is used. Please refer to anticoagulation guidance provided by the Sponsor in Appendix E, and hospital standard of care for extracorporeal treatment.

Since anticoagulation therapy is associated with changes in coagulation parameters which may be more profound in patients with liver disease, the use of anticoagulants must be monitored according to the study site's internal procedures for continuous anticoagulation of extracorporeal circuits in subjects with increased bleeding risks.

The Investigator at each site is responsible for assessing the need of each subject for anticoagulation therapy prior to ELAD initiation and during ELAD treatment, and may consult with the study site's experts (e.g., intensive care staff, nephrology staff, or perfusionists) to apply

the study site's internal standards for anticoagulation of liver disease subjects on continuous extracorporeal circuits.

The most frequently used anticoagulant for ELAD treatments in the past has been heparin, monitored by PTT. Other anticoagulants used in prior ELAD trials include low molecular weight (LMW) heparin monitored by anti-factor Xa or prostacyclin. If contraindications against heparin occur (e.g., suspected heparin induced thrombocytopenia), hirudin analogs may be considered, according to the study site's internal procedures. The use of either prostacyclin or hirudin analogs should be controlled by appropriate laboratory measurements according to the study site's internal standards.

Since the performance of C3A cells may be affected by decreased ionized calcium, **citrate must not be used as an anticoagulant.**

The Investigator or designee may contact the Sponsor (Medical Monitor or designee) prior to initiation of ELAD treatment and during ELAD treatment to assist in the evaluation of the need for, and dosing of, anticoagulants for the ELAD System.

At the discretion of the Investigator, anticoagulation therapy can be discontinued at any time. All coagulation laboratory parameter results obtained during ELAD treatment will be recorded on the appropriate eCRF.

Clotting in the blood circuit may occur. If that happens, the ELAD Specialist will notify the Investigator or designee, who is responsible for determining the need for anticoagulation therapy, as discussed above.

5.5.3 Evaluations 24 Hours after ELAD Treatment Ends (ELAD-treated subjects only)

The following evaluations will be completed during the study day after the study day during which ELAD treatment ends, at least 24 hours (±6 hours) after ELAD treatment ends. These evaluations may coincide with standard End of Study Day evaluations, and if so, do not need to be repeated.

- Vital signs (blood pressure, heart rate, respiration rate, and temperature)
- Physical examination (performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution)
- Encephalopathy stage [West Haven Criteria, performed by a study physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated
- Laboratory evaluations: BUN, creatinine, glucose, sodium, potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, ALT, AST, bilirubin (total/direct), albumin, total protein, alkaline phosphatase
- Additional required laboratory evaluations: ammonia, lactate, α-fetoprotein, and LDH
- CBC: WBC count, hematocrit, hemoglobin, and platelets
- Pulse oximetry and FiO_2 and method of delivery

- PT, PTT, and INR
- MELD score will be autocalculated in accord with Section 5.2.4
- Blood collection for outside analysis (consented subjects at participating centers only) (See Section 5.6.3)
- Subject location

5.5.4 Evaluations on Study Days 14, 21, and 28 (+3 days)

On Study Days 14, 21 and 28 the following evaluations will be completed for all subjects:

- Vital signs (blood pressure, heart rate, respiration rate, and temperature)
- Physical examination (performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution)
- Encephalopathy stage [West Haven Criteria, performed by a study physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated
- Laboratory evaluations: BUN, creatinine, glucose, sodium, potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, ALT, AST, bilirubin (total/direct), albumin, total protein, alkaline phosphatase
- Additional required laboratory evaluations: ammonia, lactate, α-fetoprotein, and LDH
- CBC: WBC count, hematocrit, hemoglobin, and platelets
- PT, PTT, and INR
- Blood collection for outside analysis (consented subjects at participating centers only) (See Section 5.6.3)
- Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented
- MELD score will be autocalculated in accord with Section 5.2.4
- Assessment of concomitant medications
- Concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial line) will be monitored continuously from Randomization and recorded
- Assessment of adverse events
- Follow-up Dietary consult (including weight)

5.5.5 Evaluations on Study Day 63 and Study Day 91 (+5 days)

On Study Days 63 and 91, the following evaluations will be completed for all subjects:

- Vital signs (blood pressure, heart rate, respiration rate, and temperature)
- Physical examination (performed by a Physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution)
- Encephalopathy stage [West Haven Criteria, performed by a study physician, Physician Assistant, or Nurse Practitioner if within the scope of practice for institution (Refer to Appendix A)] unless subject is sedated

- Laboratory evaluations: BUN, creatinine, glucose, sodium, potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, ALT, AST, bilirubin (total/direct), albumin, total protein, alkaline phosphatase
- Additional required laboratory evaluations: ammonia, lactate, α-fetoprotein, and LDH
- CBC: WBC count, hematocrit, hemoglobin, and platelets
- PT, PTT, and INR
- Blood collection for outside analysis (consented subjects at participating centers only) (See Section 5.6.3)
- Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented
- MELD score will be autocalculated in accord with Section 5.2.4
- Assessment of concomitant medications
- Concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial line) will be monitored continuously from Randomization and recorded
- Assessment of adverse events
- Follow up Dietary consult (including weight)
- EQ-5D-5L Quality of Life (QOL) questionnaire ONLY if subject is being discharged or if it is the Study Day 91 visit

5.5.6 Evaluations at Discharge

In addition to the daily labs above, on the date of discharge the following should also be performed:

- PEth test for alcohol use
- Discharge Dietary consult, including weight (within 24 hours prior to discharge); follow-up Dietary consult (including weight) with each return study visit
- EQ-5D-5L Quality of Life (QOL) questionnaire (within 24 hours prior to discharge)

5.5.7 Evaluations Following Discharge

A post-hospital discharge evaluation is any study-defined subject evaluation that takes place following discharge from the hospital where study treatment was conducted. It is defined the same across both treatment arms.

All study-scheduled, post-hospital discharge follow-up evaluations will be conducted by an Investigator who is blinded to the subject's treatment assignment unless the subject is currently hospitalized. Inpatient evaluations can be carried out by any member of the study team. For clarification, study-defined subject evaluations that would be carried out by the Blinded Investigator would include the physical exam, encephalopathy assessment (West Haven Criteria), assessment of AEs and SAEs, and recommendation of management of the subject's care (inclusive of recommendations for hospital readmission when applicable).

Study participants will be instructed not to tell the Blinded Investigator conducting the post-discharge evaluations whether or not they received ELAD treatment. This information must be reinforced to all subjects after treatment and again upon hospital discharge. They must be instructed to NOT divulge their study arm (treatment group) to any study personnel who were not a part of their care team while during the treatment phase of the study.

Decisions for hospital readmission are to be made independently of knowledge of the subject's treatment group.

Payments will be made to the subject for each post-discharge study site visit and home visit if allowed by the individual site/IRB/EC and/or regulatory agency.

The study site will notify the home visit agency at the time of subject discharge (i.e., at a minimum provide subject's name, address, contact information). The Investigator will provide the discharge orders for the home visits to commence. If the subject is discharged to a skilled nursing facility (SNF), hospice, or rehabilitation facility, the Investigator will assure discharge orders are reflective of the research to be carried out in the facility by the home visit practitioner. It is the study site's responsibility to ensure the home visit will be allowed at the facility. In both cases, discharge to home or to another facility, every effort will be made by the home visit practitioner to schedule the initial visit within one week of hospital discharge. Subsequent visits will be scheduled weekly (±2 days) thereafter through the End of Study Day 91 (based on the original date of discharge not on the day of the previous visit). All study subjects must have a valid home address and phone number where they can be reached, or the information of the facility to which they are to be transferred.

The initial home visit will be scheduled for 1 to 2 hours; subsequent visits will be scheduled for approximately 1 hour. During the visits, the home visit practitioner will administer two questionnaires and collect an alcohol biochemical marker, PEth blood spot test (Center for Substance Abuse Treatment 2006; Allen 2003; Cluver 2007; Miller 2005). The home visit practitioner's scope of practice does not extend past the aforementioned duties. During the visit if the home visit practitioner, in his/her professional opinion, deems the subject in need of immediate medical attention, the home visit practitioner will observe his/her specific agency protocol as appropriate and notify the Principal Investigator and/or appropriate healthcare provider within 24 hours of the event. Again, as with the Blinded Investigator, the home visit personnel are not to be informed of the subject's treatment group. This must be reinforced with the subjects at discharge.

Initial Weekly Visit (1 to 2 hours)

- Alcohol Timeline Followback Questionnaire (TLFB)
- Modified Treatment Services Review Questionnaire (mTSR)
- Test for alcohol use (PEth)

Subsequent Weekly Visits through End of Study Day 91 (irrespective of scheduled Study Day Evaluations) (1 hour)

- Alcohol Timeline Followback Questionnaire (TLFB)
- Modified Treatment Services Review Questionnaire (mTSR)
- Test for alcohol use (PEth)

The weekly TLFB (Sobell 2003) and mTSR questionnaires (provided separately from this protocol) will be completed (unless the subject is unable to do so due to his/her physical state). However, as with the blood test to determine if the subject has been drinking, these results will not become part of the medical record or be shared with the Principal Investigator or hospital staff.

5.5.8 Missed Visits

If a subject misses a scheduled appointment, every effort should be made to reach the subject by the study site personnel. The missed visit should be communicated to the home visit provider and a concerted effort should be made to contact the subject. If the subject does not return messages after at least 3 attempts, and a back-up contact was identified, every effort should be made to reach the back-up contact.

If the back-up contact cannot be reached by phone after at least 3 attempts, a registered letter will be sent by the study site personnel to both the study subject and to the back-up contact, requesting that they call the site to reconfirm contact information.

If no information is forthcoming within three weeks of sending the registered letters, the subject will be considered lost to follow-up. If at a later time, the subject contacts the study site, or survival information is made known, the study visit should be done at that time (or as much of it as possible; i.e. documentation of survival outcome), even if it is outside the study window. If the back-up contact responds, effort should be made to get the subject's current contact information and confirm outcome, if possible.

The site should look in their medical records for any information regarding possible death of the subject if they get no response to calls. All deaths will be documented on the eCRFs. Deaths that occur during the subject's active participation in VTL-308 will be reported as an outcome of an SAE.

5.5.9 Early Discontinuation from the Study

If a subject is removed from the study prematurely, the reason for doing so and the date of early discontinuation will be documented in the source documents and the subject's eCRF.

Investigator or study coordinator must clarify if a subject is withdrawing from the treatment part of the study and is still willing to be contacted for follow up throughout the 91-day study, as well as long term follow-up for 5 years.

5.6 LABORATORY TESTING

5.6.1 Blood Culture Samples for Microbiological Testing (ELAD and Control subjects)

Blood culture samples will be collected by the Investigator or designee to determine whether the subject's blood has become contaminated. A set of blood culture samples includes one sample to be cultured under aerobic conditions, and one sample to be cultured under anaerobic conditions.

Blood culture samples will be collected to assess for possible microbial contamination in Control subjects at the End of Study Day 2 and End of Study Day 4.

The following applies to blood culture samples collected to assess for possible microbial contamination in ELAD-treated subjects:

1. Two sets of blood culture samples (1 aerobic bottle and 1 anaerobic bottle per set) will be collected by the Investigator or designee, prior to first connection to the ELAD System, and every 48 hours of ELAD treatment (Hours 0, 48, and 96).
2. During ELAD treatment, beginning at hour 48, the two sets of blood culture samples will be collected at the same time as ultrafiltrate culture samples (See Section 5.6.2).
3. All blood culture samples will be labeled and processed as per institution policy.
4. All positive (preliminary or final) blood culture results that were collected prior to or during ELAD treatment must be brought to the attention of the Sponsor immediately:
    a. If the ELAD Specialist is on-site, the Investigator or designee must communicate the results to the ELAD Specialist, who will be responsible for promptly reporting the results to the Medical Monitor.
    OR
    b. If the ELAD Specialist is not on-site, the Investigator or designee must communicate the results via phone or text message to the Medical Monitor.
5. The Medical Monitor will promptly review the relevant information of the positive blood culture results.

5.6.2 Ultrafiltrate Culture Samples for Microbiological Testing (ELAD Subjects only)

Ultrafiltrate culture samples will be collected by the ELAD Specialist to determine whether the ultrafiltrate circuit has become contaminated. A set of ultrafiltrate culture samples include one sample to be cultured under aerobic conditions, and one sample to be cultured under anaerobic conditions.

The following applies to ultrafiltrate culture samples collected to assess for possible microbial contamination:

1. Two sets of ultrafiltrate culture samples (aerobic and anaerobic) will be collected by the ELAD Specialist after ELAD cartridges are installed in the ELAD System, just prior to starting the ultrafiltrate pump, and every 48 hours of treatment (Hours 0, 48, and 96). The ultrafiltrate culture samples are sampled from the ultrafiltrate circuit flow before the ELAD cartridges (Pre-ELAD) and after the ELAD cartridges (Post-ELAD).
2. The ELAD Specialist will label the ultrafiltrate culture samples as Pre-ELAD and Post-ELAD, date, time, and initials. The ELAD Specialist will deliver the samples to the investigator or designee for processing per institution policy.
3. During ELAD treatment, beginning at hour 48, aerobic and anaerobic ultrafiltrate culture samples will be collected at the same time as blood culture samples (See Section 5.6.1).
4. All positive (preliminary or final) ultrafiltrate culture results that were collected prior to or during ELAD treatment must be brought to the attention of the Sponsor immediately:

a. If the ELAD Specialist is on-site, the Investigator or designee must communicate the results to the ELAD Specialist, who will be responsible for promptly reporting the results to the Medical Monitor.

   OR

b. If the ELAD Specialist is not on-site, the Investigator or designee must communicate the results via phone or text message to the Medical Monitor.

5. The Medical Monitor will promptly review the relevant information of the positive ultrafiltrate culture results.

5.6.3 Blood Collection for Future Research (ELAD and Control subjects)

In addition to the collection of ultrafiltrate samples, at certain investigational sites that agree to perform additional sampling, blood samples will be collected in accord with procedures summarized in Table 1, Table 2 and Table 3 for analysis at a later time. At these sites, this procedure will only be carried out if the subject has signed an additional consent allowing this added analysis. VTL intends to use the samples to evaluate biomarkers/proteins in order to gain further understanding of ELAD's mechanism of action. Testing results could provide information for future development of a safer and more effective version of ELAD. No genetic testing will be performed, and if added later, it will not be done without prior additional informed consent from the subject. Participating centers must document collection times of samples and placement in -70ºC freezer on a log provided by the Sponsor.

5.6.4 Ultrafiltrate Collection for Future Research (ELAD Subjects only)

Ultrafiltrate culture samples will be collected by the ELAD Specialist to assess ELAD cartridge performance. Please refer to the VTL-308 Laboratory Manual for more details.

1. A sample volume of 6 mL of ultrafiltrate from the inlet line (UF In), and 6 mL of ultrafiltrate from the outlet line (UF Out) will be collected by the ELAD Specialist after ELAD cartridges are placed in the ELAD System, just prior to starting the ultrafiltrate pump and every 24 hours of treatment (Hours 0, 24, 48, 72, 96, and 120).
2. Six 1-mL samples will be collected from the ultrafiltrate withdrawal line (UF In), and six 1-mL samples will be collected from the ultrafiltrate return line (UF Out). They will be collected in separate 2mL vials.
3. In the event that ELAD treatment is discontinued early, an additional set of six 1-mL UF In, and six 1-mL UF Out samples will be collected.
4. The ELAD Specialist will ensure the samples are properly labeled per the VTL-308 Laboratory Manual.
5. The Investigator or designee is responsible for placement of the samples in the -70ºC freezer, and documenting such on the ultrafiltrate sample log provided by the Sponsor.
6. The samples will be sent to a Sponsor-designated vendor, by the Investigator or designee as outlined in the VTL-308 Laboratory Manual.
7. The Sponsor may request additional ultrafiltrate culture samples for analysis, up to a maximum of 100 mL, in any one 24-hour period.
8. There will be 100 mL of ultrafiltrate collected from the remaining residual ultrafiltrate left in the system after treatment ends.

6 ELAD SYSTEM

6.1 ELAD SYSTEM USE

The setup of the ELAD System is described in the ELAD System Operator's Manual.

A dedicated, dual-lumen, kink-resistent dialysis catheter must be placed by an experienced physician, preferably ultrasound-guided, into the right internal jugular vein (preferred site), left internal jugular, femoral, or subclavian veins. If available and accessible, in complicated cases the interventional radiology team should be involved. Catheters should be designed to deliver a minimum of 300 mL/min blood flow to the ELAD System. For insertion and care of the catheter, the Centers for Disease Control and Prevention (CDC) or the European CDC guidelines (or local equivalent) guidelines should be followed.

Extracorporeal systems often require anticoagulation therapy. Refer to Section 5.5.2.1 for additional details on anticoagulation.

It is recognized that there may be some interruptions in continuous treatment for up to 6 hours to allow for subject transport (See Section 6.6). All interruptions in ELAD treatment must be accurately recorded in the subject's source documentation.

All system-related malfunctions will be noted and reported to VTL on the ELAD System Incident Report Form for follow-up by the appropriate internal VTL team.

6.2 PRECAUTIONS WHILE ON ELAD

Subjects in this study may require procedures under standard of care that carry a higher risk if performed while a subject is heparinized or otherwise anticoagulated. These include, but are not limited to, liver biopsies, paracentesis, surgery or dental surgery, insertion of large bore intravascular catheters, and/or elective intubation. Such procedures, if elective in character, should be performed after discontinuation of anticoagulation which also may require interruption of ELAD treatment. Depending on the nature of the intervention, rechecking of the coagulation parameters and correction by administration of blood products may be required.

6.3 ELAD SYSTEM DOSAGE

The ELAD System consists of a set of four cartridges, each containing approximately 110 g of VTL C3A cells (approximately 440 g total). It is estimated that the mass of cells within four cartridges represents approximately 20% to 30% of the native liver hepatocyte mass, a residual mass believed to be necessary for survival.

6.4 RECEIPT AND ADMINISTRATION OF ELAD CARTRIDGES

Upon receipt and inspection of the ELAD cartridges by the VTL ELAD Specialist, the system will be taken to the bedside and treatment will be started as soon as possible. In all cases, treatment should be initiated according to Section 5.4.

6.5 ELAD CARTRIDGE REPLACEMENT

ELAD cartridges may need to be replaced during treatment due to ELAD cartridge performance or interruption of treatment greater than 6 continuous hours.

If scheduled End of Study Day procedures occur while the subject is awaiting new cartridges, those scheduled procedures will be carried out in accordance with the protocol. The 24-hour safety evaluations and UF culture and samples performed on treatment day 1 must be repeated if the ELAD cartridges are replaced during treatment (see Section 5.5.2, Section 5.6.2, and Section 5.6.4). In addition, if ELAD treatment is stopped for more than 6 hours, the Pre-ELAD safety assessments (See Section 5.4.2.1.1) in accord with the pertinent Exclusion Criteria 2, 3, 4, 7, 9, 10, 15, and 16 relative to the safety evaluation time point will be performed. Administration of 100 mg hydrocortisone and 50 mg diphenhydramine do not need to be repeated prior to initiation of ELAD treatment after cartridge replacement.

6.6 INTERRUPTION OF ELAD TREATMENT

ELAD treatment may be interrupted. If the interruption is for a period of *less* than 6 hours then ELAD may be restarted with the same set of cartridges. If ELAD treatment is interrupted for a period of *more* than 6 hours but less than 72 hours (e.g. to allow for the conduct of a diagnostic or therapeutic procedure or for management of an adverse event, or should there be a need to interrupt the use of anticoagulants), then a new set of ELAD cartridges must be used if treatment of the subject will continue. In order for ELAD treatment to be restarted with a second set of cartridges after more than 6 hours, the subject must meet the same safety eligibility criteria as are required prior to the initiation of the first set of cartridges (See Section 5.4.2.1.1 and Section 6.5). If there is a need to replace ELAD cartridges, the subject's overall ELAD treatment duration will be calculated based from the initial start of treatment. If the subject's ELAD treatment is interrupted for greater than 24 hours, the treatment period may be extended, not to exceed a total of 120 treatment hours. The maximum period between stopping treatment and starting with a new set of cartridges is 72 hours.

6.7 CRITERIA FOR DISCONTINUATION OF ELAD TREATMENT

ELAD treatment will be discontinued and will not be restarted if any of the discontinuation criteria listed in Section 3 are met.

6.8 ELAD SYSTEM ACCOUNTABILITY

6.8.1 Shipment of ELAD Cartridges

The ELAD cartridges will be packed in a temperature controlled, secured shipping container to maintain temperature within specifications outlined in the ELAD System Operator's Manual. The shipment will be sent to the Investigator or designee by rapid courier. Final ELAD cartridge accountability records will be maintained as described in Section 6.8.4.

6.8.2 ELAD Cartridge Inspection and Storage

The ELAD cartridges may be received at the study site by the ELAD Specialist or designee. ONLY the ELAD Specialist will open, inspect, and store the ELAD cartridges as per the ELAD System Operator's Manual. ELAD cartridge accountability will be documented as described in Section 6.8.4.

ELAD cartridges may remain stored in a temperature controlled environment for up to 60 hours according to the "Begin Use By" date and time indicated on the label. Cartridges that have been stored longer than 60 hours before they are used in ELAD treatment will be returned to VTL or designee or destroyed by the ELAD Specialist according to the institution's policies.

6.8.3 Packaging and Labeling of Cartridges

The ELAD cartridges will be labeled with the protocol number, ELAD cartridge identification, conditions for storage, subject number, Investigator's name and telephone number, "Begin Use By" date and time (60 hours allotted for transport and storage until installation into ELAD System), and "Complete Use By" (date and time after which the cartridges can no longer be used for subject treatment). Other labels must not be adhered to the cartridges to obscure any of this labeling information. The final label will be adapted to the requirements of the individual country where the study is being performed in accordance with local regulations.

6.8.4 Dispensing and Accountability of Cartridges

ELAD cartridges will be dispensed to the study site for subject use. Other labels must not obscure the VTL label, especially the cautionary statement on Investigational Use, which is required verbatim by law.

The ELAD Specialist will maintain complete accountability records of all ELAD cartridges shipped to the study site. The accountability records are provided to the study site upon completion of treatment. The ELAD cartridge accountability log will include the following:

- Subject Identification Number
- ELAD cartridge reception, inspection, and storage
- ELAD cartridge final disposition
- ELAD cartridge lot number

The ELAD Specialist will also provide the study site with copies of the shipping records of the ELAD cartridges and a copy of the Certificate of Analysis as part of the subject's source documentation.

The ELAD cartridges are to be prescribed only by the Investigator named on the Form FDA 1572 (or equivalent) and listed on the Delegation of Authority Log. Under no circumstances will the Investigator allow the ELAD cartridges to be used other than as directed by this protocol.

6.8.5 ELAD System Disposition

Maintenance and accountability of the ELAD System, non-mechanical materials (disposables) and supply cart will be maintained by the ELAD Specialists in accord with the ELAD System Operator's Manual.

All System-related malfunctions will be noted by the ELAD Specialist and reported to VTL according to internal VTL processes.

ELAD cartridges, ELAD System, and supply cart disposition is recorded on the ELAD System Monitoring Forms. This is completed by the ELAD Specialist on site at the end of subject treatment. These forms serve as verification of the accountability (including disposition) of the ELAD System, cartridges, and supplies, and this information is available to the site personnel in the EDC system.

Each ELAD System and supply cart has a unique identification number that will be used for this purpose and will include date of arrival as well as date shipped out of the investigational site.

Any issues involved with the identity, quality, durability, reliability, safety, or performance of the system or any of its components will be recorded on the ELAD System Monitoring Forms by the ELAD Specialists. Any investigational product-related subject adverse events will be reported as detailed in Section 9.

6.8.6 Cleaning of Equipment after Discontinuation of ELAD Treatment

At the conclusion of the subject's treatment and the removal of all non-mechanical (disposable) materials from the ELAD System, the ELAD Specialists will clean all surfaces of the ELAD System using the appropriate disinfectant solution(s) according to the ELAD Bedside Unit disinfection procedure. The cleaning of the equipment designated for ELAD treatment will be noted on the ELAD Declaration of Decontamination Status Form.

7 STATISTICS AND METHODS OF DATA ANALYSIS

7.1 SAMPLE SIZE RATIONALE

The sample size is based on experience from the VTI-208 study.

Analysis of the VTI-208 data suggests that in a group of subjects meeting the inclusion criteria for VTL-308 it might be reasonably anticipated that the hazard rate in the control group will be 0.004 and the hazard ratio 0.4 in favor of the ELAD group. Under the assumption of a proportional hazards model, this leads to median survival estimates of approximately 175 days and 438 days for Control and ELAD subjects, respectively.

The VTL-308 inclusion criteria have been established to reflect the same study population as that enrolled in a subset of the VTI-208 population with baseline criteria including MELD <30, age <50, INR ≤2.5, creatinine <1.3 and serum total bilirubin ≥16mg/dL, to define the ITT population, with key safety criteria also applied prior to initiation of ELAD treatment and at End of Study Day 2 (EOSD 2) in the Control group to establish the mITT population. Applying these criteria to the analysis of the primary endpoint of overall survival in the ITT population, and assuming: 1) that this prospectively-defined population behaves similarly to this subgroup of the VTI-208 study; 2) the use of a log-rank test comparing two survival curves with a two-sided significance level of 0.05; 3) exponential survival curves with proportional hazards; 4) uniform accrual with an accrual time of at least 720 days and a minimum follow-up time of 90 days; and 5) a drop-out rate of 10%, a sample size for VTL-308 of 75 subjects per group with 1:1 allocation is consistent with power of at least 0.95 with estimated median survival times of 175 and 438 days respectively.

Due to the distribution of subjects between the ITT and mITT group it may be necessary to enroll more than 150 subjects total in order to ensure that there are a minimum of 75 subjects actually treated with ELAD in the ITT population. Consequently, enrollment may continue until ELAD treatment has been initiated on 75 subjects.

The proportional hazards model modified in accord with findings from Study VTI-208 estimates that approximately 55 deaths are anticipated as the total number of events required to achieve approximately 95% power under these assumptions. If the total number of observed events in the ITT primary population is less than this number after all 150 enrolled subjects complete Study Day 91, are lost to follow up, withdraw consent, or die before that Study Day, then the study may continue until a minimum of 55 deaths are recorded. This provision is intended to ensure that the desired power is achieved in the ITT population even if the event rate is lower than would be anticipated from the reference study, VTI-208.

7.2 POPULATIONS

7.2.1 Safety

This population is defined as all subjects who are randomized and begin treatment including standard of care; analyses of this population will be based on actual treatment received. This population will include all randomized subjects assigned to the group that most accurately reflects the actual treatment that they received.

As a practical matter this means that a subject deemed an "ELAD-treatment initiation failure" that received no ELAD treatment would be assigned to the Control group. A subject that was randomized to the Control group yet received ELAD treatment would be assigned to the ELAD group.

7.2.2 Efficacy

7.2.2.1 *Intent-to-Treat (ITT)*

The ITT population is defined as all subjects who are randomized; analyses of this population will be based on assigned randomized treatment. This population will include all randomized subjects assigned to the group to which they were randomized irrespective of actual treatment. The ITT population will be the primary analysis population.

7.2.2.2 *Modified Intent-toTreat (mITT)*

The mITT population is defined as all subjects who are randomized to the ELAD treatment group that subsequently become eligible for treatment initiation and received ELAD treatment, and all subjects who are randomized to the Control group that also remained eligible for treatment in accord with EOSD 2 evaluations. Treatment assignment will be based on the treatment group to which the subject was randomized.

7.2.2.3 *Per-Protocol (PP)*

The PP population will include all randomized subjects assigned based on treatment group (ELAD or Control) ***less*** those that received <72 hours of treatment (ELAD or Control) and ***less*** those that had major protocol violations, including but not necessarily limited to the following:

- Baseline ineligibility
- Ineligible for ELAD treatment initiation but treated anyway
- Continued treatment for more than 3 days despite an increase in bilirubin in excess of 25%
- Off treatment for longer than 6 hours with the same set of cells
- Off treatment for longer than 72 hours with a second set of cells
- Treated with cartridges >60 hours from harvest (i.e., expired)
- Received different treatment than their treatment assignment

Those subjects deemed an "ELAD treatment initiation failure" that received no ELAD treatment would be excluded from this population (as they received different treatment) as would those subjects who died within the first 72 hours (Control or ELAD treatment) or those that received a total of less than 72 hours of Control or ELAD treatment for other reasons.

7.3 PRIMARY EFFICACY ANALYSIS

7.3.1 Overall Survival (OS) of AILD Subjects through at Least Study Day 91

OS will be assessed using a Kaplan-Meier survival analysis of the intent-to-treat (ITT) population utilizing a log-rank test, with follow-up Protocol VTL-308E providing additional survival data up to a maximum of 5 years that will be included, as available, through VTL-308

study termination. Model-based estimates and confidence limits will be calculated for median survival by treatment group and the hazard ratio and its confidence limits. This analysis will also be carried out on the mITT, Per-Protocol (PP) and Safety populations as sensitivity analyses. Two-tailed alpha for the log-rank test will be set at 0.05. The statistical analysis plan will outline the methods used to account for missing data in this and all other analyses.

The statistical analysis plan will also evaluate differences in standard of care between the groups that may affect subject outcome, such as differences in the administration of steroid therapy, and define analytical strategies to deal with those differences should they arise.

7.4 SECONDARY EFFICACY ANALYSES

7.4.1 Proportion of Survivors at Study Day 91

A chi-square test will be used to evaluate the proportion of subjects who survived at End of Study Day 91 based on the ITT population. Two-tailed alpha will be set at 0.05. This analysis will also be carried out on the mITT, PP and Safety populations as sensitivity analyses.

7.5 EXPLORATORY EFFICACY ANALYSIS

Exploratory efficacy analyses will be carried out as defined in the SAP. These may include, but not be limited to, analyses of efficacy outcomes based on any subgroups, such as those defined by medical history and baseline demographics and other baseline characteristics, along with region (USA vs. Europe). Additional analyses may be based on the relationship between outcomes and ELAD System performance, therapeutic interventions of interest and administration of concomitant pharmacotherapies of interest.

7.6 SAFETY ASSESSMENT

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and incidences will be compared between treatment groups using MedDRA Preferred Terms within System Organ Classes for the Safety Population. The following will also be summarized by treatment group:

- Adverse Events
- Mean Arterial Pressure (MAP)
- Clinical laboratory values
- The use of concomitant procedures including concomitant blood product administration (e.g., whole blood, packed red blood cells, platelets, fresh frozen plasma, albumin, cryoprecipitate)
- Concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial line)

7.7 DEMOGRAPHICS AND OTHER BASELINE VARIABLES

Demographic and other baseline variables include age, sex, ethnicity, height, weight, vital signs, and all safety and efficacy analyses related to the subject's condition at Baseline, including all laboratory-based evaluations.

8 STUDY DOCUMENTATION

8.1 SITE DOCUMENTATION

8.1.1 Laboratory Accreditation

Any laboratory facility to be used for analysis of routine clinical laboratory samples required by this protocol must provide evidence of adequate licensure and accreditation. Documentation of laboratory certification/accreditation must be collected throughout the study and maintained in the site's regulatory binder. Reference values and/or normal ranges for the test results must be provided to VTL. VTL must be notified immediately in writing of any changes occurring in reference values during the course of the study.

8.1.2 Financial Disclosure

The Investigator and Sub-Investigators, as identified on Form FDA 1572 (or equivalent), shall provide VTL with sufficient financial information to allow VTL to submit accurate certification or disclosure statements as required under 21 CFR 312.54. The Principal Investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for one year following the completion of the study.

8.1.3 Subject Confidentiality

All reports and subject laboratory documents submitted with the eCRFs or SAE reports will be identified only by subject initials (where allowed) and study number (coded number) to maintain subject confidentiality. All records will be kept confidential to the extent required by law.

8.2 PRE-STUDY SITE VISITS AND SITE INITIATION VISITS

Prior to commencement of the study, representatives of VTL will visit the study site to ensure adequacy of facilities to conduct the protocol, and to discuss with the Investigator the general obligations regarding studies with investigational new products. Upon satisfactory receipt of all necessary documentation (including Form FDA 1572 or equivalent and Financial Disclosure Forms), and all pertinent regulatory documents, the VTL or designated monitor will arrange for all study material to be delivered to the study site and for the scheduling of a mutually-convenient appointment. At this meeting, all personnel expected to be involved in the conduct of the study will undergo orientation to include review of the study protocol, instructions for eCRF completion, and overall responsibilities including those for system accountability and study file maintenance. Subject entry must not begin until the site has been informed by the Sponsor that they are open for enrollment.

Training of site personnel regarding study administration, subject management, and ELAD treatment will occur prior to or at the site initiation visit and be documented appropriately.

8.3 RECORDING OF SUBJECT DATA

Investigators will provide subject data, which will be collected on individual eCRFs, and will be substantiated by source documents at the clinical site and monitored by the Sponsor's clinical research associates (CRAs). The Investigator must maintain a separate subject list containing the name, address, and other contact information for each study participant so that they can be easily

contacted if necessary. This information will not be provided to VTL but will be kept in a secure location at the study center.

8.3.1 Electronic Case Report Forms (eCRFs)

The eCRF will be comprised of a series of electronic forms. Subjects are not to be identified on the eCRFs by name. Subjects will be identified by a unique subject identification number, which is assigned sequentially upon registering the subject in the EDC system. The eCRFs should be completed in a timely manner (the timeframe required by the study contract/agreement). Electronic CRFs are not considered source documents and source documentation must be provided by the site. Sufficient training will be provided on the process of entering data into the eCRFs.

It is the obligation of the Investigator to review each page of the eCRFs for accuracy, completeness, and legibility before signing the designated and appropriate forms as the study authority. Electronic CRFs are to be completed by appropriately trained study personnel. Data recorded on eCRFs should be supported by information recorded in the subject's source documentation (e.g., laboratory reports, hospital records, x-ray reports). Electronic CRF completion may be formally delegated to other study personnel, but they must be identified on the Delegation of Authority Log.

Prior to initiation of the study, the study monitor will conduct an initiation meeting. The appropriate use and completion of all eCRFs will be discussed at that time. Additionally, training on the eCRF system will be done with each study site by the EDC vendor responsible for the system.

Completed eCRFs will be reviewed by the monitor to ensure data accuracy, completeness, and consistency. Any discrepancies found during the eCRF review are to be clarified by the Investigator (or his/her designated staff). This includes eCRF reviews at the site by the Sponsor or its designee, or during data validation and quality assurance review of the data by data management and other functions. A complete audit trail of the original entries, changes and deletions, session dates and times, and the identity of the eCRF user who performed the operation will be maintained by the system.

8.3.2 Records Retention

The Investigator must maintain copies of all documents and records relating to the conduct of the trial. This documentation includes, but is not limited to, the protocols and amendments, eCRFs, advertising for subject participation, adverse event reports, subject source data, correspondence with health authorities and ECs/IRBs, fully executed consent forms, Investigator curricula vitae, monitor visit logs, subject screening and tracking logs, investigational product accountability records, laboratory reference ranges, and laboratory certification/ accreditation or quality control procedures. Subject files and other source documents, and eCRF data must be kept for a minimum time period, as specified below. VTL must be consulted if the Investigator wishes to assign the files to someone else, remove them to another location, or is unable to retain them for the specified period.

The Investigator must maintain all trial records for a period of at least two years following the date on which the investigational product is approved by FDA or other applicable regulatory agency for marketing for the purposes that were the subject of the clinical investigations. If no

application is to be filed, records must be retained until two years following the date that the study is discontinued and the FDA or other applicable regulatory agency is notified. If the application is not approved by the FDA or other applicable regulatory agency for such indication, records must be retained for two years after notification by VTL, of the FDA or other applicable regulatory agency decision (or longer if local regulations require). VTL should be notified in writing at least 30 days prior to the disposal of any study records related to this protocol.

8.3.3 Concomitant Medications, Procedures and Therapies

All concomitant medications administered to the subject from Randomization through Study Day 91, or until subject discontinuation within this 91-day period, will be recorded in the subject's source documents and transcribed into the eCRF in the EDC system. All blood products and procedures administered to the subject from Randomization through Study Day 91, or until subject discontinuation within this 91-day period, will be documented in the subject's source documents and recorded on the Concomitant Therapy eCRF. Use of blood products known to artificially alter serum bilirubin in the 24 hours prior to Randomization will be recorded in the eCRF. If an adverse event requiring concomitant therapy (i.e., medications, blood products, or procedures) occurs up to and including Study Day 91, or until subject discontinuation within this 91-day period, it must be recorded on the appropriate Medication, Blood Product Administration, or Therapies and Procedures eCRF. Use of other defined medications of interest will be captured for the 6 weeks preceding Randomization

The following medications are typically administered for the indications shown in parentheses, and are considered medications of interest:

- Albumin [treatment of hepatorenal syndrome (HRS), hypotension, following large-volume paracentesis, etc.];
- Antibiotics [treatment of infections diagnosed immediately before or while in the study, following gastrointestinal bleeding (prevention of infection following bacterial translocation), or as prophylaxis or treatment of spontaneous bacterial peritonitis (SBP)];
- Beta-blockers [prophylaxis or treatment for bleeding gastroesophageal (GE) varices, following GE banding, etc.]
- Blood products [packed red blood cells (PRBCs), platelets, fresh frozen plasma (FFP), cryoprecipitate for treatment of clinical bleeding, anemia, coagulopathy, etc.];
- Diuretics (treatment of ascites);
- Heparin (or alternative anticoagulation therapy) [for controlling clot formation during ELAD treatment as well as prophylaxis for deep vein thrombosis (DVTs)];
- H_2 receptor antagonists [treatment of gastrointestinal bleeding or prophylaxis of stress-related mucosal disease (SRMD) bleeding];
- Lactulose [treatment of hepatic encephalopathy (HE)];
- Midodrine (treatment of HRS);

- N-acetyl cysteine (treatment of AAH)
- Nutritional supplements;
- Octreotide (treatment of HRS);
- Pentoxifylline (treatment of AAH);
- Rifaximin (treatment of HE);
- Steroids (treatment of AAH);
- Trace metal supplements (treatment of deficiencies of calcium, magnesium, etc.);
- Vasopressors (treatment of hypotension and/or HRS);
- Vitamins (treatment for vitamin deficiencies e.g., pyridoxal phosphate, thiamine, etc.).

As ELAD may provide some degree of liver function, modification of the dosage of drugs known to be subject to liver metabolism should be carefully considered by the treating physician in accord with institutional standard of care. Subjects in this study may require procedures under standard of care that carry a higher risk if performed while a subject is heparinized or otherwise anticoagulated (See Section 6.2).

8.4 MONITORING OF THE STUDY

Throughout the course of the study, VTL or the designated monitor will make periodic contact with the Investigator. This will include electronic and on-site visits at appropriate and necessary intervals. During these visits, eCRFs will be reviewed for completeness and accuracy, and the study conduct will be reviewed for adherence to the protocol. As part of the data review, it is required that source documents (e.g., hospital records, office records) will be made available for review by the study monitor. The monitor will also perform cartridge and supply accountability checks, and will review the Investigator's study file to ensure completeness of documentation. The Investigator or appointed delegate will be available to the monitor during these on-site visits and will provide necessary study documents for inspection and will respond to all inquiries that may arise as part of this review. On completion of the study, the monitor will arrange for a final review of the study files, after which the files should be secured for the appropriate time period as specified in Section 8.3.2. The Investigator will also permit inspection of the study files by authorized representatives of the Institutional Review Board/Ethics Committee (IRB/EC), VTL representatives or their designees, the FDA, or other applicable regulatory agencies.

8.5 SITE CLOSURE

Throughout the course of the trial, the Investigator shall make every reasonable effort to maintain the enrollment rate of appropriate subjects at a level previously determined. Should the enrollment rate lag, VTL may elect to terminate the study at that site. VTL also has the right to terminate the study at any time for non-adherence to the protocol, unavailability of the Investigator or his study staff to VTL monitoring representatives, or for administrative reasons.

The Sponsor reserves the option to terminate the participation of a Principal Investigator or the study at any time. Reasons for terminating the study or participation by the Principal Investigator include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies with the investigational product indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- The Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the study
- The Institutional Review Board/Ethics Committee/Research Ethics Board (IRB/EC/REB) or Data and Safety Monitoring Board (DSMB) decides to terminate or suspend approval for the study or the Investigator
- The Investigator asks to withdraw from study participation

9 PHARMACOVIGILANCE AND SAFETY REPORTING

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, without any judgment about causality. An adverse event can arise from any use of the product (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

It is also defined as any untoward effect, or worsening in severity, or increase in frequency of a sign, symptom, or clinically-significant laboratory abnormality, that occurs after Randomization, regardless of its relationship to the investigational product. This applies to both Control and ELAD-treated subjects.

On the eCRFs, the syndrome or diagnosis should be recorded and not each sign, symptom, and laboratory abnormality associated with that syndrome or diagnosis. Adverse events will be captured on the eCRFs from Randomization through Study Day 91 or until subject discontinuation within this 91-day period. If the subject (ELAD or Control) withdraws consent from the study at any time during the Study, the subject will no longer be followed by the site through Study Day 91. All efforts should be made to ensure subject continues to consent for safety and follow up. All adverse events, regardless of severity and whether or not ascribed to the study treatment, will be recorded in the appropriate section of the eCRF. Adverse events will include all complications including those associated with: the control treatments; surgical procedures; therapeutic or diagnostic procedures (e.g., paracentesis, etc.); catheters [distinguish between those related to the ELAD versus other central venous catheters, peripherally inserted central catheters (PICC), etc.], blood products (e.g, transfusion reactions, etc.), other concomitant drugs (e.g., heparin), in addition to adverse events associated with the ELAD System (VTL C3A cells in ELAD cartridges; ELAD System; and ELAD Procedure), or other causes. The onset, duration, severity, action taken, relationship to treatment, and outcome of all adverse events will be documented in the eCRF. Investigators should use appropriate medical judgment in the characterization of a serious adverse event for subjects with AILD. A medical or surgical intervention required to treat a recognized sign, symptom, laboratory abnormality, or clinical condition associated with AILD may not necessarily be considered a serious adverse event. Please refer to the Investigator's Brochure for the most current ELAD System subject experiences. Additionally, Appendix D includes information regarding how to assess relatedness to ELAD; whether it should be attributed to the VTL C3A cells, the ELAD System, or to the ELAD Procedure. Please refer to this appendix prior to assigning a causal relationship to any reported events.

The Investigator will report serious adverse events (defined consistent with a reportable adverse event in accord with local and regional regulations) to the Sponsor within 24 hours of knowledge of the event in accordance with this section. The Investigator will also report the event(s) to the IRB/EC within 24 hours of knowledge of the event(s) if required by local regulations.

Subjects experiencing adverse events or clinically-significant laboratory abnormalities will be assessed and appropriate evaluations performed until all parameters have returned to baseline levels, or are consistent with the subject's then-current physical condition.

9.1 ADVERSE EVENTS

The safety of all subjects enrolled in this study will be monitored throughout the study. Safety monitoring will include history and physical examination with vital signs, adverse event reporting, and laboratory evaluations. Adverse events will be collected and recorded for subjects from Randomization through Study Day 91, or until subject discontinuation within this 91-day period.

ELAD is a combination biologic and medical device product that is regulated as a medicinal product in the US and EU where the study is being conducted. However, adverse events need to be assessed and reported following both the drug and medical device regional requirements.

The Investigator is responsible for assessment of all AEs/SAEs for severity and relationship to the assigned treatment. All SAEs must be reported by the Investigator to the VTL Designee within 24 hours of knowledge. The VTL Designee will report SAEs that meet the criteria for expedited reporting (per the Investigator's Brochure) to the appropriate health authorities in accord with regional requirements. The Investigator must submit these expedited reports to their IRB/EC in accord with local requirements. Further detail is provided below.

9.2 DEFINITIONS

The following definitions are guided by applicable international regulations and guidelines.

Adverse Event - (Also referred to as an adverse experience) can be any unfavorable and unintended sign [e.g., an abnormal, clinically-significant laboratory finding (see Investigator's Brochure)], symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the investigational product (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

An adverse event can also be defined as 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 product.

Note: This definition includes events related to the investigational product (Biologic, Device, and Procedure).

Adverse Device Effect - Adverse event related to the use of an investigational medical device. This includes any event resulting from insufficiencies or inadequacies in the instructions for use (ELAD System Operator's Manual), the deployment, the implantation, the installation, the operation, or any malfunction of the investigational product, and any event that is a result of a use error or intentional misuse.

Device Deficiency – This is inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

Suspected Adverse Reactions - An adverse event for which there is a reasonable possibility that the drug caused the adverse event. A "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event (See Section 9.6).

Unexpected - An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of investigational products.

This definition relies entirely on a listing of the adverse events or suspected adverse reactions in the Investigator's Brochure as the basis for determining if newly acquired information generated from clinical trials or reported from other sources is unexpected. The suspected adverse reactions listed in the Investigator's Brochure (i.e., "expected") are those observed with the investigational product and for which a causal relationship between the event and the product is suspected or confirmed. These events are listed along with the adverse events experienced by subjects in the Control group in some tables, but it is clear which ones occurred in ELAD-treated subjects. Thus, adverse events that would be anticipated to occur as part of the disease process are considered unexpected for the purposes of reporting because they would not be listed in the Investigator's Brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the Investigator's Brochure. **Therefore, all deaths are to be reported to the Sponsor**.

Serious Adverse Event (SAE) - An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

1. Death,
2. Serious deterioration in the health of the subject, that either results in:
    a. A life-threatening illness or injury, or
    b. A permanent impairment of a body structure or a body function, or
    c. In-patient hospitalization or prolonged hospitalization, or
    d. Medical or surgical intervention to prevent life-threatenting illness or injury or permanent impairment to a body structure or a body function,
3. Led to fetal distress, fetal death or a congenital abnormality or birth defect.

Note 1: Planned hospitalization for a pre-existing condition, or a procedure required by the clinical investigational plan (CIP), or protocol, without serious deterioration in health, is not considered a serious adverse event.

Note 2: This includes **device deficiencies** that might have led to a serious adverse event if, a) suitable action had not been taken, or b) intervention had not been made, or c) if circumstances had been less fortunate. These are handled under the SAE reporting system.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of an SAE. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Life-threatening – for the purpose of reporting adverse events, refers to an event in which the subject was, in the view of the initial reporter, at immediate risk of death at the time of the event as it occurred (i.e., it does not refer to any event which might have resulted in death if it were more severe).

Serious Adverse Device Effect (SADE) – An adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Unanticipated Serious Adverse Device Effect (USADE) – A serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the Investigator's Brochure.

9.3 LABORATORY ABNORMALITIES AS ADVERSE EVENTS

Many laboratory abnormalities observed during the course of a study will be encompassed under a reported adverse event describing a clinical syndrome (e.g., elevated BUN and creatinine in the setting of an adverse event of "renal failure"). In these cases (e.g., an AE of "renal failure"), the laboratory abnormality itself (e.g., elevated creatinine) does not need to be recorded as an adverse event. Refer to the Investigator's Brochure for hematology and serum chemistry results that occur while the subject is on ELAD treatment that should be reported as AEs (e.g., decreased platelet count or increased INR relative to baseline). However, isolated laboratory abnormalities (e.g., hyponatremia) should be reported as adverse events if they are considered to be clinically-significant by the investigator. Criteria for a "clinically-significant" laboratory abnormality are:

- A laboratory abnormality that leads to a treatment-limiting toxicity (e.g., thrombocytopenia that results in study treatment discontinuation or interruption)
- A laboratory abnormality that results in therapeutic intervention (e.g., protamine for a prolonged PTT)
- Other laboratory abnormality judged by the Investigator to be of any particular concern (e.g., detection of fibrin split products).

Significant elevations in α-fetoprotein level is an expected laboratory change in subjects on ELAD treatment. This should not be reported as an adverse event.

9.4 REPORTING OF SERIOUS ADVERSE EVENTS

All SAEs defined as being reportable adverse events in accord with 21 CFR 312.32 [that also includes the reporting of suspected unexpected serious adverse reactions (SUSARs)], European database of Suspected Unexpected Serious Adverse Reactions (Eudraviligance – Clinical Trial Module), and serious adverse device effects (SADEs) in accord with 21 CFR 812, in addition to

all other local regulations, that occurs in any subjects participating in this clinical trial, must be reported as described below:

As soon as feasible, but at least within 24 hours of knowledge of a new SAE, the Investigator must report the event to the VTL designee. The SAE reporting form should be completed and submitted irrespective of the volume of information available about the event, though the Investigator is expected to provide as much information as possible.

Information to be sent to VTL (or designee) includes, where applicable:

- Pertinent subject medical history
- Concomitant medications
- Physical examination results, vital signs and laboratory reports if available
- Treatment(s) administered
- Outcome of the event

SAEs should be reported from Randomization through the last day of study participation or through Study Day 91.

Follow-up information to serious adverse events must be provided promptly as it becomes known to the Investigator and submitted to the VTL designee.

The approving IRB/EC must be notified by the Investigator of any fatal, life-threatening and/or serious adverse events regardless of cause on a timely basis according to local regulatory requirements. In addition, a written report of all SAEs will be submitted by the Investigator per local IRB/EC requirements. All subjects with serious adverse events should be followed clinically and by the appropriate diagnostic evaluations until the event (1) resolves, (2) an outcome is reached, or (3) the event is otherwise explained or stabilized.

Subjects withdrawn from the study due to an SAE will be followed by the Investigator until the outcome is determined and, when appropriate, additional written reports and documentation will be provided to the VTL designee.

All SAEs that occur after the discontinuation of treatment with the ELAD System, regardless of relatedness to the study treatment, must be reported to the VTL designee if the onset of the SAE occurred from Randomization through the last day of study participation or through Study Day 91.

All suspected unexpected serious adverse reactions (SUSARs) related (related is defined as possibly related, probably related, and related) to the investigational product which occur during this study are subject to expedited reporting.

All adverse events, regardless of severity, and whether or not related to the study treatment, will be recorded in the appropriate section of the eCRF.

All adverse events beginning after the discontinuation of treatment with the ELAD System, whether serious or non-serious and regardless of relatedness to the study treatment, must be reported to the Sponsor if the onset of the adverse event occurred during the subject's study

participation (from Randomization through the last day of study participation or through Study Day 91).

All serious adverse events that are unexpected (defined as an adverse event not listed in the Investigator's Brochure) and related (related is defined as possibly related, probably related, and related) to the investigational product that occur from Randomizaton through the last day of study participation or through Study Day 91 must be reported to the Sponsor in a timely fashion.

Any death or liver transplant occurring from Randomization through the last day of study participation or through Study Day 91, must be reported to the Sponsor regardless of how much time has elapsed since the last exposure to investigational product treatment. The cause of death must be reported as an SAE, since death is an outcome and not an SAE. A death occurring after Study Day 91 that is not reasonably associated with investigational product treatment does not require completion of an SAE reporting form. However, both of these events should trigger completion of applicable VTL-308E eCRFs for the subject (see Section 13).

If progression of the underlying disease (i.e., the condition being treated with the investigational product) might be reasonably anticipated given the nature and severity of the underlying disease, then progression of the underlying disease per se will not constitute an adverse event. However, if the progression of the underlying disease meets the criterion for "serious" categorization of adverse events (e.g., the underlying disease results in premature death or prolonged hospitalization), then it should be reported as an SAE.

9.5 CLASSIFICATION OF ADVERSE EVENTS BY SEVERITY

The Investigator must categorize the severity of each adverse event according to the following guidelines:

- MILD: Awareness of sign or symptom, requires no special medical management or attention
- MODERATE: Tolerable, but requires medical management
- SEVERE: Intolerable, requires medical management

9.6 CLASSIFICATION OF ADVERSE EVENTS BY RELATIONSHIP TO STUDY TREATMENT

For assessing relatedness of an event, the meaning of "reasonable possibility" is made clear by providing the following examples of types of evidence that would suggest a causal relationship between the investigational product and the adverse event. Associated with the use of the investigational product means that there is a reasonable possibility that the event may have been caused by the investigational product. Examples of evidence of a causal relationship to the investigational product are the following:

- A single occurrence of an event that is uncommon and known to be strongly associated with investigational product exposure (e.g., anaphylaxis, angioedema, Stevens-Johnson Syndrome)
- One or more occurrences of an event that is not commonly associated with investigational product exposure, but is otherwise uncommon in the population exposed to the investigational product (e.g., tendon rupture).

- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of investigational product treatment) that indicates those events occur more frequently in the investigational product treatment group than in a concurrent or historical control group

The relationship of each adverse event to the study treatment will be assessed by the Investigator after careful consideration, and according to the following guidelines:

Not Related

This category is applicable to those adverse events which are clearly due to extraneous causes (underlying disease, clinical state, concurrent drugs, environment, etc.) and do not meet the criteria for relationship listed under Possibly Related, Probably Related, and Related.

Possibly Related

This category applies to those adverse events judged to be perhaps related to the study investigational product administration. An adverse event may be considered Possibly Related when it meets at least one of the following criteria:

- It follows a reasonable temporal sequence from application of the study treatment;
- It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
- It follows a known or expected response pattern to the study treatment.

Probably Related

This category applies to those adverse events judged with a high degree of certainty to be related to the study treatment. An adverse event may be considered Probably Related if it meets at least two of the following criteria:

- It follows a reasonable temporal sequence from application of the study treatment;
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
- It disappears or decreases on cessation or reduction in study treatment dose. There are exceptions when an adverse event does not disappear upon discontinuation of the study treatment, yet study treatment relatedness clearly exists (e.g., coagulopathy, sepsis, etc.);
- It follows a known pattern or expected response to the study treatment.

Related

This category applies to those adverse events judged to be incontrovertibly related to study treatment. Any adverse event may be assigned to this category if it meets at least three of the following criteria:

- It follows a reasonable temporal sequence from application of the study treatment;
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
- It disappears or decreases on cessation or reduction in study treatment dose. There are exceptions when an adverse event does not disappear upon discontinuation of the study treatment, yet study treatment relatedness clearly exists (e.g., coagulopathy, sepsis, etc.);
- It follows a known pattern or expected response to the study treatment.
- It reappears or worsens when the study treatment is re-administered.

To standardize the reporting of AEs, all of the Investigator terms used to describe complications will be collapsed into primary terms as defined by MedDRA. The incidence of adverse events for each study group, therefore, will be organized and summarized by system organ class, preferred term, relatedness, and degree of severity. Please see Appendix D regarding information about relatedness, including specific attribution to the VTL C3A cells (biologic), the ELAD System (device), or to the ELAD procedure.

9.7 SPONSOR AND INVESTIGATOR REPORTING REQUIREMENTS

With regard to regulatory compliance, the specific AE and SAE reporting requirements of Investigators and Sponsors participating in this study are governed by the local and regional regulations of each Regulatory Authority authorizing the conduct of the trial, and by the ICH. This protocol reflects the shortest reporting timelines in these collective regulations, hence adhering to compliance at all investigational sites.

In certain European countries where ELAD is regulated as both a biologic/advanced therapy and a device, VTL is required to report all SAEs that have occurred in the trial in compliance with the EU MEDDEV 2.7/3 guidelines on Serious Adverse Event reporting. In order to minimize any potential compromise to the trial whose primary efficacy endpoint is overall survival and to maintain the blind in study conduct, those SAEs that have an outcome of death, or in rare cases where death is listed as an event term, will not be reported in the MEDDEV reports. This exception in SAE reporting is consistent with the recommendations in ICH Guidance E2A Clinical Safety Data Management regarding potential compromise of the clinical investigation if the blind is broken.

Similarly, reporting of deaths and associated statistics in the periodic safety reports, including the Development Safety Update Report, required for submission by the Sponsor in compliance with local and regional regulations where the trial is being conducted, will be masked to further minimize potential compromise to the trial.

9.8 UNANTICIPATED DEVICE ADVERSE EFFECTS

Unanticipated device adverse effects will be reported by the Investigator and by the Sponsor as required by local regulations. In addition, the ELAD Specialist will record all device-related malfunctions on the ELAD System Clinical Incident Report Form, whether they appear to impact the subject and/or ELAD treatment or not, and follow-up by VTL will be conducted. Any broken or malfunctioning components caused by shipping or moving the system and/or supply cart are also captured on the form. The ELAD Specialist will notify his/her supervisor of all device and/or component deficiencies during treatment within a timely manner, in addition to filling out the ELAD System Clinical Incident Report Form. Similarly, an ELAD System Clinical Incident Report and notification to the supervisor is required if the ELAD Specialist notes an increase in the need to replace any specific component(s) during the subject's treatment. Once reported, appropriate action, if required, will be taken by the Sponsor.

All device issues that do not impact the subject directly are reported by the ELAD Specialists on the ELAD System Clinical Incident Report Form and follow-up at VTL is conducted by the Risk Evaluation Team. Any deficiencies listed on the form will be discussed with the ELAD Specialist's supervisor within 24 hours. Any incidents believed capable of doing harm to subjects, even if they did not in the case reported, will be conveyed to the Risk Evaluation Team for immediate assessment and consideration of expedited regulatory and IRB/EC reporting. All unanticipated adverse device effects will be reported as SAEs if they meet the reporting requirements, as described in Section 9.4.

9.9 DATA AND SAFETY MONITORING BOARD (DSMB)

An independent DSMB will be constituted to include at least one expert hepatologist, and one intensivist. An independent statistician will provide the data for DSMB review and consult the DSMB for statistical questions. The DSMB will review and interpret safety data from the study on a regular basis. The DSMB membership and responsibilities will be documented in a charter that will be prepared prior to the first DSMB review meeting. The DSMB reviews will include summaries of safety data, such as laboratory data (with special attention to liver function tests), serious adverse events, and treatment discontinuation. After each safety review, the DSMB will make recommendations to VTL regarding continuing, modifying, or terminating the trial.

10 HUMAN SUBJECTS AND INFORMED CONSENT

10.1 INSTITUTIONAL REVIEW BOARD/ETHICS COMMITTEE

This protocol, the proposed informed consent form, any advertisement for subject recruitment and any subject instructional materials must be reviewed and approved by the appropriate IRB/EC prior to the start of the study. The proposed informed consent form and any proposed advertisement must also be agreed to by VTL. A copy of the IRB/EC approval letter of the protocol, any amendments, the informed consent form and any advertisements or subject instructional materials must be supplied to VTL prior to starting the study. During the course of the study, the Investigator shall make timely and accurate reports to the IRB/EC on the progress of the trial, at intervals not exceeding one year, as well as satisfying any other local IRB/EC regulations regarding reporting. Protocol deviations/procedure violations will be reported to the IRB/EC as required. Copies of all reports and correspondence with the IRB/EC must be provided to VTL. Furthermore, at the completion or early termination of the study, a final report should be made to the IRB/EC by the Investigator within the applicable IRB/EC time frames.

The IRB/EC must review any requests for deviations, and reports of deviations, if the deviation affects subject's rights, safety and well-being, or the scientific integrity of the clinical investigation if local regulations require. Please check with your IRB/EC regarding their specific reporting rules.

Any significant changes or revisions in the study protocol or any changes that may alter subject risk must be approved by VTL (and may require FDA or other regulatory agency review and approval) and must be approved in writing by the IRB/EC prior to implementation. A protocol change intended to eliminate an apparent immediate hazard may be implemented immediately provided that VTL is immediately notified and an amendment is subsequently provided by VTL and approved by the IRB/EC. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the IRB/EC. Such deviations must be documented and reported to the sponsor and the IRB/EC as soon as possible.

The Investigator's Brochure, protocols, case report forms, informed consent form and other subject information, or other clinical investigation documents will be amended as needed throughout the clinical investigation. The sponsor will notify the Principal Investigator, or the coordinating Investigator of such amendments. The amendments to the protocol and the subject's informed consent form shall be notified to, and approved by, the IRB/EC and regulatory authorities, if required. The amendment number and date of amendments will be documented.

It is the Investigator's obligation to maintain an IRB/EC correspondence file, and to make this available for review by VTL representatives as part of the study monitoring process.

10.2 INFORMED CONSENT

The proposed informed consent form, which must be in compliance with the regulations, must be reviewed and approved by VTL prior to initiation of the study. The proposed informed consent form must contain a full explanation of the purpose, nature and duration of the study, a description of the procedures, the possible advantages, risks, alternative treatment options, and a statement of confidentiality of subject study records, a statement regarding voluntary compensation and availability of treatment in case of injury, an explanation of whom to contact

about the research, the subjects rights, and notification that participation is voluntary and refusal will involve no penalty or loss of medical benefits. These requirements will be in accordance with US Federal Regulations as detailed in 21 CFR 50.25 and the Declaration of Helsinki and any applicable local regulations. It should also indicate, by signature, that the subject, or legal guardian/legally authorized representative, permits access to relevant medical records by the Sponsor and/or the Sponsor's duly appointed agent and by representatives of the US FDA or other applicable regulatory authorities. Additionally, Investigators in states and countries with specific regulations regarding subject's rights have a responsibility to follow and document their fulfillment of those regulations.

The Investigator will be responsible for obtaining written informed consent from potential subjects or their subject representative(s) prior to any study-specific screening required for entry into this study. During the consent process, the Principal Investigator or appropriate designee(s) will verbally inform all subjects or their legally-authorized representative(s) about the purpose, nature and duration of this clinical study. The Investigator or appropriate designee will also inform all subjects regarding the methods to which they will be subjected and the experimental nature of the treatment, as well as the potential risks and benefits that may result from utilization of study treatment. Subjects will be informed that they are free to refuse participation in this clinical study. If they should participate, it will be made clear that they may withdraw from the study at any time without prejudicing further care. Subjects will also be informed of alternative methods of treatment should they not wish to participate in this clinical study. The person obtaining informed consent from the subject and the subject or their legally-authorized representative must sign and date the informed consent document.

A signed copy of the informed consent will be provided to the subject, and a signed copy will be maintained with the subject's eCRFs, or in the study documentation notebook. The original will be retained by the Investigator.

Subjects participating in this protocol will provide written informed consent at the time of the screening visit indicating their understanding of the study. Due to the critical nature of the subject's condition, they may not be able to understand or execute the informed consent at the time of Screening. The subject's legally authorized representative must then sign the subject informed consent at the time of subject entry. The reason the subject was unable to personally provide consent will be indicated on the consent form signed by the subject's legally authorized representative. Should the subject's condition improve such that they are able to understand the study and provide personal consent, they will be asked to give their consent to continue participation. This consent will be documented on a separate form. If the subject is able to understand the study and provide informed consent, but is unwilling to continue their participation, the subject must be withdrawn from the study. The source documents must note the subject's decision, and this information must be accurately captured in the subject's eCRFs.

Consent includes authorization of participation into VTL-308E, the 5-year registry follow-up portion of the trial. See Section 13 for more information regarding this.

Should withdrawal of consent be implemented by the subject or the subject's legally-authorized representative, a written document will be presented to them for signature with the ability to check the extent to which consent is withdrawn.

11 DISCLOSURE OF DATA/PUBLICATION POLICY

All information obtained as a result of this study or during the conduct of this study will be regarded as the confidential property of VTL. Disclosures (i.e., release of information to any third party not noted herein) or any information related to the study, not previously known to be public, and/or study results shall not be made either by publication or by oral or poster presentation without the express, written consent of VTL.

VTL intends to have the results of this trial published following study conclusion. The results will appear on at least one public database, www.clinicaltrials.gov.

12 BIOHAZARD CONTAINMENT

Since the transmission of HIV and other blood-borne infectious agents can occur through contact with contaminated needles, blood or blood products, appropriate precautions must be employed by all personnel involved in this study, as currently recommended by the Centers for Disease Control. Universal Precautions information can be obtained at the following website:

http://www.cdc.gov/niosh/topics/bbp/universal.html

13 ESTABLISHMENT OF REGISTRY FOR SUBJECT FOLLOW-UP – VTL-308E

VTL intends to provide for long-term follow-up of subjects recruited into this trial through establishment of a registry which is described in the extension protocol VTL-308E.

13.1 VTL-308E JUSTIFICATION

VTL is conducting clinical trial VTL-308, in which subjects with AILD are treated with ELAD and standard of care, or with standard of care alone to assess the safety and efficacy of ELAD treatment.

A hypothetical risk exists that over an extended period of follow up there may be an increased incidence of tumor formation in subjects treated with ELAD. However, the underlying disease processes in subjects with alcoholic hepatitis also leads to an increased risk of tumor formation in the liver and elsewhere. Worldwide, hepatocellular cancer is the fifth most common cancer in men and eighth most common cancer in women, varying in accordance to prevalence of hepatitis B and C infections, and well over 80% of all primary liver cancers are caused by chronic hepatitis infections (Bosch and Diaz 2004).

The VTL-308 trial monitors subject safety up to 91 days after Randomization. To assess whether there is an increased risk of late tumor formation in subjects treated with ELAD, this registry protocol segment of VTL-308 extends the safety monitoring period to 5 years.

VTL-308E is designed to provide a long-term assessment of subject's outcome in relationship to tumor (in particular liver tumor), liver transplant, and overall mortality. The protocol includes both control and ELAD-treated subjects to assess the significance of any findings.

13.2 OBJECTIVES

The intent of this protocol is to determine the status of VTL-308 study subjects at 6 months (± 2 weeks), 9 months (± 2 weeks), 1 year (± 2 weeks), and then annually for 4 additional years (± 2 months) following their participation in the study to assess:

- Survival
- Incidence and characterization of tumor (in particular hepatocellular tumor)
- Incidence of liver transplant
- Quality of life using a standard, validated questionnaire

Subjects who withdraw prior to Study Day 91 and who continue to consent to be followed in the VTL-308E study will be contacted according to the VTL-308E procedures at each of the remaining parent study scheduled visits (e.g. Study Day 28, Study Day 63, Study Day 91). VTL may request additional unscheduled contacts during the extension period in order to support survival analyses, particularly around the time of completing the parent study.

13.3 SUBJECT POPULATION

Since this is a follow-up segment to the VTL-308 study, only subjects participating in VTL-308 will be considered for enrollment into this protocol. Subjects lost to follow-up in VTL-308 will be considered as the same, lost to follow-up, for this extension segment of this protocol (VTL-308E).

13.4 RESEARCH DESIGN AND METHODS

While consenting for VTL-308, each subject will be consented to participate in the follow-up VTL-308E protocol as an extension of the protocol. Once consented, they will provide contact information that can be used for 5 years of follow-up (see Section 5.2.1). This will include a current address and telephone number as well as that of a contact person who may know their whereabouts should they no longer be able to be reached at the phone number or address provided. This contact information (e.g. address, telephone number, contact person) will not be sent to VTL or to their data management contractors. It will be available to study personnel at the investigational site only.

Additionally, subjects will be asked to notify their study Investigator if they are diagnosed with cancer at any time during the 5 years following their VTL-308 participation regardless of when the diagnosis is made in reference to their annual follow-up call. They will be given the contact information necessary to make the notification.

The date of Randomization will be logged on an eCRF. Based on that date, at 6 months, 9 months, 1 year, and then annually for 4 additional years following their date of Randomization, subjects will be contacted by phone by the investigative site. VTL will keep a master log of the coded subjects and their anniversary dates and will send a reminder to the sites within 2 weeks of the time the call should be made. The site will be provided with eCRFs that include the specific questions to ask.

The questions involve the following:

If the subject responds:

When the subject is reached by phone, or if the subject calls the site, he/she will be asked the following:

1. Since your last contact with study staff, have you had a liver transplant?
   If yes, obtain the date of the transplant.
2. Since your last contact with study staff, have you been diagnosed with cancer (that is different than any cancer you may have had while in the trial)? If yes, proceed with the following questions:
   a. What type of cancer were you diagnosed with?
   b. What was the extent of your metastatic disease (if applicable)? (To be asked as *has your cancer spread? If so, where?*)
3. Have you changed or do you anticipate changing your contact information? If so, please update it now.
4. Have you changed or do you anticipate changing your contact person or their contact information? If so, please update it now.

5. The EQ-5D-5L Quality of Life questionnaire will be completed.

If the call recipient states the subject has expired:

If another person answers and states the subject is deceased, the investigative site's designated caller will ask the following:

6. When did the subject expire?
7. What was the cause of death?
8. Was an autopsy performed? If yes, where can a copy of the report be obtained?
9. Ask if signed approval can be obtained to access the subject's medical records that have the pertinent information (particularly if cancer was present) if the information is not in the medical record at the investigational site. If approval is given, the coordinator will send out the appropriate medical records release form for a legally appropriate family member to sign. A prepaid FedEx return envelope will be included.
10. Was the subject diagnosed with cancer after being in the VTL-308 study? If so:
    a. What type of cancer was the subject diagnosed with?
    b. What was the extent of the subject's metastatic disease (if applicable)? Again, ask in terms the caller can understand.
11. Had the subject had a liver transplant? If so, obtain the date of the transplant.

If the subject is deceased, it will be noted on the eCRF and no further follow-up will be initiated. Vital Therapies must be made aware of this to update the call reminder list. Call the office at +1-858-673-6840 and ask for any of the VTL-308 staff. Deaths, incidences of cancer, and liver transplants will not be reported as SAEs, but should be reported to Vital Therapies as soon as feasible.

Subject is not home/available:

If the subject is unavailable, the caller will find out when to call back to speak to the subject, or what a better phone number might be to reach the subject. A call-back number can be left for the subject.

A message can be left on a subject's voicemail or answering machine/service only if it gives no medical information or relationship to the reason of the call. For instance, "This call is for John Doe. Please call Jane at 555-555-6655 when you get this message." No reference to a clinic, doctor's office, etc. should be left unless the subject has previously stated this is allowed.

Subject not located or calls not returned:

If a subject is no longer at the number given, continues to be unavailable, or does not return messages for at least 3 attempts, the person listed as the contact person should be notified for information.

If the contact person cannot be reached by phone (at least 3 attempts), a registered letter will be sent to both the study subject and to the contact person requesting contact information for them both.

If no information is forthcoming within 3 weeks of sending the registered letters, the subject will be considered lost to follow-up. No further attempts will be made to reach the subject or the contact person. VTL should be notified of the lost-to-follow-up status by calling the office at +1-858-673-6840 and asking for any of the VTL-308 staff.

If at a later time, the subject or the contact person responds, the study visit should be done at that time, even if it is late. The protocol will resume on the following anniversary, so ensure that VTL is made aware of the late contact to keep the subject on the call reminder list.

The site should look in their medical records for any information regarding possible death of the subject if they get no response to calls. All deaths will be noted on the eCRF.

Prior to calling a subject annually, the previous year's information will be reviewed to assure no subject contact is inadvertently made if the subject has expired and we have been made aware of it.

For subjects lost to follow-up, whether during or after the 91-day parent study, VTL may engage a third party vendor that specializes in identifying survival status (e.g. through a search of public records). In such cases, this vendor will provide documentation of the survival status to study site, who will then enter this new information in the eCRF.

13.5 MAINTAINING CONFIDENTIALITY

All efforts to maintain confidentiality of subject identification will be upheld. VTL will have the investigative site personnel call all subjects and maintain the contact information. Identifiable subject data will not be stored at VTL nor will it be made available to VTL.

A coding system will be in place using the coded patient identifiers used in VTL-308 (the subject number). Only the investigative site will have the corresponding subject names, phone numbers, addresses, and contact information in the event the subject cannot be reached. There will be no social security or medical record numbers stored.

A cover page with the subject identification coded will be completed at the site. This cover page will have the subject's full name, telephone number, and address. It will also have the name, telephone number, and address for their back-up contact should they be unreachable. This cover page, and any information contained within, will not leave the site or be made available to VTL or to the data management company.

Site staff must be aware of the importance of keeping subject data confidential. In accordance with ICH *Guideline for Good Clinical Practice*, confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

Monitor(s), auditor(s), the IRB/EC, and the regulatory authority (ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.

Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential.

14 CRITERIA FOR STUDY DISCONTINUATION

This study shall be terminated following completion of study participation of all study subjects enrolled, or at the discretion of VTL, or the Data and Safety Monitoring Board (DSMB). If during review of safety information it is determined that the study represents higher risk than potential benefit, the DSMB will inform the Sponsor if action is required, per the DSMB Charter. In addition, if an SAE or ELAD System Clinical Incident is observed of such a nature that it represents an immediate, life-threatening risk to ELAD subjects, enrollment into the study will be suspended, the occurrence investigated, and IRBs/health authorities notified expeditiously. Study enrollment can only be reinitiated if deemed appropriate after review and release by the DSMB and health authority notification.

15 INVESTIGATOR AGREEMENT

I have read protocol VTl-308 (incorporating VTL -308E) and its appendices and agree to adhere to all requirements in the conduct of the study.

In signing below I agree:

- To assume responsibility for the proper conduct of the study at my site
- To conduct the study in compliance with this protocol, any future amendments, and with any other study procedures provided by Vital Therapies, Inc.
- That I am aware of, and will comply with Good Clinical Practices (GCP) and all applicable regulatory requirements
- To ensure that all persons assisting me with the study are adequately informed about the study and of their study-related duties as delegated by me
- Not to implement any changes to the protocol without written agreement from Vital Therapies, Inc. and prior review and written approval from the Ethics Committee/Institutional Review Board (EC/IRB) except where necessary to eliminate an immediate hazard to study subjects

I acknowledge that failure to adhere to these stipulations may constitute a breach of governmental regulations, may invalidate the data and may result in termination of the study at my site.

Principal Investigator's Signature	Date

Principal Investigator's Printed Name

Institution's Name

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Table 1. Schedule of Evaluations and Procedures at Screening for Randomization

Procedures	Screening[1]	Randomization (Start of Study Day 1)
Identify Subject/Notify Vital Therapies	X	
Informed Consent	X	
Demographics (DOB or age, race, ethnicity, gender, height, weight)	X	
Medical History	X	
Physical Exam[2] and Vital Signs[3]	X	
Inclusion/Exclusion Criteria	X	
Encephalopathy Stage (West Haven Criteria)[2]	X	
Ultrasound [11]	X	
Serology for active viral hepatitis A, B, and C[4]	X	
Laboratory Evaluations[5]	X	
Complete Blood Count (CBC)	X	
Blood for outside analysis (consented subjects at participating centers ONLY) [6]	X	
PT/control PT/PTT/INR	X	
Baseline Laboratory Evaluations[12]	X	
Test for alcohol use (PEth)	X	
Pulse Oximetry and FiO_2	X	
Blood Culture samples (×2)[6]	X	
Sputum and urine Culture and Sensitivity[6]	X	
Peritoneal Fluid Culture[6] (only if paracentesis has not been done within 7 days prior to Screening)	X	
Serum Pregnancy Test (if applicable)	X	
MELD Score and Maddrey Score autocalculated[7]	X	
Enroll Subject/Notify Vital Therapies		X[8]
Adverse Events		X
Concomitant Medications[10]		X
Concomitant Blood Product Administration		X
Concomitant Fluid Administration		X
Subject Location[9]		X

1 All Screening evaluations and procedures can be obtained from standard of care if previously performed within 24 hours of Randomization unless otherwise specified.

2 Performed by a study physician, physician assistant or nurse practitioner if within their scope of practice for institution.

3 Blood pressure, heart rate, respiration, temperature.

4 Serologies are to be completed at Screening if not previously performed during this hospital admission.

5 BUN, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, sodium, potassium, chloride, carbon dioxide (or bicarbonate), calcium, magnesium, phosphate, albumin, total protein, alkaline phosphatase. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin.

6 Blood sample, urine sample, sputum sample, and peritoneal fluid sample for Culture and Sensitivity (C&S) testing, and blood for outside analysis may be collected at any time after informed consent (unless indicated per standard of care) but before the End of Study Day 2 for Control subjects or before start of treatment for subjects randomized to ELAD.

7 A MELD Score and Maddrey Score will be autocalculated using these data (see Section 5.2.4). The MELD Score must be calculated using values obtained at the same time point, and from blood draws taken within 6 hours of each other.

8 Subject eligibility will continue to be monitored up until the time of initiation of Randomization. Randomization must occur within 24 hours of Screening, otherwise Screening evaluations must be repeated. (See Section 5.2.3).

9 Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented.

10 Steroid use is to be collected for the 6 weeks prior to hospital admission, if possible.

11 Ultrasound must be performed no more than six weeks prior to the current hospitalization and be indicative of and have a temporal relationship to the subject's current clinical condition (See Section 5.2.3.2).

12 Ammonia, lactate, albumin, α fetoprotein, lactic dehydrogenase (LDH).

Table 2. Schedule of Evaluations and Procedures for CONTROL Subjects

Control Subject Procedures	End of Study Day 2 Matched safety eligibility check (±6 hrs)	Daily on End of Study Day 1[1] to End of Study Day 7	End of Study Day 14, 21, 28 (+3 days)	End of Study Day 63 and 91 (+ 5 days)
Physical Exam[3]		X	X	X
Vital Signs[4]	X	X	X	X
Exclusion Criteria check	X[2]			
Weight		X (SD 7 outpatient only)	X[14]	X[14]
Encephalopathy Stage (West Haven Criteria) [3]		X	X	X
Laboratory Evaluations[5]	Cr, T bili	X	X	X
CBC	Platelets	X	X	X
PT/PTT/INR	INR	X	X	X
Pulse Oximetry, FiO_2, delivery method (inpatients only)		X		
Blood culture samples (×2)[6]	X[6]	X[6]		
MELD scores to be calculated[7]	X	X	X	X
Blood for outside assessment[8]		X	X	X
TLFB and mTSR Questionnaires[9]		X	X	X
Test for alcohol use (PEth)[10]		At hospital discharge and at all home health visits		
EQ-5D-5L QOL Questionnaire		At hospital discharge and Day 91 only		X[15]
Continuous monitoring:				
Adverse Events[11]		X	X	X
Concomitant Medications[12]		X	X	X
Concomitant Therapies[12]		X	X	X
Subject Location[13]		X	X	X
Dietary consultation[14]		X	X	X

1 First morning Standard of Care Labs after Randomization. Study Day 1 may be less than 24 hours in duration; Daily assessments must be performed on each Study Day a minimum of 12h apart, if patient discharged prior to end of SD 7 they must return for SD 7 evaluations (+2d).

2 Exclusion Criteria 2, 3, 4, 7, 9, 10, 15, and 16.

3 Performed by a study physician, physician assistant or nurse practitioner if within their scope of practice for institution.

4 Blood pressure, heart rate, respiration rate, and temperature.

5 Blood Urea Nitrogen (BUN), creatinine (Cr), glucose, sodium, potassium, chloride, carbon dioxide (CO_2), calcium, magnesium, phosphate, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (total/direct), albumin, total protein, alkaline phosphatase, lactic dehydrogenase (LDH), ammonia (NH_3), lactate and α fetoprotein. Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin.

6 Drawn only at End of SD2 (matched safety eligibility check) and End of SD 4.

7 A MELD Score will be calculated using these data (see Section 5.2.4). The MELD Score must be calculated using values obtained at the same time point, and from blood draws taken within 6 hours of each other.

8 Only participating sites and consented subjects; draw at Screening, Days 3, 5, 7 (and/or 24 hrs post ELAD), 14, 28, and 91. If blood draw is missed at Screening, it must be drawn before End of Study Day 2.

9 TLFB and mTSR obtained weekly by home visit practitioners for subjects that are outpatients only. Study staff not responsible for obtaining TLFB and mTSR.

10 Blood alcohol testing will be done by home visit personnel if subjects are outpatients. Study site does one Blood alcohol testing (PEth) at hospital discharge only.

11 AEs will be monitored continuously from after Randomization throughout Study Day 91 and recorded in the eCRFs.

12 Concomitant medications including concomitant blood product administration (e.g., whole blood, packed red cells, platelets, fresh frozen plasma), and concomitant therapies (e.g., intubation, tracheostomy, CVVH/CVVHD, arterial blood gas) will be monitored continuously from Randomization through Study Day 91.

13 Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented.

14 Dietary consultation within 72 hrs of Randomization, at discharge, and all outpatient visits (including weight).

15 EQ-5D-5L Quality of Life Questionnaire (Appendix C) at Day 91 and hospital discharge only.

Table 3. Schedule of Evaluations and Procedures for ELAD Subjects

Treated Subject Procedures	End of SD1 (Hr 24)[1]	ELAD Treatment					Daily on End SD2 to End SD7[1, 20]	End of SD 14, 21, 28 (+ 3 days)	End of SD 63 and 91 (+ 5 days)
		Safety Eligibility (-6 Hrs to ELAD Start)[21]	Hr 1-3[21]	Hr 6-10 and 14-18[21]	Q 24 Hr on ELAD	24 Hr Post ELAD Stop[1] (±6 hours)			
Physical Exam[3]	X					X	X	X	X
Vital Signs[4]	X	X	X	X		X	X	X	X
Exclusion Criteria check		X[2]							
Weight								X[17]	X[17]
Encephalopathy Stage (West Haven Criteria) [3]	X					X	X	X	X
Laboratory Evaluations[6]	X	T Bili[7], Cr[7]				X	X	X	X
CBC	X	Platelets[7]		Platelets		X	X	X	X
PT, PTT[18], INR	X	INR[7], PTT		X		X	X	X	X
Pulse Oximetry, FiO_2,delivery method	X					X	X[10]		
Blood culture samples (×2)		X			X[8]				
Ultrafiltrate Cultures (×2)		X[5]			X[8]				
Ultrafiltrate Sample to assess cartridge performance		X[5]			X				
Blood for outside assessment[9]						X	X	X	X
MELD scores to be calculated[11]	X	X				X	X	X	X
TLFB and mTSR Questionnaires[12]							X	X	X
Test for alcohol use (PEth)[13]	At hospital discharge and all home health visitis								
EQ-5D-5L Quality of Life Questionnaire	At hospital discharge and Day 91 only								X[19]
Adverse Events[14]	X						X	X	X
Concomitant Medications[15]	X						X	X	X
Concomitant Therapies[15]	X						X	X	X
Subject Location[16]	X					X	X	X	X
Dietary Consultation[17]							X	X	X

1 First morning Standard of Care Labs after Randomization. Study Day 1 may be less than 24 hours in duration. Daily assessments (including 24-hour post-ELAD stop) must be performed on each Study Day, a minimum of 12h apart If patient discharged prior to end of SD7 they must return for SD 7 evaluations (+2d).

2 Exclusion Criteria 2, 3, 4, 7, 9, 10, 15, and 16.

3 Performed by a study physician, physician assistant or nurse practitioner if within their scope of practice for institution.

4 Blood pressure, heart rate, respiration rate, and temperature.

5 Just before ELAD pump start (hour 0).

6 Blood Urea Nitrogen (BUN), creatinine (Cr), glucose, sodium, potassium, chloride, carbon dioxide (CO_2), calcium, magnesium, phosphate, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (total/direct), albumin, total protein, alkaline phosphatase, lactic dehydrogenase (LDH), ammonia (NH_3), lactate and α fetoprotein . Bilirubin measurements must be taken at least 12 hours after any procedure known to artificially alter serum bilirubin.

7 Results of total bilirubin, creatinine, INR and platelets must be available and reviewed by study physician prior to initiating ELAD treatment.

8 Blood culture and Ultrafiltrate culture samples drawn at the sample time at Hour 48 and 96 of ELAD Treatment Period only.

9 Only participating sites and consented subjects; Screening (or before ELAD treatment start), Days 3, 5, 7 (and/or 24 hrs post ELAD), 14, 21, 28, 63 and 91.

10 FiO2 and pulse oximetry on inpatients only.

11 MELD Score calculated using these data (see Section 5.2.4). The MELD Score must be calculated using values obtained at the same time point, and from blood draws taken within 6 hours of each other..

12 TLFB and mTSR obtained weekly by home visit practitioners for subjects that are outpatients only. Study staff not responsible for obtaining TLFB and mTSR.

13 Blood alcohol testing will be done by home visit personnel if subjects are outpatients. Study site does one Blood alcohol testing (PEth) at hospital discharge only.

14 AEs will be monitored continuously from Randomization throughout Study Day 91 and recorded in the eCRFs.

15 Concomitant medications including concomitant blood products and concomitant therapies will be monitored from Randomization through Study Day 91.

16 Documentation of all transfers, discharges, readmissions and admissions within or outside institution. All emergency room visits must also be documented.

17 Dietary consultation within 72 hrs of Randomization, at discharge, and all outpatient visits (including weight).

18 If heparin is administered, additional PTT's will be assayed in accord with the standard anticoagulation therapy protocol at the site.

19 EQ-5D-5L Quality of Life Qestionnaire (Appendix C) at Day 91 and hospital discharge only.

20 Additional End of Study Day Visits, beyond End of Study Day 7, will need to be added as necessary if the start of ELAD Treatment is delayed beyond the End of Study Day 3.

21 To be repeated if ELAD cartridges are replaced.

Table 4. Schedule of Evaluations and Procedures for Standards of Care (SOC)

Important Note: This summary table does not take the place of reading the protocol. It is essential that these SOC guidelines be read and followed for study consistency across investigative sites. All study-related procedures done prior to Randomization, if done to follow the SOC described here, must have prior subject informed consent.

Standards of Care (SOC) for Treated and Control Subjects	Screening	Randomization (Study Start) through Hospitalization	Post Hospital Discharge	Comments
Steroids	Document current and prior steroid use, and pertinent clinical indications and rationale.	Administer steroids in accord with AASLD guidelines (see Section 5.4.1.2) Full documentation of steroid administration and tapering schema must be obtained in accord with Section 5.4.1.2)	Steroid use post discharge must be monitored and recorded through End of Study Day 91	
Ascites (tense and refractory) For specific type, see below and see protocol	If ascites is present, do abd paracentesis to rule out SBP. If fluid removed exceeds 5 L, administer colloid infusion (8 g of serum albumin/liter of ascitic fluid removed). See protocol for testing to be done on ascitic fluid.	Salt-restricted diet/diuretic regimen. If history of SBP or GI bleed, use antibiotic therapy per protocol.		It is essential that the guidelines in Section 5.4.1.3 be reviewed when treating ascites. See list of drugs contraindicated with ascites
Tense Ascites		Salt-restricted diet/diuretic regimen. A dual diuretic regimen of spironolactone and furosemide is recommended. If tense ascites recurs, paracenteses can be repeated at any time.		
Refractory Ascites Defined as fluid overload that 1) Is unresponsive to sodium-restricted diet and high-dose diuretic treatment or 2) Recurs rapidly after therapeutic paracentesis.		Serial therapeutic paracenteses are effective in controlling ascites even in subjects with no urinary sodium excretion if they are performed approx every two weeks.		

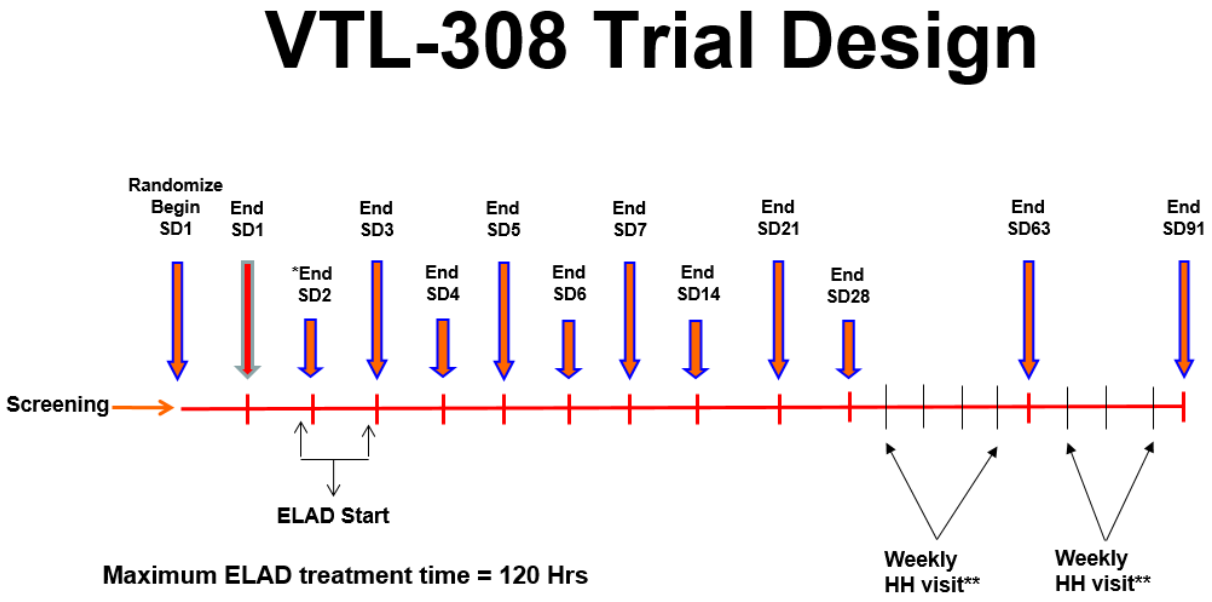
Standards of Care (SOC) for Treated and Control Subjects	**Screening**	**Randomization (Study Start) through Hospitalization**	**Post Hospital Discharge**	**Comments**
Gastroesophageal Varices See categories below				
Medium/Large Varices that have not bled		Place on non-selective β-blockers at the maximum tolerated dose. May treat with endoscopic variceal ligation, but this procedure and the follow-up endoscopies should be conducted after subject completes Study Day 7 (Control) or ELAD treatment (Treated) whenever possible.		
Actively Bleeding Varices	Must be stable with no bleeding and no further need for blood products for 2 days prior to Randomization.	Treat according to the AASLD guidelines: blood transfusion; short course antibiotics (e.g. PO norfloxacin 400 mg bid × 7 days; if the antibiotics cannot be admin PO, IV ceftriaxone 1 g/day for 7 days - or equivalents); drug therapy (e.g., vasopressin, terlipressin, etc); endoscopy using EVL/sclerotherapy, balloon tamponade, or TIPS, if needed. Subjects treated for recurrent variceal bleed while in study to be treated with non-selective β-blockers and EVL to prevent recurrence. TIPS may be considered for appropriate subjects.		
Active bleeding within two weeks prior to Randomization	See above	Oral norfloxacin 400 mg bid for 7 days (or equivalent); if the antibiotics cannot be administered PO, IV ceftriaxone 1 g/day for 7 days (or equivalent) is to be administered.		
Hepatic Encephalopathy		Nutritious diet (protein intake between 1.0 to 1.5 g/kg/day), and will be maintained on an adequate daily dose of lactulose to maintain two to three soft bowel movements per day. Selective digestive decontamination, for example, rifaximin, may also be given.		

Standards of Care (SOC) for Treated and Control Subjects	Screening	Randomization (Study Start) through Hospitalization	Post Hospital Discharge	Comments
Hepatorenal Syndrome (HRS)		Type I HRS will be considered for SOC in accord with local practice, e.g., albumin/octreotide/midodrine or terlipressin/albumin regimen. If treatment is initiated during Screening, eligible subjects can be randomized when stable and the regimen is continued during study. Treatment of Type II HRS is driven by the clinical needs of the subject. Renal replacement therapy may be useful in subjects who do not respond to vasoconstrictor therapy, and who fulfill criteria for renal support. VTL requires that subjects who develop Type I HRS after randomization but prior to the start of ELAD treatment as well as while on ELAD treatment must be discontinued from ELAD treatment and are to be continued on SOC. VTL also requires that subjects on ELAD treatment who require renal replacement therapy/hemodialysis are to be discontinued from ELAD treatment before renal replacement therapy/hemodialysis is started.		
Hyponatremia (sodium level lower than 130 mmol/L)		Hypovolemic hyponatremia is treated by stopping diuretics, and fluid administration as needed per clinical needs. Hypervolemic hyponatremia is treated with fluid restriction if practical.		
Spontaneous Bacterial Peritonitis	**All** ascitic subjects will get paracentesis prior to Randomization (if not previously obtained within 7 days of Randomization)	If SBP is suspected, paracentesis is to be performed. Follow the algorithm: • IV ceftriaxone × 7 days or norfloxacin BID for 7 days (or equivalent) in subjects who have GI bleeding. • Subjects surviving an episode prior to the study or had SBP was while in the study will start long-term prophylaxis with daily norfloxacin (or trimethoprim-sulfamethoxazole or equivalent); if indicated, long-term antibiotic prophylaxis can be started at **Randomization** or following an episode of SBP occurring bet. Study Day 1- 91. • Subjects with cirrhosis and ascites but no GI bleed are to start on long-term prophylaxis with		

Standards of Care (SOC) for Treated and Control Subjects	Screening	Randomization (Study Start) through Hospitalization	Post Hospital Discharge	Comments
		daily norfloxacin (or trimethoprim-sulfamethoxazole or equivalent) if ascitic fluid protein is < 1.5g/dL and at least 1 of the following is present: serum creatinine ≥ 1.2 mg/dL, blood urea nitrogen ≥ 25 mg/dL, serum sodium ≤ 130 mEq/L, and Child-Pugh ≥ 9 points with bilirubin ≥ 3 mg/dL.		
Thrombocytopenia		For a platelet count < 20,000/mm^3 without clinical manifestations of bleeding during study treatment (ELAD and Control) will receive platelet transfusions based on the judgment of the clinical team. If the platelet count is < 20,000/mm^3 and clinical manifestations of only localized contained bleeding, (ELAD and Control) subjects are to receive platelets. Subjects with platelet count of < 20,000/mm^3 and generalized bleeding are to be promptly assessed, and if on ELAD, considered for treatment discontinuation.		
Nutritional Support		Dietary consultations within 72 hours of Randomization. If not available, the Investigator must provide dietary/nutritional supervision. Continuous Dietary follow-up throughout and at discharge.	Dietary to see at each follow-up visit	
ICU Discharge		When physiologic status has stabilized or when physiological status has deteriorated and further interventions are not warranted. Subjects with alcohol withdrawal should receive ICU care until their detoxification stage has stabilized (i.e. absence of seizures and delirium requiring intense monitoring). They should be hemodynamically stable prior to discharge (i.e. not requiring intense monitoring for vasopressor admin or mechanical respiratory support). For discharge from the ICU, a general guideline is that the subject is no longer in need of vasopressors, mechanical ventilation, or continuous extracorporeal renal treatment.		

Standards of Care (SOC) for Treated and Control Subjects	Screening	Randomization (Study Start) through Hospitalization	Post Hospital Discharge	Comments
Hepatic Hydrothorax		First-line therapy of hepatic hydrothorax consists of dietary sodium restriction and diuretics while TIPS is considered to be second-line treatment when it becomes refractory. Chest tube insertion is contraindicated in a subject with hepatic hydrothorax.		
Post-Hospital Care		Notify Home Visit Agency to arrange post-discharge visits	Weekly home visits by outside agency	

Figure 2. Main Study Timeline



* Pre-Treatment Safety Eligibility assessments (Controls at ESD2; ELAD subjects within 6 hours of ELAD start.

** Home Health (HH) visits will start weekly after hospital discharge.
- Subject will need to attend scheduled site SD visit AND complete weekly HH visits after hospital discharge.

Appendix A. West Haven Criteria

The evaluation of severity of persistent hepatic encephalopathy is based on the West Haven Criteria for semi-quantitative grading of mental status, referring to the level of impairment of autonomy, changes in consciousness, intellectual function, behavior, and the dependence on therapy.

Grade 0: (Sub-clinical hepatic encephalopathy) – Lack of detectable changes in personality or behavior. Minimal changes in memory, concentration, intellectual function, and coordination. Asterixis is absent.

Grade 1: Trivial lack of awareness; Euphoria or anxiety; Shortened attention span; Impaired performance of addition. 67% of cirrhotic patients may have 'minimal hepatic encephalopathy'.

Grade 2: Lethargy or apathy; Minimal disorientation for time or place; Subtle personality change; Inappropriate behavior; Impaired performance of subtraction.

Grade 3: Somnolence to semi-stupor, but responsive to verbal stimuli; Confusion; Gross disorientation.

Grade 4: Coma (unresponsive to verbal or noxious stimuli).

Appendix B. Tool for Determining Capacity to Consent

Evaluation of Capacity to Consent to Participate in Research

Subject Number: _____ **Date:** _____

Study: Study: VTL-308 "A Randomized, Open-label, Multicenter, Controlled Pivotal Study to Assess Safety and Efficacy of ELAD® in Subjects with Alcohol-induced Liver Decompensation (AILD)"

Is Subject able to understand the nature and purpose of the study?	☐ Yes ☐ No
Is Subject able to understand the risks and benefits of the study?	☐ Yes ☐ No
Subject understands all that is required of him/her in this study?	☐ Yes ☐ No
Subject is able to make an informed and rational decision regarding Participation?	☐ Yes ☐ No

Comments:

_____

_____

_____

_____

_____

Based on the evaluation performed by: _____ _____

Clinician's Name Subject Number

Is deemed to **have / not have** *(circle one)* the capacity to provide consent for participation in this study.

_____ _____

Clinician's Signature Date

Appendix C. EQ-5D-5L Quality of Life Questionnaire

Health Questionnaire

English version for the US
(Other language versions are available from VTI as needed)

SCRIPT FOR TELEPHONE INTERVIEW

GENERAL INTRODUCTION

It is suggested that the telephone interviewer follows the script of the EQ-5D. Although allowance should be made for the interviewer's particular style of speaking, the wording of the questionnaire instructions should be followed as closely as possible. In the case of the EQ-5D descriptive system on page 2, the exact wording must be followed.

It is recommended that the interviewer and respondent have a copy of the EQ-5D in front of them as it is administered over the telephone. This enables the respondent's answers to be entered directly on the EQ-5D by the interviewer on behalf of the respondent (i.e. the appropriate boxes on page 2 are marked and the scale on page 3 is marked at the point indicating the respondent's 'health today'). If the respondent asks for clarification, the interviewer can help by re-reading the question verbatim. The interviewer should not try to offer his or her own explanation but suggest that the respondent uses his or her own interpretation.

If the respondent has difficulty regarding which box to mark, the interviewer should repeat the question verbatim and ask the respondent to answer in a way that most closely resembles his or her thoughts about his or her health today.

INTRODUCTION TO EQ-5D

(Note to interviewer: please read the following)

We are trying to find out what you think about your health. I will first ask you some simple questions about your health TODAY. I will then ask you to rate your health on a measuring scale. I will explain what to do as I go along but please interrupt me if you do not understand something or if things are not clear to you. Please also remember that there are no right or wrong answers. We are interested here only in your personal view.

EQ-5D DESCRIPTIVE SYSTEM - PAGE 2: INTRODUCTION

First I am going to read out some questions. Each question has a choice of five answers. Please tell me which answer best describes your health TODAY. Do not choose more than one answer in each group of questions.

(Note to interviewer: it may be necessary to remind the respondent regularly that the timeframe is TODAY. It may also be necessary to repeat the questions verbatim.)

EQ-5D DESCRIPTIVE SYSTEM - PAGE 2: TASK

MOBILITY

First I'd like to ask you about mobility. Would you say that:

1. You have no problems walking?
2. You have slight problems walking?
3. You have moderate problems walking?
4. You have severe problems walking?
5. You are unable to walk?

(Note to interviewer: mark the appropriate box on the EQ-5D questionnaire)

SELF-CARE

Next I'd like to ask you about self-care. Would you say that:

1. You have no problems washing or dressing yourself?
2. You have slight problems washing or dressing yourself?
3. You have moderate problems washing or dressing yourself?
4. You have severe problems washing or dressing yourself?
5. You are unable to wash or dress yourself?

(Note to interviewer: mark the appropriate box on the EQ-5D questionnaire)

USUAL ACTIVITIES

Next I'd like to ask you about your usual activities, for example work, study, housework, family or leisure activities. Would you say that:

1. You have no problems doing your usual activities?
2. You have slight problems doing your usual activities?
3. You have moderate problems doing your usual activities?
4. You have severe problems doing your usual activities?
5. You are unable to do your usual activities?

(Note to interviewer: mark the appropriate box on the EQ-5D questionnaire)

PAIN/DISCOMFORT

Next I'd like to ask you about pain or discomfort.Would you say that:

1. You have no pain or discomfort?
2. You have slight pain or discomfort?
3. You have moderate pain or discomfort?
4. You have severe pain or discomfort?
5. You have extreme pain or discomfort?

(Note to interviewer: mark the appropriate box on the EQ-5D questionnaire)

ANXIETY/DEPRESSION

Finally I'd like to ask you about anxiety or depression. Would you say that:

1. You are not anxious or depressed?
2. You are slightly anxious or depressed?
3. You are moderately anxious or depressed?
4. You are severely anxious or depressed?
5. You are extremely anxious or depressed?

(Note to interviewer: mark the appropriate box on the EQ-5D questionnaire)

ELAD®

EQ VAS - PAGE 3: INTRODUCTION

(Note for administrator: If possible, it might be useful to send a visual aid (i.e. the EQ VAS) before the telephone call so that they can have this in front of them when completing the task).

Now, I would like to ask yu to say how good or bad your health is TODAY.

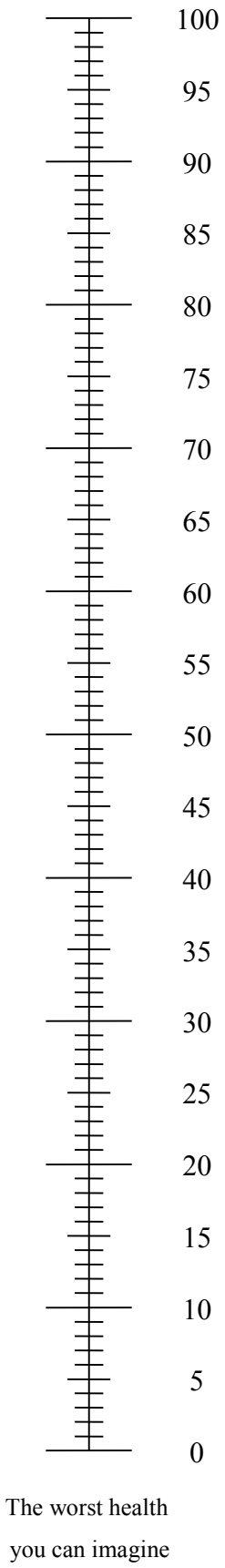
I'd like you to try to picture in your mind a scale that looks a bit like a thermometer. Can you do that? The best health you can imagine is marked 100 (one hundred) at the top of the scale and the worst health you can imagine is marked 0 (zero) at the bottom.

EQ VAS - PAGE 3: TASK

I would now like you to tell me the point on this scale where you would put your health today.

(Note to interviewer: mark the point on the scale at the point indicating the respondent's 'health today')

Thank you for taking the time to answer these questions.



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

MOBILITY

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

SELF-CARE

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

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

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

PAIN / DISCOMFORT

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

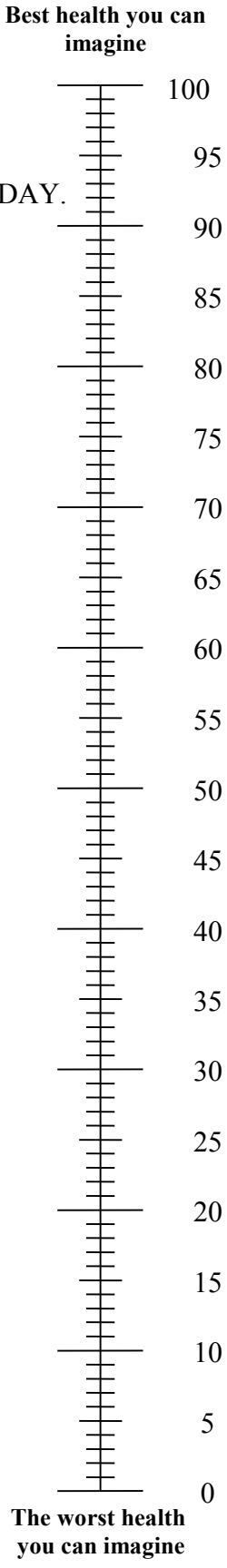
ANXIETY / DEPRESSION

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

We would like to know how good or bad your health is TODAY.

- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Appendix D. Clinical Trial Pharmacovigilance Investigator Guide

Introduction

The purpose of this document is to provide relevant background information and proposed criteria to determine whether an adverse event (AE) or serious adverse event (SAE) should be attributed to the VTL C3A cells (biologic), the ELAD System (device), or to the ELAD Procedure.

ELAD-Related Adverse Events

VTL C3A Cells in ELAD Cartridges: Overview

The active drug product within the ELAD C3A cell cartridge are the VTL C3A cells, which are derived from a subclone of the human hepatoblastoma cell line HepG2. VTL C3A cells are monitored during the manufacturing process for the production of liver-specific proteins (e.g., albumin and transferrin) and for cell viability (oxygen utilization and glucose consumption). Oxygen utilization and glucose consumption by the VTL C3A cells are monitored closely during the course of patient treatment.

The 0.2-µm hollow-fiber membrane that physically separates the VTL C3A cells from the patient ultrafiltrate allows diffusion of inflammatory cytokines, toxins, system source oxygen and system source glucose towards the VTL C3A cells and the simultaneous delivery of secreted macromolecules and other cellular products into the patient's ultrafiltrate.

Hepatocyte functions used to assess VTL C3A cell function include: α-fetoprotein, albumin, factor V, monoethylglycine-xyliide (surrogate marker of cytochrome P450 enzyme activity), transferrin, transforming growth factor-α, and urea production; galactose and glucose consumption; oxygen utilization rate; and lactate production to glucose consumption ratio are displayed in the Investigator's Brochure. Post-treatment ELAD cartridges maintained viability, made liver specific proteins, and metabolized galactose and lidocaine at levels similar to pre-treatment ELAD cartridges containing VTL C3A cells released from manufacturing (see Investigator's Brochure).

The in-line cell filter (0.2-µm hollow fiber filter) prevents any VTL C3A cells that may have escaped from the ELAD cartridges from passing into subjects. Histological examination of a sample of ELAD cartridges revealed no broken fibers (>50,000 observations).

Adverse Events: VTL C3A Cells (Biologic)

Adverse events (AEs) or SAEs that may be attributed to VTL C3A cells should be considered when there appears to be clinically-significant changes in hepatic function (synthesis, conjugation, and secretion) or allergic reactions while a subject is undergoing ELAD treatment.

The following point should be noted when assessing changes in hepatic function while subjects are on ELAD treatment:

- Increased levels of α-fetoprotein have been observed in ELAD-treated subjects in prior studies and are considered to be a surrogate marker of exposure of the plasma ultrafiltrate to VTL C3A cells. Levels of up to approximately 10×10^7 ng/dL have been reported while subjects are on ELAD.

Expected Adverse Events: VTL C3A Cells[1] (Biologic)

Based on a review of safety data from past clinical studies and investigator assessments of AEs in the VTI-208 study, the following AEs could potentially be related to VTL C3A cell function:

Acidosis	Coagulopathy	Hypersensitivity	Lactic acidosis
Alkalosis	Device occlusion	Hypoglycemia	Metabolic acidosis
Allergic reaction	Fungal infection	Hypotension	Pyrexia
Anaphylactic reaction	Hemolysis	Increased ammonia levels	Rash maculopapular
Cartridge malfunction	Hepatic enzyme increased	Increased AST, ALT, and LDH levels	Thrombocytopenia

[1] Listed alphabetically

ELAD System (Device)

ELAD System: Overview

The current ELAD System, depicted in the Investigator's Brochure, first draws blood from the subject via a dual-lumen catheter placed in a large vein using an extracorporeal pumping unit, and then separates the plasma fluid (ultrafiltrate) from cellular components by using a specifically-designed ultrafiltrate generator cartridge. While the cellular components are returned to the subject via the venous access, the ultrafiltrate is circulated at a high-flow rate through four metabolically-active ELAD cartridges.

The cartridge that houses the VTL C3A cells contains approximately eight thousand hollow fibers made up of a semi-permeable polysulfone material that has a nominal pore size of 0.2-µm (Brotherton 2007). During ELAD treatment, an extracorporeal pumping system pumps the subject's ultrafiltrate through the hollow fibers of the cartridge wherein the semipermeable membrane permits a bidirectional flow between the cells (grown between the hollow fibers) and the ultrafiltrate (contained in the lumen of the hollow fibers). Toxins, nutrients and dissolved oxygen pass from the ultrafiltrate to the cells, while the potentially-beneficial macromolecules and other substances synthesized by the cells simultaneously pass into the patient's ultrafiltrate.

After circulation through the cartridges, the ultrafiltrate passes through a 0.2-µm pore size filter, is recombined with the cellular components of the subject's blood, and is returned to the patient through the dual-lumen catheter. The toxins metabolized by the VTL C3A cells are thereby returned to the subject to be excreted by the renal or gastrointestinal system. This circulation is maintained continuously for the duration of ELAD treatment, typically between three and five days.

Adverse Events: ELAD System (Device)

Adverse events should be attributed to the ELAD System when malfunctions of the bedside unit or contamination of catheters, tubing sets or filter components arise while the subject is undergoing ELAD treatment.

Expected Adverse Events: ELAD System[1] (Device)

Based on a review of safety data from past clinical studies and investigator assessments of AEs in the VTI-208 study, the following AEs could potentially be related to the ELAD System:

Abdominal infection	Coagulopathy	Hypocalcaemia	Pyrexia
Activated partial thromboplastin time prolonged	Catheter site haemorrhage	Hypofibrinogenaemia	Rectal haemorrhage
Air embolism	Disseminated intravascular coagulation	Hypotension	Thrombocytopenia
Allergic reaction	Haemorrhage	Hypothermia	
Anemia	Hemolytic anaemia	Infection	
Anaphylactic reaction	Hypersensitivity	Lung Infection	

1 Listed alphabetically

Note: Infection (contaminated ultrafiltrate cultures): In one subject in study VTI-206, ultrafiltrate cultures taken from the ultrafiltrate lines before and after passage through the ELAD cartridge (but prior to the cell filter) grew out Streptococcus viridans and indicated bacterial contamination introduced during ELAD cartridge or other component replacement. An SAE of sepsis occurred three days after stopping ELAD treatment and was considered to be possibly related to ELAD treatment (positive ultrafiltrate cultures); however, the causal role of the positive cultures in the sepsis was confounded by an episode of gastrointestinal bleeding (frequently associated with bacteremia) just before the onset of sepsis, concurrent clinically significant ascites associated with spontaneous bacterial peritonitis, and a urinary tract infection. While it is possible that an infection in the ultrafiltrate loop might lead to a septic response in the subject due to transmission of exotoxin/endotoxin through the cell filter, it is highly unlikely that bacteria in the ultrafiltrate loop would lead directly to a positive blood culture due to the nominal pore sizes of the cell filter. Blood cultures taken on the third day of ELAD treatment were negative.

ELAD Procedure

ELAD Procedure: Overview

The ELAD Procedure is considered to occur while the subject's blood is circulating through the ELAD System and also includes the time the subject is on the System when interruptions during treatment take place (due to switch out of the ELAD cartridges, clotting of catheters, replacement of catheters, etc.).

Adverse Events: ELAD Procedure

Adverse events should be attributed to the ELAD procedure when systemic events occur in subjects while their blood is being circulated through the ELAD System. Since usage of heparin during the ELAD procedure is based on the investigators' clinical judgment and varied across prior studies, there is no distinction between the expected adverse events related to the ELAD procedure for those subjects who do or do not receive heparin.

Expected Adverse Events: ELAD Procedure[1]

Based on a review of safety data from past clinical studies and investigator assessments of AEs in the VTI-208 study, the following AEs could potentially be related to the ELAD procedure:

Abdominal pain	Coagulopathy	Hyperglycaemia	Metabolic acidosis
Abdominal discomfort	Device related infection	Hyperkalaemia	Mouth haemorrhage
Abdominal distension	Device related sepsis	Hypernatraemia	Multi-organ failure
Acute respiratory distress syndrome	Disseminated intravascular coagulation	Hypersensitivity	Oedema peripheral
Agitation	Ecchymosis	Hypertension	Oliguria
Allergic reacton	Edema	Hypocalcemia	Pain
Anaemia	Edema peripheral	Hypocoagulable state	Pneumonia
Anuria	Electrolyte imbalance	Hypofibrinogemaemia	Pulmonary oedema
Application site bleeding	Enecphalopathy	Hypokalaemia	Renal failure acute
Ascites	Epistaxis	Hypomagnesaemia	Respiratory arrest
Asthma	Fever	Hypophospataemia	Tachycardia
Atrial fibrillation	Fluid overload	Hypotension	Thromboctyopenia
Bacteremia	Gastrointestinal haemorrhage	Hyopthermia	Vessel puncture site haematoma
Blood lactate dehydrogenase (LDH) increased	Gingival bleeding	Hypovolemia	Wound secretion
Blood pressure decreased	Haematemesis	Ileus	
Catheter site hematoma	Haematuria	Infection	
Catheter site hemorrhage	Hemoglobin decreased	Injection site haemorrhage	
Catheter site pain	Hemolysis	Ischemic hepatitis	
Catheter site reaction	Haemorrhage	Leukocytosis	
Coagulation disorder	Hepatic encephalopathy	Melaena	
Coagulation prolonged	Hepatic failure	Mental status changes	

1 Listed alphabetically

Expected Adverse Events: Extracorporeal Procedures

Certain adverse events are typically observed in patients undergoing treatment with extracorporeal procedures. Anemia, coagulopathy, and thrombocytopenia tend to be the most common adverse events observed during treatment with extracorporeal procedures. Specific details about each of these adverse events are presented in the following sections.

Anemia

Data from prior ELAD clinical studies (PS-0698; CR-202, VTIC-301, VTI-201, VTI-206 and VTI-208) indicate that hemoglobin levels tend to decrease after ELAD treatment is started. The reduced hemoglobin levels may be related to mechanical hemolysis, which is occasionally observed in patients undergoing other extracorporeal procedures (Boehning 2014). Patients with chronic liver disease may be predisposed to mechanical hemolysis during ELAD treatment due to abnormal RBC membranes, RBC membrane strength and durability, maintenance of RBC cell volume, and intermediary metabolism affecting RBC homeostasis. Anemia in the prior studies may also have been related to dilution, clotting in the catheters or dual-lumen, and stress during generation of the plasma ultrafiltrate. Additional information regarding anemia with extracorporeal procedures is provided in the Investigator's Brochure.

Coagulopathy

Extracorporeal circulation increases the risk of hemorrhagic and thrombotic complications because blood is exposed to foreign non-endothelialized surfaces; cellular and acellular systems are activated/induced by exposure to foreign non-endothelialized surfaces and cause release of various intermediate products into the systemic circulation that induce hemorrhagic or thrombotic complications (Shann 2008). Patients with chronic liver disease have defective synthesis of coagulation factors (pro- and anti-coagulation factors) that can confound assessments of hemorrhagic and thrombotic complications observed in subjects undergoing ELAD treatment with or without concurrent anticoagulation. Additional information regarding coagulopathy with extracorporeal procedures is provided in the Investigator's Brochure.

Thrombocytopenia

Thrombocytopenia is a potential side effect with extracorporeal procedures; the blood and dialyzer membrane interaction can cause significant thrombocytopenia through the activation of the complement (Olafiranye 2011). Thrombocytopenia in chronic liver disease patients undergoing ELAD treatment is confounded by concurrent coagulopathy, concomitant heparin use, and ongoing hypersplenism. Additional information regarding thrombocytopenia with extracorporeal procedures is provided in the Investigator's Brochure.

Clinically-Significant Laboratory Changes While on ELAD Treatment

In general, laboratory changes in these critically-ill subjects should not be recorded as AEs. However in certain cases abnormal laboratory changes should be reported as AEs. The suggested laboratory values for hematology, serum chemistry, and urinalysis parameters that occur while the subject is on ELAD treatment that should be reported as AEs are listed in the Investigator's Brochure.

If the subject develops a laboratory abnormality meeting these criteria, a clinical diagnosis should be provided with the reported laboratory abnormality, if applicable. For example, if the subject developed hematuria in the presence of >1.0 g/dL of hemoglobin, hematuria should be listed as the clinical event and include in the details of the AE that a decrease in hemoglobin was associated with the hematuria.

If the laboratory abnormality was not associated with a clinical event, the laboratory abnormality should be listed as an AE and indicate that no clinical findings were present. For example, hyponatremia is not usually associated with any specific clinical diagnosis but is found in

subjects with clinically significant decompensated liver disease, so the specific diagnosis would not be included.

The specific notable laboratory changes for hematology, serum chemistry, and the urinalysis while on ELAD to be reported as AEs are as follows:

Hematology

- Hemoglobin >1.0 g/dL decrease from Baseline in the absence of gastrointestinal bleeding or other clinically overt sources of bleeding (e.g., hematuria, central line bleeding) or hemoglobin levels <7.0 g/dL
- WBC counts $<1.5 \times 10^3$/mL in the absence of sepsis or an uncontrolled infection
- Platelet counts <20,000 x 10^3/mL and note if there is evidence of impaired clotting or clinical bleeding or oozing

Serum Chemistry

- Abrupt (within 48 hours) reduction in kidney function defined as an absolute increase in serum creatinine of 0.3 mg/dL [≥26.4 μmol/L or increase of serum creatinine of 50% or more (1.5-fold) from baseline]
- Hyperglycemia (serum glucose >350 mg/dL)
- Hyperkalemia (serum potassium >7.0 mEq/L)
- Hypocalcemia (serum calcium <3.5 mmol/L)
- Hypoglycemia (serum glucose <50 mg/dL)
- Hypokalemia (serum potassium <2.0 mEq/L)
- Hypomagnesemia (serum magnesium <1.0 mEq/L)
- Hyponatremia (serum sodium <120 mEq/L)
- Hypophosphatemia (serum phosphate <0.3 mmol/L)
- Lactate >4 mmol/L

Urinalysis

- Presence of significant increase in RBCs in the urine not related to the subject's renal disease, Foley catheters, etc.
- Urine hemoglobin levels of 3+ or 4+, if not present at these levels at Baseline

Expected Adverse Reactions for ELAD Treatment

According to the literature and previous protocols using either artificial or bio-artificial liver support systems, AEs in the following five categories might be expected to be encountered during treatment.

General Risks of Fractionated Plasma Filtration

- Higher risk of bleeding due to anticoagulation and/or unfavorable effects on the coagulation system. Specifically, in liver failure subjects, where hemostasis is disturbed, the use of plasma filtration or dialysis can precipitate bleeding.
- Hypotension
- Acute transient complement activation, leukopenia, thrombocytopenia, eosinophilia
- Hemolysis due to mechanical destruction of RBCs during whole blood perfusion through the ultrafiltrate generator
- Clotting within the extracorporeal blood circuit and in the ultrafiltrate generator
- Hypersensitivity: for example, an acute reaction may be observed similar to that known as first use syndrome reaction observed in patients undergoing dialysis
- Loss of valuable substances, including proteins, hormones, nutrients, electrolytes due to either dilution or binding to components of the tubing set
- Temperature disorders (e.g. hypothermia) (Biggers 1977; Chiu 2006; VitaGen, CR-202 CSR, 2004).

General Risks Associated With Using Heparin-Based Anticoagulation

- Heparin-induced thrombocytopenia
- Bleeding due to heparin (Hirsch 2001)

General Risks Associated With Using Central Venous Catheters

The general risks associated with central venous catheters may occur during insertion, while they are indwelling, and during extraction (Kusminsky 2008).

- Insertion complications (pneumothorax, malpositioning, vascular injuries, dysrhythmias, neurological complications, lymphatic complications, and guide wire loss)
- Indwelling complications (infection, thrombosis, occlusion, vascular erosion and perforation, as well as catheter fracture and embolization
- Extraction complications (air embolism, catheter breakage or separation from hub, and catheter knotting)

General Risks Associated With Administration of Albumin

- Rash/allergic reactions
- Hypotensive episodes/shock
- Fever
- Risk of transfusion-associated infections
- Disturbances of plasma protein pattern with associated consequences, such as hyper- or hypocoagulability, immunodysfunction, and renal tubular injury (Torchia 2000; Albumin (Human) 5%)

Specific Risks Associated With Cellular Therapy

In addition to the general risks of liver support systems described above, specific risks are theoretically possible in using biological systems containing cells (Tsiaoussis 2001):

- Hypoglycemia
- Changes in levels of substances either made by or metabolized by the cells
- Metabolic acidosis/alkalosis
- Theoretical risk of tumor formation

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Appendix E. Anticoagulation Guidelines for the VTL-308 Study

Anticoagulation Guidelines During ELAD Treatment

In general, standard anticoagulation protocols of the Investigational institution for the administration of extracorporeal procedures should be applied during the administration of ELAD treatment. However, there may be a requirement for more rigorous monitoring.

As a general guideline, subjects who are administered ELAD that have a baseline PTT of <50 seconds or an activated clotting time (ACT) of <150 seconds may require heparin in order to prevent rapid clotting in the ELAD extracorporeal blood circuit. In such cases, the initial dose applied may be as small as 200-400 Units of non-fractionated heparin per hour into the arterial line of the blood circuit, and a control sample should be taken within 1 hour of initiation of heparin treatment in order assess the impact of the heparin dosing on coagulation status. If the PTT is >80 seconds, or the ACT is >200 seconds, the dose should be reduced, e.g. by half and a control sample should be taken within 1 hour of the adjustment of the heparin dose. If the PTT is still <50 seconds and the ACT is still <150 seconds, the dose should be increased, e.g. doubled and a control sample should be taken within 1 hour of the adjustment of the heparin dose.

The goal is to maintain PTT between 50 and 80 seconds or the ACT between 150 and 200 seconds in the case of non-fractionated heparin use.

For alternative anticoagulants, such as hirudin, or fractionated heparin, institutional protocols for CVVH therapy may be applied, but must be monitored rigorously.

CITRATE MUST NOT BE USED as an anticoagulant during ELAD treatment.

A formal heparin-based protocol for extracorporeal therapy of liver patients can be found in an article by Hillebrand et al[1].

1. Hillebrand, et al. Formal heparin anticoagulation protocol improves safety of charcoal-based liver assist devices. ASAIO J. 2006 May-Jun;52(3):334-42.