UCSF DEPT. OF ANESTHESIA & PERIOPERATIVE CARE
Clinical Research Protocol
MILD HYPOTHERMIA TO PREVENT ACUTE KIDNEY INJURY IN LIVER TRANSPLANTATION (MHALT)

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April 29, 2020

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing University of California, San Francisco, Department of Anesthesia and Perioperative Care with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: UCSF IRB # 17-22384

Protocol Title:
Mild Hypothermia to Prevent Acute Kidney Injury in Liver Transplantation (MHALT)

Protocol Date: April 2020

Michael P Bokoch — April 29, 2020

Investigator Signature — *Date*

Michael P. Bokoch, MD, PhD – Assistant Professor of Clinical Anesthesia

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TABLE OF CONTENTS

1 BACKGROUND 12
1.1 Overview of Non-Clinical Studies 12
1.2 Overview of Clinical Studies 12
2 STUDY RATIONALE 12
2.1 Risk / Benefit Assessment 13
3 STUDY OBJECTIVES 14
3.1 Primary Objective 14
3.2 Secondary Objectives 14
4 STUDY DESIGN 15
4.1 Study Overview 15
5 Criteria for evaluation 16
5.1 Primary Efficacy Endpoint 16
5.2 Secondary Efficacy Endpoints 16
5.3 Safety Evaluations 17
5.4 Other Evaluations 19
6 SUBJECT SELECTION 19
6.1 Study Population 19
6.2 Inclusion Criteria 19
6.3 Exclusion Criteria 19
7 Concurrent Medications 20
8 STUDY TREATMENTS 20
8.1 Method of Assigning Subjects to Treatment Groups 20
8.2 Blinding 20
8.3 Formulation of Test and Control Treatments 20
9 STUDY PROCEDURES AND GUIDELINES 22
9.1 Clinical Assessments 22
9.2 Clinical Laboratory Measurements 23
9.3 Research Laboratory Measurements 23
10 EVALUATIONS BY VISIT 23
10.1 Visit 1 (Day 1, before and during LTx) 23
10.2 Visit 2 (Follow-up prior to hospital discharge, window of 2 ± 1 weeks after LTx) 24
11 ADVERSE Experience REPORTING AND DOCUMENTATION 24
11.1 Adverse Events 24
11.2 Serious Adverse Experiences (SAE) 25
12 DISCONTINUATION And Replacement of subjects 26
12.1 Early Discontinuation of Study Treatment 26
12.3 Withdrawal of Subjects from the Study 26
12.4 Replacement of Subjects 27
13 PRotocol Violations 27

14 DATA SAFETY MONITORING 27

15 STATISTICAL METHODS AND CONSIDERATIONS 27

15.1 Data Sets Analyzed 28
15.2 Demographic and Baseline Characteristics 28
15.3 Analysis of Primary Endpoint 28
15.4 Analysis of Secondary Endpoints 28
15.5 Interim Analysis 29
15.6 Sample Size and Randomization 30

16 DATA COLLECTION, RETENTION AND MONITORING 30

16.1 Data Collection Instruments 30
16.2 Data Management Procedures 30
16.3 Data Quality Control and Reporting 31
16.4 Archival of Data 31
16.5 Availability and Retention of Investigational Records 31
16.6 Monitoring 31
16.7 Subject Confidentiality 31

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS 31

17.1 Protocol Amendments 32
17.2 Institutional Review Boards and Independent Ethics Committees 32
17.3 Informed Consent Form 32
17.4 Publications 33
17.5 Investigator Responsibilities 33

LIST OF ABBREVIATIONS

ABG	arterial blood gas
AE	adverse event
AKI	acute kidney injury
AKIN	Acute Kidney Injury Network
ALF	acute liver failure
CFR	Code of Federal Regulations
CRF	case report form
CRRT	continuous renal replacement therapy
CTSI	Clinical & Translational Science Institute
DCD	donor after cardiac death
DMC	Data Monitoring Committee
DNDD	donor after neurologic determination of death
EBL	estimated blood loss
ECD	esophageal cooling device
ELISA	enzyme-linked immunosorbent assay
EMR	electronic medical record
FDA	Food and Drug Administration
FFP	fresh frozen plasma
GCP	Good Clinical Practice
GI	gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICA	International Club for Ascites
ICF	informed consent form
ICU	intensive care unit
IEC	Independent Ethics Committee
I-R	ischemia-reperfusion
IRB	Institutional Review Board
IVC	inferior vena cava
ITT	intention-to-treat
LTx	liver transplantation
NGAL	neutrophil gelatinase-associated lipocalin
OG	orogastric
PI	Principal Investigator
pRBC	packed red blood cells
RCT	randomized controlled trial
RRT	renal replacement therapy
SAE	serious adverse experience

sCR	serum creatinine
SSI	surgical site infection
TEE	transesophageal echocardiography

PROTOCOL SYNOPSIS

TITLE	Mild Hypothermia to Prevent Acute Kidney Injury in Liver Transplantation (MHALT)
SPONSOR	University of California, San Francisco
FUNDING ORGANIZATION	Department of Anesthesia and Perioperative Care
NUMBER OF SITES	3
RATIONALE	Patients undergoing liver transplantation (LTx) are at extremely high risk of perioperative acute kidney injury (AKI). AKI has enormous impact on outcomes and cost after LTx. 68% of patients at UCSF sustain some degree of AKI and 12% develop AKI Stage 3 (a three-fold increase in sCr from baseline or need for dialysis) after LTx. Thirty-day and 1-year mortality, graft survival, and progression to chronic kidney disease correlate with the presence and severity of AKI. Clearly, any intervention that reduces the incidence or severity of AKI in this population would have tremendous impact on short and long-term outcomes and cost. This trial will study mild hypothermia in LTx for prevention of AKI. The protocol is based on strong preliminary data from rodent studies showing that hypothermia protects renal function in ischemia-reperfusion models. Recent work from our department has shown that mild hypothermia in deceased organ donors improves graft function after kidney transplantation. In light of these preliminary data, we propose a trial of mild hypothermia to prevent AKI in LTx.
STUDY DESIGN	This is a single-blind, randomized controlled trial
PRIMARY OBJECTIVE	Assess whether or not mild hypothermia has a protective effect on early AKI after liver transplantation.
SECONDARY OBJECTIVES	1. Demonstrate the feasibility of mild hypothermia during liver transplantation. 2. Assess whether or not mild hypothermia reduces early biomarkers of AKI during liver transplantation such as neutrophil gelatinase-associated lipocalin (NGAL)
NUMBER OF SUBJECTS	230
SUBJECT SELECTION CRITERIA	Inclusion Criteria: 1. Age ≥ 18 years 2. LTx from a donor after neurologic determination of death (DNDD) Exclusion Criteria: 1. LTx from a donor after cardiac death (DCD) 2. LTx for acute liver failure (ALF) 3. Living-donor LTx 4. Simultaneous liver-kidney transplantation

	5. Preoperative renal replacement therapy (RRT) 6. Preoperative intubation and/or mechanical ventilation 7. Portopulmonary hypertension
TEST TREATMENT AND METHOD OF ADMINISTRATION	Mild hypothermia (34-35 °C) during liver transplantation Mild hypothermia will be induced using an esophageal cooling device (ECD), as well as standard measures after the induction of general anesthesia until blood flow is completely restored to the liver. The anticipated median duration of mild hypothermia will be 4-5 hours. When feasible, the right kidney will also be packed with ice-cold sponges to enhance cooling of the renal parenchyma during the anhepatic phase of the operation.
CONTROL TREATMENT AND METHOD OF ADMINISTRATION	Normothermia (36.5-37.5 °C) during liver transplantation Normothermia will be maintained using an ECD as well as standard measures after the induction of general anesthesia and throughout the liver transplant operation.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to one year. **Screening:** less than 1 day **Treatment:** less than 1 day (during liver transplantation) **Follow-up:** 1 year The total duration of the study is expected to be 36 months. 24 months for subject recruitment and 12 for final subject follow-up.
CONCOMMITANT MEDICATIONS	Allowed: N/A Prohibited: N/A
EFFICACY EVALUATIONS	
PRIMARY ENDPOINT	• Incidence of acute kidney injury within 72 hours after liver transplantation
SECONDARY ENDPOINTS	• Distribution of the stages of acute kidney injury within 72 hours after liver transplantation • Duration of intensive care unit stay • Duration of hospital stay • One-year survival rate • Need for renal replacement therapy within 7-days, at 30-days, and at 1 year after transplant • Persistent renal dysfunction at 90-days and 1-year after transplant • Change in serum NGAL levels from baseline to 2 hours after reperfusion of the portal vein • Change in urine NGAL levels from baseline to 2 hours after reperfusion of the portal vein
OTHER EVALUATIONS	• Percentage of intraoperative time within target temperature range

	• Time to reach normothermia upon initiation of re-warming (mild hypothermia arm)
SAFETY EVALUATIONS	• Number of units of blood products transfused (packed red blood cells, fresh frozen plasma, platelets, and cryoprecipitate) • Incidence of surgical site infections within 2 weeks of LTx • Intraoperative vasopressor doses
PLANNED INTERIM ANALYSES	When 50% of patients have completed the study, an interim analysis for efficacy will be conducted by an independent data safety monitoring board (DSMB).
STATISTICS Primary Analysis Plan	Presence or absence of AKI, and the different categories of AKI (Stage 1, 2, or 3 or no AKI), will be summarized as frequency and percentage. The primary outcome (presence or absence of AKI) will be compared between the two arms by a two-sample proportion test. The frequency of different categories of AKI (Stage 1, 2, or 3 or no AKI) will be analyzed by a chi-square test. Intention-to-treat analysis will be performed.
Rationale for Number of Subjects	The incidence of AKI in LTx patients at UCSF is 68%. Considering one interim analysis for efficacy, we estimate a sample size of 101 subjects per arm is needed to detect a 30% reduction in AKI with 80% power at overall $\alpha = 0.05$ (two-tailed). To allow for approximately 10% study drop-out or inability to complete the study protocol, we plan to enroll a total of 230 subjects.

1 BACKGROUND

This trial proposes a mild hypothermia intervention (34-35 °C) that is pragmatic, simple, and non-invasive. Cooling will be performed using an Esophageal Cooling Device (ECD) model EnsoETM (Attune Medical) as well as standard measures (forced-air blankets, conductive table cooling). The ECD is a Food and Drug Administration (FDA) approved device that is placed in a manner identical to a standard orogastric (OG) tube.[1-3] The ECD will help maintain the core temperature of the anesthetized patient between 34-35 °C during LTx. This temperature range is only slightly lower than that typically seen in LTx due to passive heat loss. When possible, the surgeon will also apply local cooling (ice-cold sponges on the surgical field) to the right kidney during the anhepatic phase of the operation. After blood flow is fully restored to the liver, the ECD and standard measures (forced-air, fluid, and table warmers, plus a heated anesthesia circuit) will be used to actively rewarm subjects.

1.1 Overview of Non-Clinical Studies

Extensive rodent work by Dr. Claus Niemann, one of the investigators for the current trial, and colleagues has shown the benefits of mild hypothermia for protecting visceral organs against ischemia-reperfusion (I-R) injury. Prior work showed that mild hypothermia (33-34 °C) is protective against hepatic ischemic injury in both obese and lean rats.[4-7] In soon to be published rodent work, Dr. Niemann's group has shown that mild hypothermia also protects the kidney from injury and inflammation induced by renal artery clamping. Other researchers have also shown that mild-moderate hypothermia reduces acute kidney injury due to I-R.[8]

1.2 Overview of Clinical Studies

Therapeutic hypothermia has long been investigated as a way to preserve end-organ function from ischemic insult, by reducing oxygen consumption, free radical production, and inflammation.[9] Targeted temperature management has been recommended to preserve neurologic function after cardiac arrest[10] (although recent data has questioned this finding)[11] and during circulatory arrest for aortic arch repair.[12] Cold renal perfusion may reduce AKI during open aortic aneurysm repair.[13] Recently, Dr. Niemann's group has also shown that mild hypothermia in brain-dead organ donors improves graft function after kidney transplantation.[14]

The ECD is a US FDA approved device for induction and maintenance of hypothermia through a minimally invasive approach.[2] The device has been shown to be effective and safe for induction and maintenance of hypothermia after out-of-hospital cardiac arrest[1] and in a series of intensive care unit (ICU) patients.[3].

2 STUDY RATIONALE

As in the general hospitalized population, AKI has enormous impact on outcomes and cost after surgery. Numerous, mostly pharmacologic interventions (e.g., dopamine, *N*-acetylcysteine), have been investigated for renal-protecting properties in high-risk surgery - and none have shown clear benefit.[15,16] Most of these studies suffer from heterogeneous

populations (i.e., a mix of cardiac, vascular, and other high-risk surgery), imprecise definitions of AKI, and a lack of good biomarkers. Patients undergoing LTx are a specific population at extremely high risk of perioperative AKI. Thirty-day and 1-year mortality,[17-20] as well as graft survival,[21,22] correlate with the presence and severity of AKI. While modern surgical technique (partial inferior vena cava cross-clamp) reduces the incidence of AKI,[23,24] 68% of patients at UCSF sustain some degree of AKI and 12% develop AKI Stage 3 (a three-fold increase in sCr from baseline or need for dialysis) after LTx. Clearly, any intervention that reduces the incidence or severity of AKI in this population would have tremendous impact on short and long-term outcomes and cost.

Given the preliminary data from pre-clinical and clinical studies described above (see Section 1.1 and 1.2), there is good rationale for a trial of mild hypothermia during LTx as a strategy to prevent AKI. The ECD, an FDA-approved device for cooling or warming of patients through a minimally invasive approach, will facilitate rapid cooling of patients to target temperature (test treatment), maintenance of normothermia (control treatment), and re-warming of patients prior to extubation (test treatment). Without the ECD, the current standard-of-care measures for intraoperative temperature control (forced air blankets, conductive table heater/coolers, fluid warmers, and a heated-humidified anesthesia circuit) are unable to achieve the precision needed for this trial.

2.1 Risk / Benefit Assessment

As described above, AKI leads to worse outcomes for patients. The severity of AKI, even after adjustment for multiple cofounders, correlates with increased mortality, hospital length of stay, and cost.[25] Even AKI Stage 1, characterized by increases in sCr as small as 0.3-0.4 mg/dL, is associated with a 70% increase in risk of death. In cases where sCr rises $\geq$ 2 mg/dL, the odds ratio for mortality approaches 50 and excess costs exceed $20,000 per patient. After liver transplant surgery in particular, AKI has enormous impact. Thirty-day and 1-year mortality, as well as graft survival, correlate with the presence and severity of AKI (see above Section 2 for references).

Given the major negative outcomes associated with AKI after liver transplantation, the potential benefit of a positive result in this study is extremely high. Preventing or reducing the severity of AKI in any given participant would clearly result in direct benefit by lowering the risk of outcomes such as mortality, re-hospitalization, graft loss, or chronic kidney disease. The results of the study will help define optimal care for future liver transplant patients, and possibly for other surgical patients at high risk of AKI (e.g., vascular and cardiac surgery). For society at large, a positive result of this trial would likely result in significant monetary savings in terms of health care costs and resource utilization. By comparison, the risks of the study are mild and include (1) risks of the ECD and (2) side effects of hypothermia.

Even though it is removed at the end of surgery, it is possible that placement of the ECD could lead to more discomfort, sore throat, or difficulty swallowing due to the larger size of the device compared with a standard OG tube. However, the ECD is smaller and softer than a transesophageal echo (TEE) probe that is routinely placed during LTx. The

incidence of painful swallowing after TEE insertion is 0.1%,[26] and the risk of discomfort after ECD placement is expected to be even lower. To minimize the risk of discomfort, ECDs will be well-lubricated and only placed by study doctors experienced in the placement of OG tubes and TEE probes (attending liver transplant anesthesiologists). While placement of the ECD could theoretically cause gastrointestinal bleeding from varices, placement of larger probes for TEE is generally considered safe during LTx and routinely performed by 86% of liver transplant anesthesiologists in the US.[27,28]

Mild intraoperative hypothermia has few potential risks.[29] Currently 23% of patients experience temperatures < 35 °C during LTx at UCSF (data not shown), although not in a precisely controlled fashion as proposed in this trial. Hypothermia can exacerbate coagulopathy and increase transfusion requirements, but this is mainly described at temperatures below our proposed range of 34-35 °C. Increased bleeding was NOT seen in a large randomized controlled trial (RCT) of cooling to 33 °C in intracranial aneurysm surgery.[30] Nevertheless, to mitigate the risk of coagulopathy from hypothermia, the attending liver transplant surgeon will be asked to rate the degree of coagulopathy on a qualitative scale at various points in the operation (see below). If mild hypothermia is felt to be contributing to a coagulopathy that is unsafe for the patient, either the attending surgeon or anesthesiologist may request abortion of the hypothermia protocol at any point in the operation and rewarming of the patient will be initiated. Cardiovascular instability was not observed in a secondary analysis of the aneurysm trial mentioned above[31] or in the trial of brain-dead kidney donors.[14] Hypothermia has been reported to increase surgical site infections and bacteremia,[29,30] but we are confident that this can be prevented by active warming prior to case end and the current antibiotic protocols for LTx.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to assess whether or not mild hypothermia has a protective effect on early AKI after liver transplantation. We hypothesize that mild hypothermia will protect against intraoperative AKI during LTx. We will measure the proportion of patients that develop AKI in the early post-transplant period (≤ 72 hours) as our primary efficacy outcome. This time window is likely to reflect AKI occurring due to intraoperative events,[22,32] and therefore has potential to be modulated by the study intervention. Serum creatinine measurements will be recorded by observers blinded to the treatment arm, and the presence and severity of AKI adjudicated according to the latest criteria.[33,34]

3.2 Secondary Objectives

The secondary objectives are:

- **Assess the feasibility of a mild hypothermia protocol during LTx.** Cooling will be performed using an ECD as well as standard measures. When possible, the surgeon will also apply local cooling (ice-cold sponges on the surgical field) to the right kidney during the anhepatic phase of the operation. After blood flow is fully

restored to the liver, the ECD and standard measures will be used to actively rewarm subjects. Feasibility will be assessed by the proportion of patients successfully completing the protocol, and the percentage of time spent within the target temperature range.

- **Measure biomarkers of AKI during and after mild hypothermia or normothermia in LTx.** NGAL, a protein elevated in AKI,[35] has been described by Dr. Niemann's group as an early biomarker of AKI during LTx.[24] Serum NGAL indicates glomerular injury, while urine NGAL is a marker of distal tubular injury.[36] Blood and urine samples will be collected for NGAL determination at baseline and two hours after reperfusion of the portal vein.

4 STUDY DESIGN

4.1 Study Overview

This is a multi-center, single-blind, RCT. 230 subjects are planned. Each subject will have an ECD placed after induction of general anesthesia and undergo LTx with one of two temperature management protocols (Fig. 1). Each subject will receive only one of the two experimental treatments. Subjects will be randomizaed to one of the two treatments. Evaluations will be taken at baseline, and at several intraoperative time points. Data from the electronic medical record will be analyzed post-operatively.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used (Fig. 1):

- **Experimental treatment** – Mild hypothermia (34-35 °C). Cooling will be initiated after induction of anesthesia and maintained throughout the anhepatic phase of LTx (Fig. 1). In all feasible cases the surgeon will cover the peritoneal surface over the right kidney, which is exposed during the operation, with ice-cold sponges to enhance cooling of the renal parenchyma. Shortly (15 minutes) after the portal vein is reperfused, the ECD and other standard measures (forced-air, fluid, and table warmers, plus a heated anesthesia circuit) will be used to actively re-warm the patient (expected warming rate ≥ 1 °C/hour). The goal is to achieve normothermia by case end.
- **Control** – Normothermia (36.5-37.5 °C). After induction of anesthesia, the ECD and standard warming measures will be used to maintain normothermia throughout the operation.

Total duration of subject participation will be approximately 6-10 hours, which is the duration of surgery. Subjects will be followed up remotely via the electronic medical record (EMR) for 1 year after LTx.

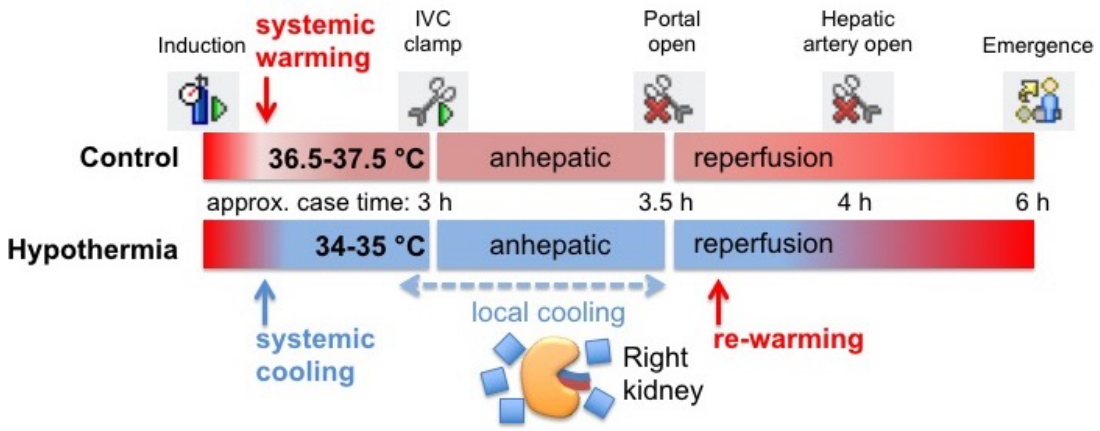

Figure 1. Scheme of overall study design showing temperature management protocols.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the incidence of AKI within 72 hours after LTx. The International Club for Ascites (ICA) 2015 criteria, a revision of the KDIGO criteria for patients with cirrhosis, will be used to define AKI.[33] The definitions are as follows:

- **AKI**: increase in sCr ≥ 0.3 mg/dL within a 48 hour time window, or a percentage increase ≥ 50% from baseline, or initiation of RRT.
- **Baseline sCr**: the most recent value of sCr prior to LTx

A predefined subgroup analysis of the primary outcome will be performed in patients undergoing liver transplantation for hepatocellular carcinoma (HCC) with Model for End-stage Liver Disease exception points.

5.2 Secondary Efficacy Endpoints

A. The distribution of the stages of AKI within 72 hours after liver transplantation. The ICA 2015 criteria will be used to define the stages of AKI as follows:

- **AKI Stage 1**: increase in sCr ≥ 0.3 mg/dL, or an increase in sCr ≥ 1.5-fold and ≤ 2-fold from baseline.
- **AKI Stage 2**: increase in sCr > 2-fold and ≤ 3-fold from baseline.
- **AKI Stage 3**: increase in sCr > 3-fold from baseline, or sCr ≥ 4.0 with an acute increase of ≥ 0.3 mg/dL, or initiation of RRT

B. Duration of intensive care unit (ICU) stay.

- AKI may worsen volume overload, electrolyte abnormalities, and metabolic acidosis, all of which can lead to prolongation of ICU stay.

C. Duration of hospital stay.

D. One-year survival rate.

- AKI after LTx is a negative predictor of mortality.[17,20]

E. Incidence of post-operative renal replacement therapy within 7-days, at 30-days, and at 1 year after transplant.

- LTx is a predictor of developing chronic renal failure.[37]

F. Persistent renal dysfunction at 90-days and 1-year after transplant.

- Presence of a reduction in GFR by ≥ 25 mL/min or ≥ 50% from baseline (Kellum et al.)
- We are aware that patients may not have lab values available from dates exactly 30-days, 90-days, and 1-year after the date of transplantation surgery. In order to standardize our data collection, we have developed rules to determine the appropriate serum creatinine values to record. The rules are as follows:

  <u>1 year follow up: preferences (in order of preference) for selecting serum creatinine value to record:</u>

  1. Serum creatinine value within 1 month after 1-year follow-up date.
  2. Serum creatinine value within 1 month before 1-year follow-up date.
  3. Serum creatinine value collected at a date after 1-year follow-up date that is beyond 1 month but closest to the follow-up date.

  <u>30 and 90 day follow up:</u>

  - The serum creatinine value recorded must have been collected on or post the 30 and 90 follow-up dates. Select the serum creatinine value on or closest to the 30- and 90-day follow-up dates.

G. Change in serum NGAL levels from baseline (start of surgery) to 2 hours after reperfusion of the liver graft (opening of the portal vein).

- NGAL has been shown to be an early marker of AKI in LTx,[24] and as such has the potential to be modulated by the study intervention

H. Change in urine NGAL levels from baseline (start of surgery) to 2 hours after reperfusion of the liver graft (opening of the portal vein).

- NGAL has been shown to be an early marker of AKI in LTx,[24] and as such has the potential to be modulated by the study intervention

5.3 Safety Evaluations

A. **Intraoperative coagulopathy assessment** – while it is unlikely that mild hypothermia (34-35 °C) will significantly impair the coagulation cascade or platelet function to a significant degree,[30] we will ask the attending surgeon to qualitatively rate the degree of clinical coagulopathy as visualized on the

abdominal wall at two intraoperative time points: (1) shortly after incision when the abdominal wall retractors are first placed, and (2) 30 minutes after reperfusion of the hepatic artery. Coagulopathy will be rated as minimal, mild, moderate, or severe.

B. Blood product transfusions and estimated blood loss – While previous studies of mild hypothermia during intracranial aneurysm surgery did not show increases in surgical bleeding or blood product transfusions,[30] we will record the number of packed red blood cell (pRBC), fresh frozen plasma (FFP), platelet, and cryoprecipitate units transfused during surgery, as well as the volume of autologous cell salvage blood (i.e. "Cell Saver", which is standard practice at UCSF in LTx operations) administered to the patient. The estimated blood loss (EBL) will be calculated by the Cell Saver technician and recorded.

C. Vasopressor doses – Prior studies of mild intraoperative hypothermia have not shown increased vasopressor requirements,[31] and vasopressor use is extremely common during LTx. As a safety evaluation, we will record the total vasopressor doses in the pre-anhepatic, anhepatic, and reperfusion phases of LTx.

D. Arterial blood gases – The acid-base status of the patient undergoing LTx, as measured by the base deficit on arterial blood gas (ABG) analysis, reflects the overall perfusion of the patient as well as function of the liver graft. ABGs are typically drawn at several time points during the LTx operation and these values will be recorded.

E. **Incidence of the following adverse events (AEs)**:

- **Significant bleeding in conjunction with evidence of severe, refractory coagulopathy**
  - Defined as significant bleeding that is not attributable to vascular injury in the presence of ongoing severe clinical coagulopathy despite ***aggressive treatment*** (fresh frozen plasma 15 mL/kg, cryoprecipitate 10 units x 2 doses, and the administration of antifibrinolytics). While coagulopathy is a clinical diagnosis during LTx, we would consider persistent INR > 4 or fibrinogen < 75 mg/dL after the ***aggressive treatment*** above as laboratory evidence of severe coagulopathy.
- **Severe upper gastrointestinal (GI) bleeding at the time of ECD insertion**
  - It is common to have mild upper GI bleeding after insertion of a standard orogastric tube during liver transplant, so only blood return of > 500 mL will be considered an AE.
- **Severe hypothermia**
  - < 33 °C for greater than 30 minutes

- Oropharyngeal, dental, or esophageal trauma
- Perforation or damage to the ECD
- Intraoperative cardiac arrhythmias requiring treatment [31] – excluding bradycardia typically seen at the time of reperfusion of the liver graft.

- Intraoperative myocardial ischemia or infarction.
- Discomfort (oropharyngeal pain, dysphagia, odynophagia) - patients will be asked about these symptoms post-operatively
- Surgical site infections (SSIs) – prior to discharge from the hospital after LTx, or within two weeks of LTx (whichever comes first).[38]

5.4 Other Evaluations

A. Fluid totals – Fluid intake (crystalloid, colloid, and blood products) and output (estimated blood loss, urine output) will be tallied and recorded at defined time points in each case: start of surgery (skin incision), clamping of the hepatic artery, 15 minutes post-reperfusion of the of the portal vein, completion of the hepatic artery, completion of the bile duct, and end of surgery (skin closure).

B. Time at target temperature range – The percentage of time within the target core temperature range (34-35 °C for the Experimental Treatment, and 36.5-37.5 °C for the Control) as measured by bladder temperature, will be calculated.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of liver disease who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Male or female ≥ 18 years of age at the time of LTx.
2. Undergoing LTx from a donor after neurologic determination of death (DNDD).
3. Written informed consent obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

1. Undergoing LTx from a donor after cardiac death (DCD)
2. Undergoing LTx for acute liver failure (ALF)
3. Undergoing LTx from a living donor
4. Undergoing simultaneous liver-kidney transplantation
5. Subject has a diagnosis of portopulmonary hypertension (defined as mean pulmonary artery pressure ≥ 25 mmHg, pulmonary vascular resistance > 3 Woods Units, and pulmonary artery wedge pressure < 15 mmHg in the setting of portal hypertension without another known cause).

6. Subject received RRT, including hemodialysis or continuous renal replacement therapy, within a week prior to liver transplantation.
7. Subject is intubated and/or mechanically ventilated prior to entering the operating room LTx.
8. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

7 CONCURRENT MEDICATIONS

Not applicable.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Up to 230 eligible patients will be randomly assigned to the Experimental Treatment (mild hypothermia) or Control (normothermia) groups in a 1:1 ratio using a block randomization scheme with a randomly chosen block size of 6 or 8 for each block. The randomization scheme was developed by Randomize.net and is accessed through a secure web portal.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to patients. The following study procedures will be in place to ensure single-blind administration of study treatments.

Access to the randomization code will be strictly controlled. The randomization code will not be revealed until the patient has entered the operating room for liver transplantation. The study protocol will be completed in its entirety while the patient is in the operating room under general anesthesia. Therefore, it will be impossible for the patient to know the treatment assignment until the protocol is complete.

No attempt will be made to blind the study doctors (anesthesiologists), who are investigators in the study, to the treatment assignment, as they will be the ones administering and monitoring the temperature protocol during LTx.

Research staff assigned to adjudicate the Primary Outcome (presence or absence of AKI within 72 h of LTx) will be blinded to the treatment assignment and the intraoperative anesthesia record showing the patient's core temperature. Only the preoperative and postoperative values of sCr will be necessary to adjudicate the Primary Outcome.

The study blind will be broken on completion of the clinical study and after the study database has been locked.

8.3 Formulation of Test and Control Treatments

After induction of general anesthesia, the ECD will be connected to the Gaymar Medi-Therm III Hyper/Hypothermia Machine and tested for leaks according to the

manufacturer's instructions. Then, it will be lubricated and placed into the esophagus of the subject in a manner identical to that of the standard OG tube used in LTx surgery.

The standard bladder temperature probe used in LTx operations will be connected to the PATIENT PROBE jack of the Gaymar Medi-Therm III, which will be used in AUTO/Rapid mode.

In both Study Arms (Mild Hypothermia and Normothermia), the subject will be given 2 grams of magnesium (Mg) sulfate IV at the beginning of surgery if the preoperative Mg < 2 mg/dL. This will be done because mild hypothermia may trigger a cold-induced diuresis and loss of electrolytes. Therefore, efforts will be made to keep the Mg normal (1.8-2.4 mg/dL) in both groups. Potassium will also be monitored carefully during surgery and replaced at the discretion of the attending anesthesiologist/study doctor.

Experimental treatment – Mild hypothermia (34-35 °C).

- Cooling will be initiated after induction of anesthesia and maintained throughout the anhepatic phase of LTx (Fig. 1). This will be done by selecting a Set Point temeperature of 34.8 °C[1] on the Gaymar Medi-Therm III.
- In all feasible cases the surgeon will cover the peritoneal surface over the right kidney, which is exposed during the operation, with ice-cold sponges (lap sponges soaked in ice water and wrapped around ice cubes) to enhance cooling of the renal parenchyma. Local cooling will be applied to the right kidney beginning approximately 10 minutes prior to the anhepatic phase. The anhepatic phase is defined as application of the inferior vena cava (IVC) clamp and removal of the recipient liver.
- After the portal vein is reperfused, the ECD (by selecting a Set Point temperature of 37 °C on the Gaymar Medi-Therm III) and other standard measures (forced-air, fluid, and table warmers, plus a heated anesthesia circuit) will be used to actively re-warm the patient (expected warming rate ≥ 1 °C/hour). The goal is to achieve normothermia by case end.

Control – Normothermia (36.5-37.5 °C).

- After induction of anesthesia, the ECD (by selecting a Set Point temperature of 37 °C on the Gaymar Medi-Therm III) and standard warming measures will be used to maintain normothermia throughout the operation.

At the end of LTx surgery, the ECD will be removed from the patient prior to emergence from general anesthesia, extubation, and/or transport to the ICU.

[1] The original protocol used a set point of 34.4 °C. However, we found that the core temperature dropped significantly (~0.5 °C) during the anhepatic phase (due to the cold liver graft and ice pack on the right kidney). Thus, we have modified the Set Point temperature to 34.8 °C.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a subinvestigator who is a physician at Screening (this is part of the standard anesthesia preoperative assessment prior to liver transplant surgery). New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff post-operatively.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse, oximetry and respirations will be performed prior to induction of general anesthesia at Screening and continuously while receiving the Study Protocol in the OR during LTx.

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Arterial Blood Gas

Arterial blood will be obtained and analyzed by point-of-care testing using an ABL-90 blood gas analyzer. This is standard of care during LTx at UCSF and no additional ABGs will be drawn outside of what is normally required for patient care.

9.3 Research Laboratory Measurements

9.3.1 Serum NGAL Measurements

At two time points, baseline (surgical incision) and 2 hours after reperfusion of the portal vein, blood will be collected for serum NGAL determination. At each blood draw, two serum separator tubes (gold top) will be filled. Each tube requires about 5 mL of blood, there will be 2 tubes collected per time point, and 2 time points, for a total of about 20 mL of blood (4 tubes).

After filling with blood, tubes will be inverted 5-times to mix clot activator with blood. Samples will sit undisturbed for 30 min to allow clot to form. Samples will be centrifuged at 1,000 x *g* for 15 minutes. Serum will be pipetted into Eppendorf tubes in 2 mL aliquots and frozen at ≤ -20 °C until analysis, which will be performed *en batch*.

Serum NGAL determination will be performed using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, Inc.).

9.3.2 Urine NGAL Measurements

At two time points, baseline (surgical incision) and 2 hours after reperfusion of the portal vein, urine will be collected for NGAL determination. Approximately 10-15 mL of fresh urine will be aspirated from the Foley catheter collection chamber using a syringe, and dispensed into a 15-mL sterile tube (Falcon). The urine will be centrifuged at 1,000 x *g* for 15 min to pellet any solid material. The supernatant will be pipetted into Eppendorf tubes in 2 mL aliquots and frozen at ≤ -20 °C until analysis, which will be performed *en batch*.

Urine NGAL determination will be performed using an ELISA kit according to the manufacturer's instructions (R&D Systems, Inc.).

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day 1, before and during LTx)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of acute kidney injury, ascites, or hepatic encephalopathy, including diagnosis date, and prior treatments (dialysis, continuous renal replacement therapy (CRRT), large volume paracentesis).
5. Record concomitant medications.

6. Perform a complete physical examination.
7. Perform and record vital signs.
8. Perform and record oximetry.
9. Perform and record results of blood pressure testing.
10. Randomize subject (see **Section 8.1**).
11. Place ECD after induction of general anesthesia.
12. Implement intraoperative temperature protocol according to randomization (see **Section 8.3**).
13. Collect blood for ABGs (see **Section 9.2**) and NGAL determination (**Section 9.3.1**).
14. Collect urine for NGAL determination (see **Section 9.3.2**).
15. Remove ECD at the end of surgery.

10.2 Visit 2 (Follow-up prior to hospital discharge, window of 2 ± 1 weeks after LTx)

1. Record any Adverse Experiences.
2. Record changes to concomitant medications.
3. Perform complete physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. If surrogate consent was obtained for the subject, and subject has now regained capacity, re-consent the patient for use of study data.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a treatment that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational treatment, whether or not related to that investigational treatment.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study protocol, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the

guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Treatment

The relationship of an AE to the study treatment should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known side effect of treatment; or an event that follows a reasonable temporal sequence from administration of the treatment; that follows a known or expected response pattern to the suspected treatment; that is confirmed by stopping or reducing the treatment; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the treatment; that follows a known or expected response pattern to the suspected treatment; that is confirmed by stopping or reducing the treatment; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the treatment; that follows a known or expected response pattern to that suspected treatment; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study treatment.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity

- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study treatment) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Treatment

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the surgeon feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment

If a subject is discontinued from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

Subjects will also be discontinued from the study treatment if the study doctor is unable to place the ECD after three attempts.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

12.4 Replacement of Subjects

Subjects who undergo early discontinuation of the study treatment will not be replaced, and such events will be accounted for by intention-to-treat analysis.

Subjects who withdraw from the study will be replaced with a new subject that will be independently randomized.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Early termination of temperature management protocol
- Removal of ECD prior to the end of surgery
- Failure to pack the right kidney with ice
- Inability to maintain target temperature for ≥ 1 hour
- Failure to meet inclusion/exclusion criteria
- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation.

Protocol violations will be accounted for by intention-to-treat analysis. Withdrawal of the subject will not be required, as all study procedures are completed within the first visit at the time of LTx.

14 DATA SAFETY MONITORING

A Data Safety Monitoring Board (DSMB) has been established to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study. Interim reviews will be conducted by the DSMB for the purpose of monitoring study conduct and assessing patient safety at a minimum of every 6 months during the trial. Further details regarding the timing and content of the interim reviews is included in the statistical section below.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive the study treatment will be included in the safety analysis.

15.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized: race, gender, age, height and weight.

15.3 Analysis of Primary Endpoint

Both intention-to-treat (ITT) and per-protocol analysis will be performed. All randomized, non-withdrawn patients will be included in the ITT analysis. All subjects completing the systemic temperature regulation protocol as outlined in Section 8.3 will be included in the per-protocol analysis. Subjects who had a protocol violation will be excluded from the per-protocol analysis.

The primary outcome (presence or absence of AKI) will be compared between the two arms by two-sample proportion test, furthermore, a multiple logistic regression model will be utilized where AKI status (present or absence) is considered as the outcome and arms along with demographic characteristics (e.g, race, gender, age, etc.) and clinical characteristics (such as local cooling, Model for End-Stage Liver Disease score, etiology of liver disease, etc.) are considered as predictors and/or covariates.

15.4 Analysis of Secondary Endpoints

Categorical variables will be summarized as frequency and percentage, and continuous variables as median and interquartile range by arm. Categorical variables will be compared between the two arms by either chi-square or Fisher's exact test, as appropriate.[39] Continuous variables will be compared between the two arms by two-sample t-tests, or Mann-Whitney U tests if the normality assumption does not hold. Logistic regression (simple and multiple) will be used to detect variables associated with presence or absence of AKI. Patient and graft survival and time-to-event data (such as time to disease progression) will be estimated by Kaplan-Meier method and compared between the two arms by log-rank tests and cox-proportional hazard model will be applied to facilitate incorporating other covariates in the model. Statistical analysis will be performed using GraphPad Prism, STATA, and R software.

- To check if the frequencies of categories of AKI (Stage 1, 2, or 3 or no AKI) are different between the two arms, a chi-square test will be used. Similarly, a multiple proportional odds model will be applied when considering AKI in a multi-category fashion.
- Duration of intensive care unit stay and hospital stay will be summarized by median and interquartile range by arm and compared by two-sample t-tests, or Mann-Whitney U tests if the normality assumption does not hold.

- Patient survival will be estimated by the Kaplan-Meier method and compared between the two arms by log-rank tests. A Cox-proportional hazard model will be applied to facilitate incorporating other covariates into the model.
- Incidence of post-operative renal replacement therapy within 7-days, at 30-days, and at 1 year after transplant will be summarized as frequency and percentage, and compared between the two arms by either chi-square or Fisher's exact test, as appropriate.
- Incidence of persistent renal dysfunction (defined as a reduction in glomerular filtration rate by ≥ 25 mL/min or ≥ 50% from baseline) at 90-days and 1-year after transplant will be summarized as frequency and percentage, and compared between the two arms by either chi-square or Fisher's exact test, as appropriate.
- The change in serum NGAL level from baseline to 2 hours after reperfusion of the portal vein will be summarized by median and interquartile range by arm and compared by two-sample t-tests, or Mann-Whitney U tests if the normality assumption does not hold.
- The change in urine NGAL level from baseline to 2 hours after reperfusion of the portal vein will be summarized by median and interquartile range by arm and compared by two-sample t-tests, or Mann-Whitney U tests if the normality assumption does not hold.

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study treatment.

15.5 Interim Analysis

15.5.1. Interim Analysis for Efficacy

A single interim analysis for efficacy is planned for this study after 50% of the required patients (101 of the estimated 202) are accrued after accounting for withdrawn subjects. Based on the O'Brien-Fleming approach to calculate alpha spending, during the interim analysis step, if the p value is < 0.0054, the study will be stopped as the desired efficacy will have been achieved; if not, then we will continue the study. If the final p value is < 0.0492, then we will declare that the AKI status is different between the two arms.

15.5.2. Stopping Rule for Safety

Based on clinical experience during LTx at UCSF and data from the liver anesthesia quality improvement database, AEs listed in Section 5.3E of severity 3 (Severe) or 4 (Life-threatening) are expected to have a combined incidence of < 5% in study subjects. If, during routine DSMB review, an incidence of AEs of severity 3 or 4 of > 10% is observed in all study subjects, or in either treatment arm, the trial will be stopped.

15.6 Sample Size and Randomization

Considering one interim analysis for efficacy, and given the incidence of AKI in LTx patients at UCSF (68%), we estimate a sample size of 101 subjects per arm (n = 202 total) is needed to detect a 30% reduction in AKI with 80% power at overall alpha = 0.05 (two-tailed).

We will aim to enroll 230 total subjects to allow for just over a 10% drop-out rate, since some subjects may not be able to complete the protocol and will be withdrawn from the study. UCSF is a high-volume center for LTx (> 180 cases per year), the majority of which meet our inclusion criteria. Thus, we estimate it will take no more than two years to complete study enrollment. The website http://www.swogstat.org was used to perform the power calculation, and the power calculation was confirmed by the CTSI at UCSF.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study protocol.

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the investigator, but will be identified by a subject number and initials.

If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated RedCap database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the DSMB or IRB/IEC upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years after study completion.

16.6 Monitoring

Not applicable.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number and subject initials will identify all study subjects on CRFs and other documentation. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject. The Investigator must also comply with all applicable privacy regulations (e.g., Health

Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Investigator. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning patient recruitment, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization for submission to the IRB/IEC. The consent form generated by the Investigator must be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will

also comply with local regulations. The Investigator will retain an IRB/IEC-approved copy of the Informed Consent Form for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

17.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the IRB/IEC except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the DSMB or IRB/IEC any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the DSMB or IRB/IEC.
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and DSMB all changes in the research activity and all unanticipated problems involving risks to subjects or others.
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	VISIT 1 (Day 1, before and during liver transplant)	**VISIT 2 (Day 2, prior to hospital discharge, approximately 2 weeks after LTx)[A]**
Informed Consent	**X**	**X[B]**
Medical History	**X**	
Complete Physical Exam	**X**	**X**
Height	**X**	
Weight	**X**	
Vital Signs	**X**	**X**
Oximetry	**X**	**X**
Randomization	**X**	
Administration of Study Treatment	**X**	
Blood and Urine collection	**X**	
Initiate Subject Diary	**X**	
Subject Diary Review		**X**
Concomitant Medication Review	**X**	**X**
Adverse Experiences		**X**

[A] ±1 week

[B] Consent from subject will be obtained for use of study data if the patient lacked capacity and Surrogate Consent was obtained during Visit 1.

APPENDIX 2. REFERENCES

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