WP01 US Trial Protocol

A multicenter randomized controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation

IDE Ref:	G140243
Protocol Version:	WP01 Version 10.0: (24 June 2016)
National Principal Investigator:	Stuart Knechtle, MD Professor of Surgery Department of Surgery Duke University 2100 Erwin Rd, Durham, NC 27705, USA stuart.knechtle@duke.edu +1 919 613 9687
Sponsor/Manufacturer: **US Sponsor:** **US Agent:**	OrganOx Ltd. NAMSA NAMSA
Funder:	OrganOx Ltd.
Confidentiality Statement:	This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the US Sponsor, the Investigative Team, and members of the FDA/IRB unless authorized to do so.
Conflicts of Interest:	None

STUDY SYNOPSIS

Trial Title	A multicenter randomized controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation	
Short Title	WP01 – Normothermic liver preservation	
Clinical Phase	Pivotal	
Trial Design	Multicenter open-label randomized controlled trial	
Investigative Sites	Subjects will be enrolled at up to 15 investigative sites	
Trial Participants	Deceased DBD and DCD liver donors and adult liver transplant recipients	
Planned sample size	266 transplanted livers (minimum of 120 per arm), with approximately 356 randomized livers	
Follow-up duration	Subjects will be followed for 12 months post-transplant procedure. The primary endpoint analysis will be completed at 6 months post-transplant procedure.	
Planned trial period	36 months	
	Objectives	**Outcome measures/endpoints**
Primary	To compare the effect of NMP to SCS in preventing preservation-related graft injury	Severity of immediate graft injury as measured by early allograft dysfunction (EAD).
Secondary	To compare graft and subject survival between NMP and SCS livers.	Primary non-function rates: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation. Graft survival rates at 30 days, 3 months, and 6 months following transplantation. Subject survival rates at 30 days, 3 months, and 6 months following transplantation.
	To compare evidence of post-reperfusion syndrome between NMP and SCS livers on transplantation.	Assess mean arterial pressure (MAP) pre- and post-reperfusion in the context of vasopressor use.
	To compare biochemical liver function between NMP and SCS livers.	Bilirubin, GGT, ALT, AST, ALP and INR at days 1-7, day 30, month 3, and month 6 post-transplant. Lactate at days 1-7 while the subject is in ICU.
	To compare evidence of ischemia-reperfusion injury between NMP and SCS livers.	Post-reperfusion biopsies will be compared to baseline pre-reperfusion biopsies and graded according to standard histological criteria.

	To compare evidence of biliary complications between NMP and SCS livers.	Incidence of biliary investigations and/or interventions between 7 days and 6 months post-transplant.
	To assess the feasibility and safety of NMP as a method of organ storage and transportation.	Incidence of livers randomized but not transplanted and reasons for not-transplanting.
	To compare organ utilization between NMP and SCS livers.	Incidence of one or more of the following per randomized liver: (i) EAD; (ii) discard (non-transplant) of a retrieved liver; (iii) primary non-function.
	To assess the health economic implications of normothermic liver perfusion.	Logistical and healthcare costs (length of stay in ICU and hospital) and quality of life measures.
Device name	OrganOx *metra®*	
Device manufacturer	OrganOx Ltd, Oxford, UK	
Length of time device has been in use	Since February 2013 (in two European clinical studies)	

PROTOCOL SIGN-OFF

National Principal Investigator:	Signature..... Name Stuart J. Knechtle, M.D. Date August 5, 2016
Sponsor/Manufacturer's Representative:	Signature L. J. Russell Name DR L.J. RUSSELL Date 10th August 2016

INVESTIGATOR SIGNATURE PAGE

I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol and will only make changes in the protocol after notifying the US Sponsor.

I understand that I may terminate or suspend enrollment of the study at any time if it becomes necessary to protect the best interests of the study subjects as advised by the Data Safety Monitoring Board (DSMB). This study may be terminated by the Sponsor/Manufacturer or US Sponsor, with or without cause.

I agree to personally conduct or supervise this investigation and to ensure that all associates, colleagues, and employees assisting in the conduct of this study are informed about their obligations in meeting these commitments.

I will conduct the study in accordance with applicable Good Clinical Practice, and the moral, ethical and scientific principles that justify medical research. The study will be conducted in accordance with all relevant laws and regulations relating to clinical studies and the protection of patients.

I will ensure that the requirements relating to FDA review and approval are met.

I agree to maintain adequate and accurate records and to make those records available for audit and inspection in accordance with relevant regulatory requirements.

I agree to promptly report to the FDA, the IRB (per their reporting guidelines), and US Sponsor any changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without regulatory approval, except where necessary to ensure the safety of study participants.

Signature……

Name……

Date…….

CONTENTS

ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CEC	Clinical Events Committee
CFR	Code of Federal Regulations
CIT	Cold Ischemia Time
CMS	Centers for Medicare & Medicaid Services
CRF	Case Report Form
CVA	Cerebrovascular Accident
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DPMP	Donors per Million Population per Year
DRI	Donor Risk Index
DSMB	Data Safety Monitoring Board
EAD	Early Allograft Dysfunction
ECD	Extended Criteria Donor
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ERCP	Endoscopic Retrograde Cholangiopancreatography
FDA	Food & Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GST	Glutathione S-Transferase
H0	Null Hypothesis
HA	Alternative Hypothesis
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HD	Hemodialysis
HDF	Hemodiafiltration
HF	Hemofiltration
HMP	Hypothermic Machine Perfusion

HTK	Histidine-Tryptophane-Ketoglutarate
IC	Ischemic Cholangiopathy
ICU	Intensive Care Unit
IDE	Investigational Device Exemption
IFU	Instructions for Use
INR	International Normalized Ratio
IRB	Institutional Review Board
ISO	International Organization for Standardization
IVC	Inferior Vena Cava
MAP	Mean Arterial Pressure
MELD	Model for End Stage Liver Disease
MRCP	Magnetic Resonance Cholangiopancreatography
NMP	Normothermic Machine Perfusion
OPO	Organ Procurement Organization
OPTN	Organ Procurement and Transplantation Network
PNF	Primary Non-Function
PT	Prothrombin Time
PTC	Percutaneous Transhepatic Cholangiogram
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SCS	Static Cold Storage
SRTR	Scientific Registry of Transplant Recipients
UADE	Unanticipated Adverse Device Effect
UNOS	United Network for Organ Sharing
UW	University of Wisconsin Preservation Solution

1. Administrative Information

1.1 Title

"A multicenter randomized controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation."

1.2 Trial Registration

This trial protocol is registered with clinicaltrials.gov in accordance with regulations. The national clinical trial identifier is NCT02775162.

1.3 Protocol Version

1.3.1 Current Version

Version number: WP01 Version 10.0

Issue date: 24 June 2016

1.3.2 Previous Versions

Details of previous versions and amendments to this protocol are detailed in Appendix A2.

1.4 Study Funding

OrganOx Ltd

1.5 Sponsor

The trial is sponsored by OrganOx Ltd.

The US Sponsor and US Agent is NAMSA.

The Sponsor/Manufacturer will approve this protocol prior to study commencement.

1.6 Trial Personnel

1.6.1 National Principal Investigator

National Principal Investigator:

Stuart Knechtle MD; Duke University

1.6.2 Database Design

US Sponsor will be responsible for developing and maintaining the trial database and electronic case report forms. The database will have reporting functionality in order to capture data entry and verification, which the trial data manager will utilize.

1.6.3 Trial Statistician

The NAMSA statistician will be responsible for the development of the statistical analysis plan and the subsequent analysis of trial data.

1.6.4 Trial Histopathologist

Histopathology will be carried out by a central laboratory in accordance with a study laboratory manual.

1.7 Roles and Responsibilities

1.7.1 Sponsor Responsibilities

- **General responsibilities (§812.40)**
  Sponsors are responsible for selecting qualified investigators and providing them with the information that they need to conduct the investigation properly. They must also ensure proper monitoring of the investigation and IRB review and approval, submit an IDE application to FDA for significant risk device studies, and inform the IRB and FDA promptly of any significant new information about the investigation.
- **FDA and IRB approval (§812.42)**
  A sponsor cannot begin an investigation or any part of an investigation until an IRB and FDA have both approved the application or supplemental application.
- **Selecting Investigators (§812.43)**
  A sponsor is responsible for selecting investigators qualified by training and experience to investigate the device.
- **Selecting Monitors (§812.43)**
  A sponsor must select monitors qualified by training and experience to monitor the investigational study in accordance with the IDE and other applicable FDA regulations.
- **Device Control (§812.43)**
  A sponsor can ship investigational devices **only** to qualified investigators participating in the investigation.
- **Investigator Agreements (§812.43)**
  A sponsor must obtain a signed agreement from each participating investigator that includes:
    - The investigator's curriculum vitae,
    - A statement of the investigator's relevant experience, including the dates, location, extent, and type of experience, where applicable,
    - An explanation of the circumstances that led to termination of a study if the investigator was involved in an investigation or other research that was terminated,
    - A statement of the investigator's commitment to:
        - Conduct the investigation in accordance with the agreement, the investigational plan, the IDE and other applicable FDA regulations, and conditions of approval imposed by the reviewing IRB or FDA,
        - Supervise all testing of the device involving human subjects
        - Ensure that the requirements for obtaining informed consent are met.
        - Sufficient accurate financial disclosure information to allow a sponsor to submit a complete and accurate certification or disclosure statement as required under 21 CFR 54, Financial Disclosure by Clinical Investigators. A sponsor shall also obtain a commitment from the clinical investigator to promptly update this information if any relevant changes occur during the course of the investigation and for one year following completion of the study.
- **Informing Investigators (§812.45)**
  A sponsor must supply all investigators participating in the investigation with copies of the investigational plan and a report of prior investigations of the device.
- **Monitoring (§812.46)**
  Securing Compliance: A sponsor who discovers that an investigator is not complying with the signed agreement, the investigational plan, the IDE requirements, any other applicable FDA regulations, or any conditions of approval imposed by the reviewing IRB or FDA must promptly either secure compliance, or discontinue shipments of the device to the investigator and terminate the investigator's participation in the investigation. A sponsor must also require that the investigator

dispose of or return the device, unless this action would jeopardize the rights, safety, or welfare of a subject.

Unanticipated Adverse Device Effects: A sponsor must immediately conduct an evaluation of any unanticipated adverse device effect. A sponsor who determines that an unanticipated adverse device effect presents an unreasonable risk to subjects must terminate all investigations or parts of the investigations presenting that risk as soon as possible. Termination must occur no later than 5 working days after a sponsor makes this determination and no later than 15 working days after a sponsor first received notice of the effect.

Resumption of Terminated Studies: a sponsor may not resume a terminated investigation without IRB and FDA approval. A sponsor may not resume a terminated investigation without IRB approval.

- **Sponsor records (§812.140)**
  A sponsor must maintain accurate and complete records relating to the investigation. These records include:
  - All correspondence including required reports,
  - Records of shipment of the device,
  - Records of disposition of the device,
  - Signed investigator agreements including financial disclosure information,
  - Records concerning complaints and adverse device effects whether anticipated or not,
  - Any other records that FDA requires to be maintained by regulation or by specific requirement for a category of investigation or a particular investigation.
- **Sponsor Reports (§812.150)**
  A sponsor must provide the following reports in a timely manner to FDA, the IRB's, and/or the investigators.
  - Unanticipated Adverse Device Effects
  - Withdrawal of IRB Approval
  - Withdrawal of FDA Approval
  - Current List of Investigators
  - Progress Reports
  - Recalls and Device Disposition
  - Final Report
  - Use of device without Informed Consent
  - Significant Risk Device Determination

1.7.2 Investigator Responsibilities

- The investigator is responsible for protecting the rights, safety, and welfare of subjects. An investigator must conduct the investigation in accordance with the signed agreement with the sponsor, the investigational plan, the IDE regulations and other applicable FDA regulations, and any conditions of approval imposed by an IRB and FDA. (§812.100)
- While awaiting approval, an investigator may determine whether or not potential subjects would be interested in participating in an investigation, but cannot request written informed consent or allow any subjects to participate before obtaining IRB and FDA approval. (§812.110)
- An investigator is responsible for obtaining informed consent under 21 CFR Part 50.
- Supervision of device use: An investigator can permit use of the investigational device only with subjects under his/her supervision and must not supply an investigational device to any person not authorized under the regulations to receive it. (§812.110)
- **Financial Disclosure (§812.110)**
  The clinical investigator must disclose to the sponsor sufficient accurate financial information to allow the IDE applicant (or sponsor) to submit certification or disclosure of financial interests under 21 CFR

54. The investigator must update the information if any relevant changes occur during the course of the investigation and for one year following completion of the study.

- **Device Disposal (§812.110)**
  Upon completion or termination of a clinical investigation or the investigator's part of the investigation or at the sponsor's request, an investigator must return to the sponsor any remaining supply of the device or dispose of the device as the sponsor directs.
- **Records (§812.140)**
  The investigator must maintain accurate and complete records relating to the investigation. These records include:
  - All correspondence including required reports,
  - Records of receipt, use, or disposition of the investigational device,
  - Records of each subject's case history and exposure to the device,
  - The protocol and documentation (date and reason) for each deviation from the protocol,
  - Any other records that FDA requires to be maintained by regulation or by specific requirement for a category of investigation or a particular investigation.
- **Investigator Reports (§812.150)**
  The investigator must provide the following reports in a timely manner to the sponsor and/or the IRB:
  - Unanticipated Adverse Device Effects
  - Withdrawal of IRB Approval
  - Progress Reports
  - Deviations from the Investigational Plan
  - Failure to obtain Informed Consent
  - Final Report
- The **Investigators** will be responsible for:
  - Identification and recruitment of patients to the study
  - Conducting clinical procedures in accordance with the protocol and standard operating procedures
  - Data collection and completion of electronic CRFs
  - Follow-up of study participants

1.8 COMMITTEES

1.8.1 DATA SAFETY MONITORING BOARD (DSMB)

The DSMB will be appointed to monitor the conduct of the study and subject safety by periodically reviewing data from the study. The DSMB will oversee the overall safety of the current and future study subjects by protecting them from avoidable harm. The DSMB will review adverse events and other relevant study data and will make recommendations regarding continuation of the study to the US Sponsor.

1.8.2 CLINICAL EVENTS COMMITTEE (CEC)

The CEC is composed of transplant surgeons or other clinicians with relevant expertise who are not participating in this study, and who do not have significant investment with the study Sponsor/Manufacturer or US Sponsor. The CEC is charged with the review and classification of adverse events (AEs), including deaths. The CEC will establish rules outlining the minimum data required and the algorithm followed in order to classify AEs. All members of the CEC will meet regularly to review and classify AEs. All appropriate data will be reviewed by the CEC. The CEC will forward a report of event review and classifications as outlined in the CEC charter.

2. INTRODUCTION

2.1 BACKGROUND AND RATIONALE

2.1.1 LIVER TRANSPLANTATION – A SUCCESSFUL THERAPY

Liver transplantation is the only effective treatment for many patients with liver disease. For patients with liver failure, techniques for supporting liver function provide only limited and temporary benefit as a bridge to transplantation or liver regeneration in the case of acute liver failure. In contrast to kidney dialysis support in renal failure, there is no artificial means to support a patient with liver failure for an extended period. Since the first clinical case in 1963, transplantation of the liver has developed to an established and standardized procedure as cure for acute and chronic liver disease. The results of liver transplantation have greatly improved and patient survival rates of over 90% at one year and 70% at five years are routinely achieved for elective liver transplantation. As a result, liver transplantation has become the mainstay of treatment for an increasing spectrum of patients with chronic liver disease, metabolic liver disease, acute liver failure and some liver cancers.

2.1.2 LIVER FAILURE EPIDEMIOLOGY

In the US cirrhosis is the most common indication for liver transplantation (42%), other indications include cancer (22%), and acute hepatic necrosis (4%) [www.srtr.org – 2012 annual report]. The main causes for cirrhosis in the US are virus related cirrhosis and alcoholic liver disease. It is predicted that HCV-related hepatocellular carcinoma (HCC) will continue to increase during the next decade, despite the impact of new drug treatments for this infection. Meanwhile, the incidence of liver disease related to both obesity and alcohol consumption is expected to increase despite health campaigns. It is very likely that the demand for donor livers suitable for transplantation will continue to rise, exacerbating the existing organ shortage.

2.1.3 THE DONOR SHORTAGE

Over the last two decades, liver transplantation has become a victim of its own success: many more patients are referred for transplantation, but the number of suitable grafts from deceased organ donors has increased more slowly. The donor organ shortage constitutes a serious risk for patients with liver failure. It is the principal cause of increasing waiting lists and the death of patients on the waiting list worldwide. In 2012, 6,256 patients underwent liver transplantation in the USA, but 10,143 patients were added to the waiting list, and there was a mortality rate of 19% on the waiting list. This shortfall is typical of liver transplantation services around the world. In many countries, a patient is now more likely to die on the waiting list for a transplant than in the 12 months after the operation.

Great efforts have been made in recent years to increase the referral of organ donors, but an increasing proportion of deceased donors are suboptimal. These include donors declared dead by cardiovascular criteria ('donation after circulatory death', DCD) and other 'extended criteria' organ donors (older age, steatosis, etc.). There has been a much smaller increase in the number of standard criteria ('ideal') organ donors, including younger donors declared dead by neurological criteria ('donation after brain death', DBD). This is reflected in the utilization of donor organs – in 2012, 74% of solid organ donors in the USA resulted in a liver transplant (OPTN data).

2.1.4 APPROACHES TO THE DONOR ORGAN SHORTAGE

Organ donor rates in other countries range widely: using 2011 data, the highest rate occurred in Spain with 35.3 donors per million population per year (DPMP), compared to the UK (17 DPMP) and the USA (25 DPMP). It is generally accepted that these discrepancies are due not only to cultural distinctions but also due to political and legal differences. There is debate as to whether the situation is simpler in those countries that practice "presumed consent" (opting out) [1]. Most countries, however, practice a system of "opting in," whereby consent must be sought from the family of the donor.

Due to the critical shortage of donor organs, clinicians are continually searching for ways to overcome the discrepancy between demand and availability of donor livers for transplantation. Additional sources of organs include those from living donors, organs from DCD (donation after circulatory death) donors (previously known as 'non-heart-beating donors') and other less-than-optimal ('higher risk') deceased donors.

2.1.4.1 Living Donation

Living donation is one potential means to increase the number of liver transplants, using surgical techniques developed for ´liver splitting´ (a technique for using a single liver for transplantation into two recipients). The major limitations of this technique include, first, the fact that most patients do not have a willing and suitable living donor, and, second, real concerns about the risks to the healthy donor. The reported risk of donor death is estimated at 0.2% but the risk of serious complications is much higher [2]. For these and other reasons living donor transplantation has had limited impact on the shortage of donor livers for transplantation in most countries (only 246 such transplants were performed in the USA in 2012).

2.1.4.2 Higher Risk Donor Organs

Much emphasis is now placed on optimizing the condition of those organs that are available, to enable an increased number of higher risk organs to be transplanted safely. The use of a higher risk organ does constitute a greater risk to the recipient, with a higher probability that the organ will never function and require immediate replacement (primary non-function; PNF), that it will function poorly and place the patient at risk of other complications (early allograft dysfunction; EAD) or that it will lead to later complications including multiple stricturing of the biliary tree (ischemic cholangiopathy; IC).

The serious effects of the organ shortage, with many patients dying on the waiting list, has led to increased interest in using donor livers which were formerly thought unsuitable for transplantation. The use of these ´extended criteria donors´ (ECD; also called 'marginal' or 'high risk') donor livers for liver transplantation is now seen as essential if liver transplant units are to address the demand. Several donor parameters have been identified as relative risk factors for poor outcome, including age; steatosis; DCD donation; split livers; prolonged cold ischemia time (>12 hours). These were all developed using North American data and formulated into an algorithm known as the 'Donor Risk Index' (DRI) [3], and later validated using European data [4].

2.1.4.3 Donation After Circulatory Death (DCD)

For many years (following the establishment of brain death criteria) in most countries, deceased donor organs were almost exclusively sourced from donors declared dead by neurological criteria (donation after brain death, DBD). In such cases the donor remains on ventilatory support and cardiopulmonary function is maintained until the donor has been transferred to the operating room, preliminary dissection has been performed, cannulae placed and the organs are ready for cooling. This enables organs to be removed with minimal interruption to oxygenation before cooling and preservation. In contrast, in the case of DCD donors, death is certified after cardiac arrest using cardiovascular parameters; this causes an inevitable period of oxygen deprivation between cardiac arrest and cold preservation of the organs. DCD donors have been classified as 'uncontrolled', in which death is not predicted (typically after failed resuscitation in an emergency room) and 'controlled' in which death is anticipated (typically under circumstances where life-support is withdrawn from a patient in whom continued treatment has been deemed to be futile).

In the latter situation, because death is anticipated and the transplant team can be mobilized in advance, the period of 'warm ischemia' sustained by the donor organs is usually much shorter; livers from this source have been transplanted in substantial numbers in recent years, although very selectively, but with higher rates of primary non-function and other post-operative complications than DBD livers, with worse long-term graft survival [5-7]. This increase in complications results in an increased cost incurred with DCD transplantation [8]. In contrast, livers from uncontrolled DCD are not generally used for transplantation because of a high rate of primary non-function in the experience of those centers that have attempted this [9]. It has been estimated,

however, that effective use of these potential donors would add greatly to the number of organ donors [10]. This contrasts with the situation in renal transplantation in which organs from uncontrolled DCD have been used by some centers with outcomes that compare well with those from controlled DCD donors [11]. Following renal transplantation, a period of 'delayed graft function' is acceptable because it is possible to support the patient with dialysis whilst the transplanted organ recovers from the ischemic injury; an equivalent delay in initial function of a transplanted liver is fatal without urgent re-transplantation.

2.1.4.4 Viability Assessment

The safety and utility of DCD liver transplantation would be greatly improved by a reliable test to quantify the ischemic/preservation injury and assess viability in order to predict the outcome after transplantation. Even after careful pre-retrieval donor selection, up to 45% of retrieved livers from DCD donors are discarded due to doubts about viability [12]. In the US in 2013 6,449 of 7,137 (90%) of all deceased donor livers retrieved were actually transplanted, falling to 360 of 492 (73%) for livers from DCD donors (SRTR data). In addition to these losses, many DCD donor organs are declined without being retrieved because of concerns about risk factors. An effective means of pre-transplant viability assessment would not only allow greater use of higher risk donors but also minimize the risk of primary non-function by identifying and excluding non-viable organs before subjecting a patient to the risk of surgery.

2.1.4.5 Ex Vivo Reconditioning

A further strategy in the quest to use higher risk donor organs successfully is that of 'reconditioning' after retrieval – using techniques to reverse the injury sustained by the organ before and during the process of retrieval and treating the organ in such a way as to minimize the immediate damage that occurs after transplantation (ischemia-reperfusion injury). Treatment of the organ during preservation has major logistic and ethical advantages over any attempt to achieve the same effects by treating the donor (in many countries therapeutic interventions before declaration of death that are of no potential benefit to the donor are not permitted). Many cytoprotective strategies have been tested in experimental models of transplantation and several have been shown to have therapeutic potential, including various antioxidants, inflammation inhibitors, vasodilating agents, inhibitors of chemotaxis or neutrophil infiltration.

At the moment the flow of oxygenated blood ceases, the supply of oxygen, cofactors, and nutrients stops along with the means of disposal of metabolic waste products. Anaerobic metabolism continues (at a temperature-dependent rate), leading to depletion of energy stores, mainly adenosine tri-phosphate (ATP), with a concomitant build-up of an acidotic milieu. ATP is required for energy-dependent cellular functions, including the integrity of sodium/potassium pumps that maintain electrolyte balance across cell membranes and ATP depletion leads to loss of trans-cellular electrolyte gradients, cell swelling, influx of free calcium, and the subsequent activation of phospholipases. The breakdown of ATP during ischemia also generates substrates for the production of reactive oxygen intermediates on reperfusion and initiates the cascade of ischemic injury. Prevention of ATP depletion is therefore an important target of innovative preservation methods. It has been shown that providing an oxygen supply to the organ can prevent ATP depletion and preserve viability following cardiac arrest in a porcine liver transplant model [13].

2.1.5 Cold Storage

Organs retrieved for transplantation undergo injury at several consecutive stages: 1) warm ischemia prior to preservation, 2) cold preservation injury, 3) ischemic rewarming during surgical implantation and 4) reperfusion injury. These consecutive events lead to a cumulative cellular injury that may not be compatible with recovery after transplantation.

Standard clinical practice involves flushing and cooling the liver in situ with preservation solution; University of Wisconsin [UW] solution is used most commonly although Histidine-Tryptophane-Ketoglutarate [HTK] solution is also widely used. Typically several litres of cold preservation fluid are used both in situ and after removing the organ from the donor and before packing for transport and storage. Additional cooling may be provided by

topical frozen saline slush both in situ and ex situ. After retrieval, the organ is placed in sterile plastic bags for transportation and stored in an ice-box in preservation solution until transplantation. Although the available preservation solutions differ in chemical composition, the function is essentially the same: to prevent cellular swelling and death caused by fluid shifts as the membrane ion-exchange pumps cease operating in the cold environment. Although cold preservation slows metabolism by 1.5- to 2-fold for every 10°C drop in temperature, considerable metabolic activity still occurs at 1°C. This leads to accumulation of metabolic products which act as substrates for metabolism that takes place when the organ is re-perfused with oxygenated blood – the basis of the ischemia-reperfusion phenomenon [14]

In organs retrieved from DCD donors the deleterious effects of cold ischemia are superimposed on the injury sustained during warm ischemia, which causes rapid depletion of ATP. There are some differences in the pattern of injury sustained during warm and cold ischemia; the latter causes initial injury to sinusoidal endothelial cells whereas warm ischemia is more damaging to hepatocytes and cholangiocytes.

Cold storage causes injury to the graft regardless of other factors. It has been known for many years that extended preservation time with cold preservation solution has a deleterious effect on organ viability [15, 16] with a clear correlation between cold-ischemia time and post-operative primary graft function. The rate of primary non-function was reported in a large registry analysis as 5.8% [17], although individual center reports show a range of incidence. Although a strong correlation has been shown between preservation injury and subsequent acute rejection [18], this has not been confirmed in other reports [19]. Preservation injury is a more critical issue in higher risk donor organs - whereas good quality livers can tolerate preservation periods even up to 18 hours, higher risk grafts must be implanted much more quickly in order to reduce the risk of potentially fatal graft dysfunction after transplantation.

2.1.6 Machine Perfusion

Early in the history of organ preservation and transplantation, pioneers in the field investigated machine perfusion. In the first half of the twentieth century, for example, Carrel perfused organs with normothermic, oxygenated serum and demonstrated gross viability for several days [20]. A number of early successful clinical liver transplants carried out by Dr. Thomas Starzl, used machine perfusion with diluted blood under cold hyperbaric conditions [21]. This technique never gained popularity, partly due to its complexity and logistic challenges but also the subsequent introduction of effective cold flush preservation solutions. Subsequent research into extracorporeal machine perfusion largely centered on liver support [22].

2.1.7 Hypothermic Machine Perfusion (HMP)

It has been demonstrated experimentally by several groups that hypothermic machine perfusion of the kidney significantly improves the preservation compared with static cold storage, in terms of immediate graft function and medium-term outcome of deceased kidneys and DCD organs specifically [23, 24]. The mechanism of benefit is not fully understood, but probably relates both to the removal of metabolic products as well as the delivery of oxygen (although in the absence of formal oxygenation or specialist oxygen carrier molecules, oxygen delivery is likely to be limited).

HMP offers the additional benefit of some (limited) assessment of pre-transplant organ viability by measurement of perfusion pressures and also perfusate αGST levels and other biomarkers as a marker of cellular injury. It also provides the potential for local therapeutic intervention. Several groups have claimed advantages with hypothermic machine perfusions in liver transplantation and the first human clinical study of ex-vivo liver HMP has been published [25, 26].

2.1.8 Normothermic Machine Perfusion (NMP)

There is accumulating evidence of the superiority of a more physiological approach using oxygenated blood at normal body temperature. Several studies demonstrate that the quality of preservation can be improved substantially by warm perfusion, by combining the avoidance of cooling with the maintenance of a supply of

oxygen and nutrition [27, 28]. These data are mainly based on organ retrieval, preservation, ex-situ-evaluation and transplantation in large animal models (pigs). In the experimental setting normothermic perfusion has been shown to resuscitate porcine livers subjected to 60 to 90 minutes of warm ischemia [14, 29]. Warm perfusion has the added advantage of allowing more effective viability assessment of the organs while on the circuit (using multiple perfusion dynamic and biochemical parameters), contrasting with the more limited parameters available on the hypothermic circuit [27]. Preclinical liver transplant experiments in the pig model from a number of centers [27, 28, 30] have shown that the normothermically preserved liver can be transplanted reliably and successfully after warm ischemic injuries that do not allow survival using cold preservation. If these results were translated into clinical practice, this would generate a large new source of donor organs.

Normothermic perfusion has been shown as feasible in the setting of human renal transplantation [31], with a potential benefit of a reduction in the rate of delayed graft function in organs retrieved from extended criteria donors [32].

2.1.9 Preclinical Investigations

The experimental precursor to the proposed normothermic perfusion system was developed more than 15 years ago and the method of perfusion of the isolated pig liver with autologous blood has since been extensively tested and refined, although the overall design of the perfusion circuit remains unchanged [33]. The circuit incorporates a centrifugal pump, membrane oxygenator, and heat exchanger. Arterial perfusion is directly pumped and the portal vein is perfused via a soft-shell reservoir using gravitational force. The addition of various substrates to the perfusion solution enables maintenance of metabolic function [34, 35].

The initial preservation experiments were carried out to compare preservation by warm perfusion with conventional cold preservation [29]. Porcine livers were retrieved and stored for a period of 24 hours, either flushed with UW solution and placed in an icebox or attached immediately to the preservation circuit. Both groups of livers were then reperfused on the circuit for 24 hours (as a surrogate for transplantation) and markers of cellular injury and of synthetic and metabolic liver function were measured. These experiments demonstrated significant superiority of normothermic machine perfusion in terms of hemodynamic, biochemical and histological parameters.

Subsequent experiments investigated the use of oxygenated, normothermic perfusion in an experimental setting that reflected the clinical situation of DCD donor organ retrieval [14]. Perfusion with normothermic blood was again compared with static cold storage after 60 min of warm ischemia. Normothermic perfused livers demonstrated recovery of function by synthetic function, substrate utilization and perfusion hemodynamics. Furthermore these livers displayed less cellular injury as shown by hepatocellular enzymes. In contrast, cold stored livers showed no evidence of viability during reperfusion and massive necrosis on histological examination.

It is recognized that the combination of warm ischemia and conventional cold preservation leads to a poor outcome in DCD liver transplantation [7]. In the experimental setting, it is possible to institute warm perfusion with minimal exposure of the organ to cooling. However, in contrast, the logistics of clinical multi-organ retrieval in a distant donor hospital are complex and would be simplified by a period of cold preservation prior to normothermic preservation. This would enable the liver to be retrieved in the normal way, transported in an ice box and then attached to the perfusion machine once back at the base hospital. This scenario was simulated in the same experimental model by inserting a period of cold preservation prior to normothermic preservation [36]. Porcine livers were subjected to 60 minutes of warm ischemia and then assigned to either normothermic preservation for 24 hours or cold preservation in University of Wisconsin solution for 4 hours followed by 20 hours normothermic preservation to achieve a total preservation time of 24 hours [36]. Livers that underwent normothermic preservation throughout had superior bile production, metabolic activity (base deficit and greater glucose use), and less hepatocellular damage (transaminase levels), and sinusoidal endothelial cell dysfunction (hyaluronic acid). The histology of livers that had been exposed to 4 hours of cold

preservation before normothermia showed more necrosis and destruction of architecture. A similar study investigated 60 minutes of warm ischemia followed by 60 minutes of cold preservation before 23 hours of normothermic perfusion [37]. This also showed evidence of increased hepatocellular injury, sinusoidal cell injury, but no detriment in terms of protein synthesis (factor V), bile production or histological features. These studies, therefore, demonstrated the need for the warm preservation device to be transportable so that normothermic preservation can be instituted with a minimal period of cooling at the time of organ retrieval.

In order to confirm these results in a preclinical model of organ transplantation, a series of liver transplants in a pig model was performed [27]. In these experiments pig livers were cold-preserved or warm-preserved (using the same machine perfusion methodology as before) for either 5 hours or 20 hours, followed by liver transplantation. As a model of DBD and DCD clinical scenarios, organs were cold-perfused *in situ* either at the time of cessation of circulation (as in a DBD organ donation) or after 40 and 60 minutes of warm ischemia (simulating DCD organ donation). The two preservation times were selected because 5 hours is comfortably within, and 20 hours substantially beyond, the limit of the conventional cold preservation technology in pigs (in which a generally accepted limit for survival is 12 hours). Similarly the 40 and 60 minute periods of warm ischemia are considerably longer than would be acceptable in current clinical practice where warm ischemia rarely exceeds 30 minutes. Indeed, success at 40 minutes would raise the realistic prospect of transplantation of donor livers from uncontrolled DCD donors.

There was no difference in outcome between the two groups at 5 hours of preservation. After 20 hours of preservation, there were significant advantages consistently in warm compared to cold preservation of both DBD and DCD organs. These advantages applied to postoperative enzyme release and animal survival. Notably, in the 20 hour warm-preserved groups, there was no difference in survival or postoperative transaminase levels in recipients of DBD compared to DCD (40 minute warm ischemia) donor organs (86% versus 83%). At 60 minutes of warm ischemia and 20 hours normothermic preservation, however, there were no survivors.

Analysis of hemodynamic, synthetic and metabolic parameters showed that those groups of livers that subsequently went on to successful transplant were predictable before transplantation on the basis of portal flow/pressure, acid-base homeostasis and several other biochemical parameters [27]. It may be concluded that normothermic perfusion, in this context, is not only a more effective means of organ preservation than conventional cold storage, but also that this method can be configured to provide an effective means of viability assessment [38].

The prototype version of the automated clinical investigation device has been tested and demonstrated to be effective during pre-clinical studies in which human livers, discarded as unsuitable for transplantation, were perfused for 24 hours. 13 such livers were perfused with human blood and the perfusion characteristics and control algorithms have been shown to be equally applicable to human as to pig livers (manuscript in preparation). More recently, the clinical trials device has been tested, using livers declined for clinical transplantation, and all key functional aspects of the device shown to be operational, including particularly transport to the donor hospital, automation and 24 hour perfusion.

2.2 Phase 1 Clinical Trial Data

A pilot clinical trial was opened at King's College Hospital in 2012 and extended to the Queen Elizabeth Hospital, Birmingham in 2013. Between February and December 2013, 20 subjects underwent liver transplantation using donor organs preserved throughout by normothermic perfusion. Sixteen livers (80%) were from DBD and 4 (20%) were from DCD (Maastricht category III) donors. The indication for transplantation was chronic liver failure except one recipient who underwent re-transplantation for hepatic artery thrombosis. The underlying etiology of liver disease was hepatitis C virus infection (n=6), alcoholic liver disease (n=5), primary sclerosing cholangitis (n=3), primary biliary cirrhosis (n=2), α1-antitrypsin deficiency (n=1), non-alcoholic steatohepatitis (n=1), chronic autoimmune hepatitis (n=1) and other cholangiopathic disease (n=1). Two matched control subjects were identified for each test subject using pre-set criteria. The median recipient MELD score was 12 (7 – 27) in NMP vs. 14 (6 – 25) in matched controls.

Median NMP time was 9.31 (range from 3.5 to 18.5) hours. Median cold ischemia time (CIT) in the matched controls was 8.9 (range 4.2 – 11.4) hours. The period of NMP was governed by the logistic considerations (mainly other transplants). There was evidence of stable hemodynamics, synthetic and metabolic function throughout all perfusions with maintenance of pH between 7.2 and 7.4 (unsupported). Bile production commenced after the first hour and was maintained, with an upward trend, throughout NMP. Hepatic arterial and portal venous flows were consistent throughout.

Median ITU and hospital stays were similar between the test and control groups overall and when analyzed as DBD and DCD subsets. All grafts and subjects in the NMP group survived the first 30 days but 1 recipient of a DBD liver in the matched control group died on day 0 from a cardiovascular event. There was no primary non-function in either group. Three subjects (15%) demonstrated EAD in the NMP group compared to 9 (22.5%) in the control group. This difference was more pronounced in the DCD subset (1 (25%) vs. 4 (50%) subjects). EAD in the NMP group was due to: day 7 bilirubin of 211 (liver 8, donor age 77); peak AST of 2158 (liver 15 pre-retrieval AST of 1300); peak AST of 4681 (liver 16, DCD, age 53, WIT 27 minutes). There was a statistically significant difference in peak AST levels (417 vs. 902, p=0.034), numerically more pronounced in the DCD cohort (422 vs. 1894, p=0.283). In all these cases, perfusion parameters were stable with good acid-base maintenance (indicators of good outcome). Postoperatively, all subjects made good recoveries.

3. OBJECTIVES

3.1 HYPOTHESIS

Normothermic machine perfusion (NMP) is superior to static cold storage (SCS) of human liver allografts for reduction of preservation injury.

3.2 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.2.1 PRIMARY OBJECTIVE

To compare the effect of NMP to SCS in the prevention of preservation injury and graft dysfunction, as measured by early allograft dysfunction (EAD).

3.2.2 PRIMARY OUTCOME MEASURE/ENDPOINT

The severity of immediate graft injury as measured by early allograft dysfunction (EAD) [40]. The study will be powered to demonstrate a reduction in EAD from 25% to 10% in NMP versus SCS. EAD is a binary outcome defined by the presence of one of the following 3 outcomes:

1. Serum bilirubin ≥ 10 mg/dL at day 7 post-transplant
2. International normalized ratio ≥ 1.6 at day 7 post-transplant
3. ALT or AST > 2000 IU/L within the first 7 days post-transplant

	Objectives	Outcome measures/endpoints
Primary	To compare the effect of NMP to SCS in preventing preservation-related graft injury	Severity of immediate graft injury as measured by early allograft dysfunction (EAD).

Secondary	To compare graft and subject survival between NMP and SCS livers.	Primary non-function rates: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation. Graft survival rates at 30 days, 3 months, and 6 months following transplantation. Subject survival rates at 30 days, 3 months, and 6 months following transplantation.
	To compare evidence of post-reperfusion syndrome between NMP and SCS livers on transplantation.	Assess mean arterial pressure (MAP) pre- and post-reperfusion and requirement for vasopressor use.
	To compare biochemical liver function between NMP and SCS livers.	Bilirubin, GGT, ALT, AST, ALP and INR at days 1-7, day 30, month 3, and month 6 post-transplant. Lactate at days 1-7 while the subject is in ICU.
	To compare evidence of ischemia-reperfusion injury between NMP and SCS livers.	Post-reperfusion biopsies will be compared to baseline pre-reperfusion biopsies and graded according to standard histological criteria.
	To compare evidence of biliary complications between NMP and SCS livers.	Incidence of biliary investigations and/or interventions between 7 days and 6 months post-transplant.
	To assess the feasibility and safety of NMP as a method of organ storage and transportation.	Incidence of livers randomized but not transplanted and reasons for not-transplanting.
	To compare organ utilization between NMP and SCS.	Incidence of one or more of the following per randomized liver: (i) EAD; (ii) discard (non-transplant) of a retrieved liver; (iii) primary non-function.
	To assess the health economic implications of normothermic liver perfusion.	Logistical and healthcare costs (length of stay in ICU and hospital) and quality of life measures.

4. TRIAL DESIGN

This is a randomized controlled, non-blinded, clinical trial comparing SCS versus NMP for organ preservation prior to liver transplantation. Following assessment of donor and recipient eligibility and recipient informed consent, the donor liver will be randomized to either NMP or SCS. At the end of preservation, the liver will be transplanted and the enrolled recipient subject managed according to standard local practice and protocols.

Subjects are considered enrolled in the study when there is an attempt to transplant a randomized liver. Enrolled subjects who are transplanted with a randomized liver will participate in the study for a duration of 12 months. If there is an attempt to transplant a randomized liver, but the liver is ultimately not transplanted, the

subject will be followed for 30 days following the attempt for adverse events and will then be exited from the study. Subjects who are matched to a liver that is randomized, but there is no attempt to transplant the liver will not be considered enrolled. An attempt to transplant a liver is considered when there is knife-to-skin contact in the operating room during the recipient transplant procedure. A subject is considered to be transplanted when there is reperfusion of the donor liver in the recipient subject.

If the intended original recipient did not receive the randomized liver, the liver will be offered to the next recipient in accordance to the UNOS matching sequence. All attempts by the investigational team will be made to allocate the randomized liver to a local recipient who has consented and meets inclusion/exclusion criteria for the study. In situations where this is not possible, emergency use procedures may be followed in accordance to FDA regulations should the liver be randomized to the investigational device arm (NMP).

Primary and secondary outcomes will be analyzed and reported after all available transplanted subjects have completed the 6 month post-transplant follow-up visit. Subjects will continue to be followed for 12 months post-transplant for safety. Upon completion of all 12 month post-transplant follow-up visits, a final clinical study report will be completed and all subjects exited from the study.

5. Participant Identification

5.1 Study Setting

Recruitment will take place in up to 15 investigational centers which are UNOS member liver transplant centers.

5.2 Trial Participants

Participants will be 18 years of age or older and active on the waiting list for liver-only transplantation at any of the participating transplant centers.

5.2.1 CMS Reimbursement

Based on the target study population, approximately 30% of subjects enrolled will utilize CMS/Medicare reimbursement during their participation. It is not anticipated that the device under investigation will treat a Medicare population different than the demographics found in the investigators' general population for this same condition, including populations eligible for Medicare due to age (e.g., 65 years or older), disability, or other eligibility status. The demographics of recipients outside of age alone are found to be similar between Medicare and Non-Medicare populations. According to the American Liver Foundation, at least 30 million people, or one in 10 Americans, have some form of liver disease. More than one million Americans are infected with hepatitis B and four million Americans have hepatitis C. In addition, about 21,000 Americans are diagnosed with primary liver cancers, one of the few cancers on the rise in the U.S. The Centers for Disease Control and Prevention (CDC) stated that more than 75 percent of adults with hepatitis C are born between 1945 and 1966 and it is estimated that one in every 33 “baby boomers” has viral hepatitis and are five times more likely to have hepatitis C. While these numbers indicate a rise in the incidence of liver disease, it is anticipated the number of liver transplants will also rise in both the Medicare and Non-Medicare populations. The number of liver transplants has increased by 10% in all ages and 7.3% in the Medicare population in the last ten years. The recipient outcome measures are not only related to the underlying conditions of the recipient but also the donor. Several demographic measures (e.g., age, weight, race/ethnicity, functional status, geography, etc.), cause of death, function of liver, and recovery time and logistics will impact the outcome of the recipient. Thus the results of the study are expected to be generalizable to the Medicare eligible population and a logistic regression model will be used to examine the possible explanatory effect of Medicare recipient status.

5.3 Eligibility Criteria

All eligibility criteria must be met at the time of a recipient being matched to a donor and the donor liver receiving a randomization assignment.

5.3.1 Donor Criteria

5.3.1.1 Donor Inclusion Criteria

1. DBD donor aged 40 years or greater
2. DCD donor aged 16 years or greater
3. Liver allograft from donation after brain death (DBD) or donation after circulatory death (DCD) donors

5.3.1.2 Donor Exclusion Criteria

1. Living donor liver
2. Liver intended for split transplant
3. Liver which Investigator is unwilling to randomize to either arm

5.3.2 Recipient Criteria

5.3.2.1 Recipient Inclusion Criteria

1. Subject is 18 years of age or greater
2. Subject is registered as an active recipient on the UNOS waiting list for liver transplantation
3. Subject, or legally authorized representative, is able and willing to give informed consent and HIPAA authorization
4. Subject is able and willing to comply with all study requirements (in the opinion of the Investigator)

5.3.2.2 Recipient Exclusion Criteria

1. Subject **requiring all of the following** at the time of transplantation:
    a. Oxygen therapy via a ventilator/respirator
    b. Inotropic support
    c. Renal replacement therapy
2. Subject has acute/fulminant liver failure (UNOS status 1A)
3. Subject undergoing simultaneous transplantation of more than one organ (e.g., liver and kidney)
4. Subject is pregnant (as confirmed by urine or serum pregnancy test) or nursing
5. Concurrent enrollment in another clinical trial. Subjects enrolled in clinical trials or registries where only measurements and/or samples are taken (NO TEST DEVICE or TEST DRUG USED) are allowed to participate.

6. Trial Procedures

The participant timeline is illustrated in Appendix A1. The following section provides details of this timeline and all study procedures.

6.1 Recruitment

The emergency nature of liver transplantation means that once a potential recruit/study subject is called in for a transplant there will only be a 3-4 hour window for the consent and screening process to occur. This does not allow sufficient time for the potential subject to fully consider his/her participation in the study. For this reason, all patients who fulfil the entry criteria and who are on the UNOS waiting list for liver transplantation at the participating centers should be provided with full information either during a routine clinic appointment, inpatient admission, or a mailed copy of the Informed Consent Form in accordance with local IRB policy. Informed consent will then be requested at the time the patient is admitted for transplantation.

Alternatively, depending on local factors (including the size of waiting list) it may logistically be possible for patients to be informed and to provide consent while on the waiting list (in advance of the donor organ becoming available).

6.2 Informed Consent

6.2.1 Recipient Consent

Prior to being enrolled in the clinical study, subjects (or their legally authorized representative) must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them and per the requirements in 21 CFR Part 50.

Detailed information will be given both verbally and in the informed consent document that includes information about the study and the consent form will be prepared and given to the subject. This document will contain all the elements required by the 21 CFR Part 50.25 regulation for subject information and informed consent, and any additional elements required by local regulations. The document must be in a language understandable by the subject. Subjects are free to withdraw consent at any time, irrespective of their initial consent.

After reading the informed consent document, the participant (or their legally authorized representative) must sign and date the current approved version of the informed consent form before any study specific procedures are performed. Written and verbal versions of the informed consent form will be presented to the participants detailing the exact nature of the study, the implications and constraints of the protocol, and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. The participant will be allowed as much time as possible to consider the information, and the opportunity to question the Investigator, or other members of the clinical or research team, to decide whether to participate in the study.

The study coordinator at each center will maintain a list of consenting subjects.

Any use of the device without obtaining informed consent shall be promptly reported (no later than 5 working days) to the US Sponsor and the IRB.

A copy of the signed consent document must be offered to the subject. The original signed consent document will be retained by the Investigator.

The Investigator will not perform any exams or testing specifically required only for the clinical study until valid consent has been obtained.

The Investigator may inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

6.2.2 Donor Consent

Authorization from the donor family will be obtained per standard practices from the retrieving Organ Procurement Organization (OPO).

During the course of the study, donor details will be kept anonymous (specific study identification codes will be used for each study donor). Donor data will only be made available to authorized staff of the study Sponsor, its authorized representatives, and the FDA.

6.3 Screening and Eligibility Assessment

6.3.1 Recipient Assessment

All consented subjects on the transplant waiting list in participating centers will be screened for suitability for transplantation; beyond fulfilling the inclusion/exclusion criteria listed above, further screening assessment is not required as part of the trial and the subject will be followed per standard of care. Upon the offer of a suitable donor organ, consent will be requested as described in section 6.2.1, if it has not already been obtained.

6.3.2 Donor Assessment

On reciept of an organ offer, the local recipient investigative site staff will ascertain baseline demographic information from the offering OPO to assess eligibility of the liver for inclusion in the trial. The decision whether to transplant a liver in this study remains solely the responsibility of the Investigator.

6.4 Randomization and Blinding

6.4.1 Sequence Generation

Donor livers will be randomly assigned to NMP or SCS with 1:1 allocation as per a computer generated randomization schedule using variable block randomization using the following stratification factors: participating (recipient) center and by donor type (DBD or DCD). The randomization schedule will be created by the study statistician, and will remain confidential.

6.4.2 Allocation Concealment Mechanism

Allocation concealment will be ensured by use of central computerized randomization. Allocation will not be revealed until the subject has been recruited to the trial and donor and recipient inclusion/exclusion criteria have been recorded. Random permuted block length will be used; block sizes will not be disclosed.

6.4.3 Implementation

Prior to randomization, the local Investigator will confirm the availability of the investigational *metra®* device. Once informed consent has been verified from the potential recipient of an organ and recipient and donor inclusion and exclusion criteria has been confirmed, the local recipient investigative site staff will proceed with randomization. Recipients will be considered enrolled in the study once a randomized liver has been assigned and there is an attempt to transplant the randomized liver.

6.4.4 Blinding/Masking

While it is not possible to blind the local Investigators to the method of organ preservation, Histopathologists at the central laboratory interpreting the biopsy specimens will remain blinded to the randomization group to the extent possible.

6.5 Pre-Study Baseline Assessments

Pre-study baseline assessments to be completed include the following:

6.5.1 Donor Demographics

Retrieving OPOs/investigational sites will collect donor information per the Organ Procurement and Transplantation Network (OPTN) Policy.

In addition to the OPTN Policy requirements listed in Section 2.11.B, the following will be collected:

1. Age

2. Sex
3. Race
4. Ethnicity
5. Cause of death (CVA, hypoxia, trauma, other)
6. Type of donor (DBD, DCD)
7. Donor height
8. Donor risk index (DRI) [3]
9. Last and peak serum AST
10. Last and peak serum sodium
11. Last and peak GGT
12. Length of ICU stay
13. BMI

6.5.2 Recipient Demographics

Recipient demographics to be recorded will include, but not be limited to, the following:

1. Age
2. Sex
3. Race
4. Ethnicity
5. Etiology of liver disease
6. Indication for transplant
7. MELD score
8. UNOS status
9. BMI
10. Relevant medical history
11. Relevant social history

6.5.3 Quality of Life Questionnaire (EQ-5D-5L)

Recipient will complete the Quality of Life Questionnaire (measured by means of the EQ-5D-5L).

6.6 Trial Interventions

6.6.1 NMP Group (Investigational metra® Device)

If the liver is randomized to the NMP group, arrangements will be made to transport the device to the donor hospital (see section 7.6). The recipient coordinator will also arrange for 3 units of donor-type red blood cells to be available for use in the OrganOx *metra®* device. Following the routine retrieval procedure at the donor hospital the liver will be placed in ice-cold perfusion solution (according to local protocol) on the back-table, and prepared for cannulation. The procedure for preparing the device for use and placing the organ on the device is described in detail in the *metra®* Instructions for Use (IFU) document. The device is then transported to the recipient transplant center per local logistics. The procedure for removing the liver from the device is also described in the IFU. Transplantation and reperfusion of the liver proceed as per the usual practice of the transplanting center. The duration of machine perfusion will be dictated by logistics and local policy, but should not be less than 4 hours or more than 24 hours. At the end of the perfusion, a sample of perfusate will be taken for analysis of free hemoglobin and will be processed in accordance with the laboratory manual for analysis.

If cannulation proves impossible, the liver will be transported using standard static cold storage as described below. Results will be analyzed in the randomized group per the principals of intention-to-treat.

At the request of the Principal Investigator from the site, a representative from OrganOx that is trained on the OrganOx *metra®* may:

- Provide technical expertise on the use and functionality of the OrganOx *metra®* device and testing that will be conducted as part of the study
- Be present during the procurement of the donor liver and recipient procedure
- Prepare the system for use and assist with use of the OrganOx *metra®* device

6.6.2 SCS Group

Following the routine retrieval procedure, the liver will be placed in ice-cold perfusion solution (according to local protocols) on the back-table, followed by storage in cold perfusion solution within an ice box. The organ will be transported to the recipient center per local logistics, and removed from storage prior to transplantation for standard back-table preparation. The duration of cold storage will be dictated by logistics and local policy.

6.6.3 Recording of Operative and Perfusion Parameters

The procurement and transplant research teams will record the following data:

6.6.3.1 Donor Timings

6.6.3.1.1 DBD Donor Timings

The following times will be recorded for DBD donors:

1. Brain Death declaration (date and time)
2. Cessation of donor circulation (cross clamp date and time)
3. Start of cold perfusion
4. Liver removal and placement on ice
5. Removal from ice (recipient operation)
6. Portal reperfusion
7. Arterial reperfusion

6.6.3.1.2 DCD Donor Timings

The following times will be recorded for DCD donors:

1. Withdrawal of support (oxygen therapy and/or inotropic if applicable)
2. Onset of functional warm ischemia (SBP < 50 mmHg)
3. Time of Cardiac Death
4. Start of cold perfusion
5. Liver removal and placement on ice
6. Removal from ice (recipient operation)
7. Portal reperfusion
8. Arterial reperfusion

6.6.3.1.3 DBD/DCD Donor Timings Randomized to NMP

In addition to all above parameters, the following will be recorded for all donor livers randomized to NMP:

1. Initiation of normothermic machine preservation
2. Cessation of normothermic machine perfusion (cold flush)

6.6.3.2 Preservation Parameters for All Randomized Livers

The following preservation parameters will be collected for all randomized livers:

1. Degree of steatosis (graded mild, moderate, severe) – surgeon's assessment at time of retrieval
2. Quality of *in-situ* perfusion (graded poor, moderate, good)
3. Perfusion parameters for NMP livers (these are logged automatically by the device):
    a. Arterial, and IVC pressures (mmHg)

b. Arterial, portal and caval flow rates (ml/min)
c. pO_2, pCO_2 and pH
d. Blood temperature (^oC), glucose (mmol/L) and bile production (ml/h)

4. Perfusate ALT and AST (for NMP livers)
5. Lactate values and time obtained at the following time points (for NMP livers):
    a. After approximately 15 minutes of NMP
    b. Following arrival at the transplant hospital
    c. Following the end of NMP
6. Perfusion solution used for *in situ* and back-bench perfusion (all livers)
   Perfusion solution used for organ transport (SCS livers only)
7. Glucose values measured using i_STAT or similar FDA approved diagnostic device at the following time points (for NMP livers):
    a. Approximately after 15 minutes of NMP
    b. Approximately every 4 hours thereafter during perfusion

At the end of preservation a sample of perfusate/storage solution will be taken for microbiological culture (SCS and NMP groups).

6.6.3.3 Operative Parameters

These will include:

1. Total operative time: defined as time from knife-to-skin to wound closure.
2. Anastomotic time (secondary warm ischemia): defined as time between removal of organ from ice (SCS) or perfusion device (NMP) to organ reperfusion (whichever is first of portal or arterial)
3. Occurrence of post-reperfusion syndrome (defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than one minute during the first five minutes after reperfusion [41, 42])
4. Use of vasopressors prior to and after reperfusion
5. Intraoperative transfusion of blood and blood products (measured in units)
6. The use of veno-venous bypass or porto-caval shunts
7. Type of caval anastomosis: end-to-end caval replacement, piggyback (end-side or side-side)

6.6.3.4 Histological Parameters

Graft biopsies will be completed on both groups and will be taken:

1. Pre-Storage (Baseline): Before being placed for storage in either NMP or SCS
2. Post-Storage:
    a. Following storage in either NMP or SCS
    b. Immediately prior to abdominal closure

The post-storage biopsies will be compared to the pre-storage (baseline) biopsy, examined for evidence of reperfusion injury, and graded according to standard histological criteria [39, 43]. For this analysis a central core laboratory will be used where the histopathologist will be blinded to the method of storage.

6.7 Concomitant Care

All other aspects of the retrieval procedure will be carried out according to local policies and national guidelines.

Recipient management including the transplantation procedure, postoperative care, immunosuppression and other medications, and post-transplant monitoring will follow local protocols.

6.8 Study Visits

6.8.1 Inpatient Stay

Subjects will be assessed daily by their clinical team and managed according to standard local protocols (refer to Appendix A1 for Visit Windows).

6.8.1.1 Biochemical Assessments

The following biochemical outcomes will be recorded where the first measurements should be taken between 12 and 24 hours of reperfusion:

1. Daily samples for the first 7 days post-transplant, to include:
    a. Total serum bilirubin (measured in mg/dL)
    b. Serum alkaline phosphatase (ALP; measured in IU/L)
    c. Serum gamma-glutamyl transferase (GGT; measured in IU/L)*
    d. Serum aspartate transaminase (AST; measured in IU/L)
    e. Serum alanine aminotransferase (ALT; measured in IU/L)
    f. PT/International normalized ratio (INR)
    g. Serum albumin (measured in g/dL)*
    h. Serum creatinine (measured in mg/dL)*
2. Daily serum lactate (measured in mmol/L) while admitted to ICU*

Only collected if Standard of Care

6.8.1.2 Additional Assessments

The following additional data will be collected and recorded:

1. Length of stay in high level care (ICU) (days)
2. Total length of hospital stay (days)
3. Total number of ventilation days post-transplant
4. Requirement for renal replacement therapy (Hemodialysis (HD), Hemodiafiltration (HDF), Hemofiltration (HF))
5. Primary non-function: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation.

6.8.1.3 Safety Assessments

The following safety data will be collected and recorded:

1. Recipient infection (documented positive microbiological culture, pathological lesion, or clinical criteria met)
2. Biopsy-proven acute rejection episodes
3. Biliary investigations (MRCP, ERCP, PTC)
4. Biliary interventions (surgical, radiological, or endoscopic)
5. Hepatic vascular complications (e.g., hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
6. Acute Hemorrhage (requiring transfusion of two or more units of red blood cells)
7. Reoperation for graft related complications
8. Adverse event(s)

6.8.1.4 Immunosuppression

Details of induction and maintenance immunosuppression at day 7 post-transplant will be recorded.

6.8.2 Day 30 Post-Transplant Procedure

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate (refer to Appendix A1 for Visit Windows).

6.8.2.1 Biochemical Assessments

The following biochemical outcomes will be recorded at day 30 post-transplant:

1. Total serum bilirubin (measured in mg/dL)
2. Serum alkaline phosphatase (ALP; measured in IU/L)
3. Serum gamma-glutamyl transferase (GGT; measured in IU/L)*
4. Serum aspartate transaminase (AST; measured in IU/L)
5. Serum alanine aminotransferase (ALT; measured in IU/L)
6. PT/International normalized ratio (INR)
7. Serum albumin (measured in g/dL)*
8. Serum creatinine (measured in mg/dL)*

Only collected if Standard of Care

6.8.2.2 Additional Assessments

The following additional data will be collected and recorded:

1. Graft and subject survival at day 30 post-transplant
2. Requirement for renal replacement therapy (HD, HF, HDF) for more than a total of five days
3. Length of hospital stay
4. Readmissions
5. Healthcare resource use (by means of hospital admissions data and medical records)

6.8.2.3 Safety Assessments

The following safety data will be collected and recorded:

1. Recipient infection (documented positive microbiological culture, pathological lesion, or clinical criteria met)
2. Biopsy-proven acute rejection episodes
3. Biliary investigations (MRCP, ERCP, PTC)
4. Biliary interventions (surgical, radiological, or endoscopic)
5. Hepatic vascular complications (e.g., hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
6. Reoperation for graft related complications
7. Adverse event(s)

6.8.2.4 Immunosuppression

Details of maintenance immunosuppression at day 30 post-transplant will be recorded.

6.8.3 Month 3 Post-Transplant Procedure

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate (refer to Appendix A1 for Visit Windows).

6.8.3.1 Biochemical Assessments

The following biochemical outcomes will be recorded at month 3 post-transplant:

1. Total serum bilirubin (measured in mg/dL)
2. Serum alkaline phosphatase (ALP; measured in IU/L)
3. Serum gamma-glutamyl transferase (GGT; measured in IU/L)*
4. Serum aspartate transaminase (AST; measured in IU/L)

5. Serum alanine aminotransferase (ALT; measured in IU/L)
6. PT/International normalized ratio (INR)
7. Serum albumin (measured in g/dL)*
8. Serum creatinine (measured in mg/dL)*

Only collected if Standard of Care

6.8.3.2 Additional Assessments

The following additional data will be collected and recorded:

1. Graft and subject survival at month 3 post-transplant
2. Requirement for renal replacement therapy (HD, HF, HDF) for more than a total of five days
3. Readmission(s)
4. Healthcare resource use (by means of hospital admissions data and medical records).

6.8.3.3 Safety Assessments

The following safety data will be collected and recorded:

1. Recipient infection (documented positive microbiological culture, pathological lesion, or clinical criteria met)
2. Biopsy-proven acute rejection episodes
3. Biliary investigations (MRCP, ERCP, PTC)
4. Biliary interventions (surgical, radiological, or endoscopic)
5. Hepatic vascular complications (e.g., hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
6. Reoperation for graft related complications
7. Adverse event(s)

6.8.3.4 Immunosuppression

Details of maintenance immunosuppression at month 3 post-transplant will be recorded.

6.8.4 Month 6 Post-Transplant Procedure

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate (refer to Appendix A1 for Visit Windows).

6.8.4.1 Biochemical Assessments

The following biochemical outcomes will be recorded at month 6 post-transplant:

1. Total serum bilirubin (measured in mg/dL)
2. Serum alkaline phosphatase (ALP; measured in IU/L)
3. Serum gamma-glutamyl transferase (GGT; measured in IU/L)*
4. Serum aspartate transaminase (AST; measured in IU/L)
5. Serum alanine aminotransferase (ALT; measured in IU/L)
6. PT/International normalized ratio (INR)
7. Serum albumin (measured in g/dL)*
8. Serum creatinine (measured in mg/dL)*

Only collected if Standard of Care

6.8.4.2 Additional Assessments

The following additional data will be collected and recorded:

1. Graft and subject survival at month 6 post-transplant
2. Requirement for renal replacement therapy (HD, HF, HDF) for more than a total of five days
3. Readmission(s)

4. Healthcare resource use (by means of hospital admissions data and medical records).
5. Quality of life (measured by means of the EQ-5D-5L questionnaire).

6.8.4.3 Safety Assessments

The following safety data will be collected and recorded:

1. Recipient infection (documented positive microbiological culture, pathological lesion, or clinical criteria met)
2. Biopsy-proven acute rejection episodes
3. Biliary investigations (MRCP, ERCP, PTC)
4. Biliary interventions (surgical, radiological, or endoscopic)
5. Hepatic vascular complications (e.g., hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
6. Reoperation for graft related complications
7. Adverse event(s)

6.8.4.4 Immunosuppression

Details of maintenance immunosuppression at month 6 post-transplant will be recorded.

6.8.5 Month 12 Post-Transplant Procedure

This visit will, where possible, coincide with a routine outpatient appointment, but may be completed remotely via telephone. If the recipient is an inpatient, assessment will be made in hospital where appropriate (refer to Appendix A1 for Visit Windows).

1. Graft and subject survival at month 12 post-transplant
2. Requirement for renal replacement therapy (HD, HF, HDF) for more than a total of five days
3. Readmission(s)
4. Healthcare resource use (by means of hospital admissions data and medical records).

6.9 Participant Retention

All transplanted subjects completing the 12 month follow-up assessment will be regarded as having completed the study. All subjects will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments so those subjects do not incur extra financial costs (e.g., travelling costs) as a result of study participation.

Every effort should be made to secure follow-up data on enrolled subjects. A subject will be considered Lost to Follow-Up if there are three documented contact attempts made (via phone, email, or certified mail) requesting follow-up with no response.

It is understood that study subjects may withdraw consent for study participation at any time irrespective of their reasons. The Investigators may also withdraw a subject from the study in order to protect the subject's safety and/or if they are unwilling or unable to comply with the required study procedures.

In the event of a subject withdrawing from the trial, the reason for withdrawal must be documented on the eCRF.

6.10 Definition of the End of the Trial

The investigational trial will end after completion of the 12 month post-transplant follow-up visit for all available transplanted subjects in the trial and a final study report will be generated. Primary and secondary outcomes will be analyzed and reported after all available transplanted subjects have completed the 6 month post-transplant follow-up visit. Subjects will continue to be followed for 12 months post-transplant for safety.

Upon completion of all 12 month post-transplant follow-up visits, a final clinical study report will be completed and all subjects exited from the study.

The procedures for the early termination/suspension of the study at one or more clinical sites (as a consequence of safety or compliance concerns) are detailed in section 8.3.

7. THE ORGANOX METRA® DEVICE

7.1 DEVICE DESCRIPTION

7.1.1 THE ORGANOX METRA® BASE UNIT

The OrganOx *metra®* normothermic perfusion device incorporates a centrifugal pump, an oxygenator, oxygen concentrator, heat exchanger, reservoir, flow probes, pressure sensors, infusions and blood gas analyzer together with tubing and connector components. The device is comprised of three main components:

1. Reusable base unit which contains software and hardware
2. Disposable plastic circuit
3. Set of perfusion solutions suitable for 24 hours of perfusion

7.1.2 DISPOSABLE SET

The disposable set used with the core base unit of the OrganOx *metra®* contains all the disposables used with each organ recovery on the *metra®* and comprises:

1. A tubing set, including a blood reservoir, perfusion lines, a blood oxygenator/heat exchanger and centrifugal pump-head together with flow and pressure sensors.
2. A liver bowl which is pre-connected to the tubing set to contain the organ while on the device.
3. Cannulae for the celiac artery, portal vein, and inferior vena cava with easy connection attachment to the perfusion circuit.
4. A cannula and connection point for bile collection
5. Blood gas sensors for monitoring pO_2, pCO_2, and pH by means of on-line blood gas analysis.

7.1.3 PERFUSION SOLUTIONS

For the present study none of the additives necessary to perfuse and maintain the organ during the storage process, with the exception of sodium taurocholate, are included and will be sourced locally (OrganOx will provide a list of recommended suppliers in the Instructions for Use (IFU) document). These solutions include bolus injections (given at the start of perfusion) and the maintenance infusions (given throughout perfusion). The primary perfusion fluid for the liver comprises packed red blood cells, supplemented by albumin to normalize the hematocrit and osmolality.

Before connection of the liver the blood-based perfusate is supplemented with:

1. Antibiotic – e.g., Cefuroxime
2. Heparin.
3. Calcium gluconate.

During the perfusion the following are infused at a constant rate:

1. Nutrition – (Clinimix E) amino acids plus electrolytes.
2. Insulin.
3. Bile Salts.
4. Flolan® (epoprostenol Prostacyclin).

The primary fluid for perfusing the organ is packed red cells supplied per local arrangements and supplemented by albumin solution to normalize the hematocrit and osmolality. Further additions are made to

the perfusate to support the liver (in a similar mode to current preservation solutions such as Viaspan® or those used in machine preservation devices such as the Waters RM3® or the Organ Recovery Systems LifePort®). All solutions required will be attached to the circuit during set-up and before the liver is attached. The investigational study team will provide the solutions necessary for perfusion with the *metra®*. Solutions are prepared immediately before the organ is attached to the device and contain sufficient solution for 24 hours of operation, the intended maximum perfusion time for a liver on the device.

Sodium Taurocholate (bile salts) will be prepared in advance by the investigative site's pharmacy in accordance with the Instructions for Use.

7.2 Device Safety

In designing the *metra®*, OrganOx has made every attempt to maintain the current practices of organ retrieval and transplant teams, in order to minimize the risk of complications or errors that would prevent a successful retrieval. From a regulatory standpoint, it is important to note that the *metra®* is an organ preservation system and its use does not involve direct connection to either the donor or recipient at any time.

The device has been designed according to ISO 13485, the standard that stipulates the requirements for a comprehensive management system for the design and manufacture of medical devices. In addition ISO 14971 specifies a process for a manufacturer to identify the hazards associated with medical devices to estimate and evaluate the associated risks, to control these risks, and to monitor the effectiveness of the controls. As part of the development of the device an extensive risk analysis has been undertaken and the risks identified and minimized in accordance with this standard. As a result, any remaining risk can only be investigated in the context of clinical transplant studies.

OrganOx has deliberately designed the operation of the device such that it will require minimal changes to current transplant clinical practice. Also, the perfusion methodology is based on the principle that all the perfusion solutions, additives, and packed red cells must be removed from the organ prior to transplant. Therefore following the completion of the perfusion, the perfusate is flushed out of the organ with HTK solution (or the preferred preservation solution of the transplanting center).

7.3 Device Labelling

All components of the OrganOx *metra®* system (reusable base unit and disposable set) will be labelled "CAUTION Investigational device. Limited by Federal (or United States) law to investigational use". Labelling will also include the Sponsor/Manufacturer name, contact details, and a unique trial identifier.

Additional labelling requirements will be followed in accordance with OPTN Policy Section 16.0.

7.4 Device Accountability

Device accountability will be undertaken at each local site throughout the study for the reusable unit(s) and disposable sets (sterilization/assembly batch number and disposable set number). The manufacturer and lot number for each perfusion solution will also be recorded on the eCRF. The site will maintain a log of the retained unit, disposable sets, and perfusion solutions used throughout the study recording the lot number used against each subject (on the eCRF).

At the end of each procedure the OrganOx *metra®* and any unused disposable and perfusion solutions will be removed from the donor hospital and returned to the investigative site.

7.5 Device Maintenance

Device cleaning and routine maintenance will be the responsibility of the local Investigator storing the device. Full details for cleaning and routine maintenance required will be provided in the Instructions for Use (IFU), and appropriate training will be provided as part of the device training described in section 11.3.

7.6 Device Logistics

7.6.1 Logistical Considerations

A number of logistic factors must be considered in using the OrganOx *metra®*:

1. The device is large, and therefore, may not fit in normal retrieval transportation depending on local procedures.
2. The device has a battery life of 2.5 hours, and so the mode of transport should provide a means of providing AC power.
3. The device requires the manual measurement and entry of perfusate glucose every 4 hours during perfusion.

7.6.2 Logistical Arrangements for Study Sites

The OrganOx *metra®* device will be stored and maintained at the investigative site, or an approved delegated site. When an eligible donor organ is offered to the investigative site for a subject who consents to take part in the study, the recipient investigational study team will use the online randomization tool to enter required information and randomize the liver to either the treatment or control arm.

The recipient investigational study team will then contact the lead retrieval surgeon to inform him/her of the randomization assignment. If the liver has been randomized to machine perfusion, the recipient study team will arrange dispatch of the device to the donor hospital. The investigational study team will also arrange for 3 units of donor-type packed red cells to be available.

8. Data Monitoring and Safety Reporting

8.1 Data Monitoring

8.1.1 Data Safety Monitoring Board

The trial will have a Data Safety Monitoring Board (DSMB), which consists of at least four independent members including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DSMB will periodically review accruing data to safeguard the interests of the trial participants, potential participants, and future patients and assess the safety of the interventions.

A separate DSMB charter will contain full details of the committee and its roles and reporting structure.

8.1.2 Interim Analyses

Interim analyses of primary and secondary outcomes are not planned. These will only be performed if requested by the DSMB on the grounds of participant safety.

An initial safety assessment is planned to occur following the completion of the 30-day follow-up visit for the first 10 subjects enrolled in the study. The initial safety data will be submitted to the FDA to request expanded enrollment in the study.

8.2 Adverse Events

8.2.1 Definitions

An ***Adverse Event (AE)*** is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) whether or not related to the study intervention.

A ***Serious Adverse Event (SAE)*** is defined as an adverse event that:

- Led to death
- Resulted in serious deterioration in the health of the subject that results in:
    - Life-threatening illness or injury, or
    - Permanent impairment of a body structure or a body function, or
    - The need for inpatient care or prolongation of hospitalization, or
    - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.

Planned hospitalization for a pre-existing condition, or a procedure required by the trial protocol, without serious deterioration in health, is not considered a serious adverse event.

An ***Unanticipated Adverse Device Effect (UADE)*** is defined as any adverse device effect which, by its nature, incidence, severity or outcome, has not been identified in section 8.2.3.

8.2.2 Adverse Event Severity Definitions

The following definitions will be used to determine the severity rating for all adverse events:

Mild: awareness of signs or symptoms, that does not interfere with the subject's usual activity or is transient that resolved without treatment and with no sequelae.

Moderate: a sign or symptom, which interferes with the subject's usual activity.

Severe: incapacity with inability to do work or perform usual activities.

8.2.3 Anticipated Adverse Events

General

- Infection (chest, urine, blood, bile, wound, abdominal)
- Fluid collection (abdominal, pleural)
- Rejection
- Renal dysfunction
- Hepatic dysfunction
- Cardiac failure
- Respiratory failure

Events related to the disease / condition / surgery

- Early graft dysfunction
- Admission for suspected rejection
- Occurrence and treatment of abdominal or wound infection
- Respiratory failure requiring mechanical ventilation
- Hospitalization for pre-existing condition that has not deteriorated.
- Clinically significant abnormal laboratory finding or other abnormal assessments that is associated with the disease being studied (unless judged by the Investigator as more severe than expected for the subject's condition).
- Death

The Investigator will exercise his/her medical judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. However, if in the opinion of the Investigator, the frequency or severity of the event is greater than would be expected then it must be reported.

8.2.4 Device Deficiency

A device deficiency is defined as inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include device failures, device malfunctions, and use errors. Device deficiencies resulting in SAEs will be managed as detailed in section 8.2.5.

Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate will also be managed as detailed in section 8.2.5.

8.2.4.1 Device Failures

A device failure has occurred when the device is used in accordance with the IFU, but does not perform as described in the IFU and also negatively impacts treatment of the study subject.

8.2.4.2 Device Malfunctions

A device malfunction occurs when an unexpected change to the device that is contradictory to the IFU is observed, which may or may not affect device performance.

8.2.4.3 Use Errors

A device use error is an act or omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user. Use error includes slips, lapses, and mistakes. An unexpected physiological response of the subject does not itself constitute a use error.

8.2.5 Procedures for Recording Adverse Events

It is the responsibility of the local Investigator to ensure that all adverse events (AEs, SAEs, UADEs, and device deficiencies) occurring during the course of the study are recorded. This may include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the device
- Assessment of relatedness to the transplant procedure
- Severity
- Whether the AE is serious or not
- Whether the AE arises from device deficiency, device malfunction, or use error

Adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects.

It is the responsibility of the local Investigator to collect all directly observed adverse events and all adverse events spontaneously reported by the subject. In addition, each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms.

8.2.6 Reporting Procedures for All Adverse Events

It is the responsibility of the local Investigator to ensure that all adverse events which fall into the categories of SAEs and UADEs are reported to the sponsor and IRB, as required, as soon as possible after becoming aware of the event but no later than 10 working days.

The Investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect during an investigation as soon as possible, but no later than 10 working days after the Investigator first learns of the effect (812.150).

The sponsor shall immediately conduct an evaluation of any unanticipated adverse device effect. If the sponsor determines that an unanticipated adverse device effect presents an unreasonable risk to subjects, sponsor will terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination should occur no later than 5 working days after the sponsor makes this determination and no later than 15 working days after the sponsor first received notice of the effect (812.46(b)).

The sponsor shall conduct an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to FDA and to all reviewing IRB's and participating Investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (812.150).

8.3 Study Suspension or Early Termination

The DSMB or sponsor may recommend suspension or termination of the study either at an individual investigative site or the entire study for significant and documented reasons. An Investigator, IRB, or FDA may suspend or prematurely terminate participation in the study at the investigative sites for which they are responsible. If suspicion of an unacceptable risk to subjects arises during the study, or when so instructed by the IRB or regulatory authorities, the sponsor shall suspend the study while the risk is assessed. The sponsor shall terminate the study if an unacceptable risk is confirmed.

During the Conditional phase, the study will be limited to 20 enrolled subjects. Based on data collected for the first 20 subjects through 30-day follow-up, if the number of subjects experiencing an event (EAD or 30-day PNF or 30-day subject death) is greater in the NMP arm than that in the SCS arm by three the study will be suspended.

The sponsor shall consider terminating or suspending the participation of a particular study site or Investigator in the study if monitoring or auditing identifies serious or repeated deviations on the part of an Investigator.

If suspension or premature termination occurs, the terminating party shall justify its decision in writing and promptly inform the other parties with whom they are in direct communication. The Principal Investigator and sponsor shall keep each other informed of any communication received from either the institutional review board (IRB) and/or a regulatory authority.

If, for any reason, the sponsor suspends or prematurely terminates the study at an individual investigative site, the sponsor shall inform the FDA as appropriate and ensure that the IRB is notified, either by the Principal Investigator or by the sponsor. If the suspension or premature termination was in the interest of safety, the sponsor shall inform all other Investigators.

If suspension or premature termination occurs,

a) The Sponsor/Manufacturer shall remain responsible for fulfilling the obligations from the study protocol and existing agreements for following subjects enrolled in the study as applicable, and
b) The Principal Investigator or authorized designee shall promptly inform the enrolled subjects at his/her study site, if appropriate, and follow written instructions provided by the Sponsor/Manufacturer and the US Sponsor.

9. Statistics

This section provides a summary of the planned statistical analyses; additional detail will be contained in the Statistical Analysis Plan. In any apparent instances of ambiguity or disparity, the terms and procedures of the Statistical Analysis Plan (SAP) govern trial analyses.

9.1 Description of Statistical Methods

9.1.1 General Statistical Considerations

All statistical analyses will be performed using SAS version 9.3 or above (SAS Institute, Cary, N.C.) or another validated statistical software package. Unless otherwise specified, the data will be summarized in tables presenting the mean, standard deviation, and number of subjects in a group for continuous data, or presenting count and percentage analyzed using ANOVA with adjustment for categorical data, as appropriate.

In general, binary data will be assessed using the chi-squared test (or Fisher's Exact, if appropriate) or logistic regression to adjust for potential confounders. Continuous outcomes will be compared using the T-test if normally distributed, or by the Mann-Whitney U test. Time-to-event outcomes will be analyzed using survival analysis methods, such as Kaplan-Meier or Cox proportional hazards regression model with calculation of hazard ratios. Outcomes will be reported with 95% confidence intervals and p-values to 3 decimal places. Unless otherwise specified, a p-value of less than 0.05 will be regarded as statistically significant.

9.1.2 Conditional Phase Analyses

Data will be summarized on initial safety when the first 10 subjects have completed 30-day post-transplant follow-up, although no formal statistical comparisons will be performed. This summary will be strictly for FDA review to continue enrollment in the study.

9.1.3 Study Cohorts for Analyses

A modified intent-to-treat (mITT) analysis will be performed for all outcomes as the primary analysis. Transplanted subjects (a subject who has been reperfused with a donor liver) will be analyzed in the groups to which the liver received is randomly assigned, irrespective of whether the assigned method of preservation is actually used. Practically, this means that if a liver intended by randomization for machine perfusion for any reason undergoes cold storage, it will be analyzed in the assigned machine perfusion group. Because analyses require data post-transplant, the analysis will exclude recipients for whom were matched to a liver that was randomized, but were not transplanted for any reason. In the case of DCD livers in which the retrieval did not proceed, the reason will be documented along with associated DCD recovery time points. In all other cases the reason for the liver not proceeding to transplantation will be documented and a narrative summary of this data performed. In cases of emergency use, where the liver is unable to be transplanted into the initial intended recipient and is instead re-allocated to a recipient that is not enrolled in the study, data will not be collected beyond what is provided to UNOS through the UNET database, and therefore, these subjects will not be included in any outcome analyses that are performed. Recipient safety data on subject and graft survival, in these instances, will be summarized and reported separate from the study cohort on data available up to one-year post-transplant.

As additional, supportive, analyses of the outcomes, a per-protocol (PP) cohort will be considered. This cohort will include all subjects who were followed according to the protocol procedures with no major deviations (beyond not being treated in the arm they were assigned to).

9.1.4 Primary Outcome

The intervention (NMP) will be compared against control (SCS) for all primary and secondary outcomes in the mITT population.

Primary outcome, difference in EAD, will be analyzed using a logistic model with adjustment for stratification factors. The hypothesis is:

H0: $EAD_{NMP} \geq EAD_{SCS}$

HA: $EAD_{NMP} < EAD_{SCS}$

In other words, the EAD for the NMP group is less than the EAD for the SCS group.

EAD is a binary outcome defined by the presence of one of the following 3 outcomes [40]:

1. Serum bilirubin ≥ 10 mg/dL at day 7 post-transplant

2. International normalized ratio ≥ 1.6 at day 7 post-transplant
3. ALT or AST > 2000 IU/L within the first 7 days post-transplant

The primary analysis will be a logistic model, adjusted for participating (recipient) center using SAS PROC LOGISTIC or a similar procedure. Significance for the treatment effect will be assessed by the p-value associated with the F-test statistic for the treatment group assignment.

9.1.5 Secondary Outcomes

The following endpoints will be considered secondary endpoints for the study and will be summarized using the appropriate analyses described in section 9.1.1:

1. To compare graft and subject survival between NMP and SCS livers
2. To compare evidence of post-reperfusion syndrome between NMP and SCS livers on transplantation
3. To compare biochemical liver function between NMP and SCS livers
4. To compare evidence of ischemia-reperfusion injury between NMP and SCS livers
5. To compare evidence of biliary complications between NMP and SCS livers
6. To assess the feasibility and safety of NMP as a method of organ storage and transportation
7. To compare organ utilization between NMP and SCS livers
8. To assess the health economic implications of normothermic liver perfusion

Additionally, a logistic model will be used to examine the possible explanatory effects of baseline subject characteristics (in the presence of the treatment indicator variable), namely: race, age, gender, and number of liver transplants received (primary or secondary).

Secondary analysis may be stratified by recipient center and donor type (DBD or DCD) for exploratory purposes.

9.1.6 Safety Analyses

Safety data will be collected and reported on for those recipients that are matched to a randomized liver and there is an attempt to transplant the randomized liver.

9.1.7 Subgroup Analyses

Subgroup analyses will be performed for donor type (DCD vs. DBD), by donor risk index (DRI), and by duration of machine preservation in the NMP arm of the trial.

Full details of the proposed statistical analysis will be outlined in a separate Statistical Analysis Plan (SAP).

9.1.8 Poolability Analysis

Poolability analyses will be performed on the primary endpoint to assess whether results are poolable across participating centers.

A logistic model with EAD as the dependent (outcome) variable will be reported using a binary treatment indicator variable and site by treatment interaction term(s) as independent variables to assess poolability of data across sites. Absence of statistically significant interaction term(s) (using a p-value of 0.1 as the cut-off) will be taken as evidence of poolability across the sites. Any interaction term that is statistically significant will trigger an examination of the data at the relevant site to determine the underlying reason(s). Since the power associated with testing for a significant interaction is likely to be low, the difference between the two treatment groups will be reported descriptively for each investigational site. A similar approach will be used to assess poolability across donor types.

9.1.9 Missing Data

The extent and types of missing data for key study variables will be assessed as part of sensitivity analyses, and reported upon.

Withdrawals from the trial after transplantation will be documented as per section 6.10, and a narrative summary of withdrawals will be performed. Recipients in the mITT population with incomplete EAD data after transplantation will be included in primary endpoint analysis using imputation methods for missing data. The individual components of EAD will be imputed using baseline subject characteristics as well as all available components of EAD collected during study follow-up. Additional sensitivity analyses could include "completers only," last observation carried forward, and/or mixed models.

For all secondary endpoints, data will be summarized for those recipients with available data. In the instances where data is collected only if standard of care, results will be summarized for those recipients with available data where collection was standard of care. The following variables are collected only as standard of care;

a. Serum gamma-glutamyl transferase (GGT; measured in IU/L)
b. Daily serum lactate (measured in mmol/L) while admitted to ICU

9.2 Sample Size

It is expected that normothermic machine perfusion will be more effective than static cold storage in terms of EAD. Olthoff *et al.* (2010), who developed the EAD definition, showed an EAD incidence of 23%, based on data from 300 liver transplants from 3 US centers between 2004 and 2005 [40]. Estimates of EAD rates in DCD donors have been shown to be closer to 40% [44]. EAD incidence data from livers transplanted using machine perfusion is limited. Using hypothermic machine perfusion Guarrera *et al*. showed a decrease in EAD incidence from 25% to 5% in a prospective cohort pilot study of 20 livers compared to matched controls [25]. The UK pilot study of 20 liver transplants using normothermic machine perfusion showed a decrease in EAD incidence from 22.5% to 15% compared to matched controls. Based on all the available data this study has been powered to demonstrate a reduction in EAD from 25% to 10% in NMP versus SCS. The sample size estimate is based on a one-sided significance level of 0.025 and power of 90%. The pooled Z-test is the test statistic, and the assumption of the covariate distribution is that the difference between the two proportions is zero. The final sample size of 266 transplanted livers (minimum of 120 in each arm), will be achieved by randomizing approximately 356 transplanted livers (assumes approximately 25% attrition). Recipients that receive a reallocated liver but are not enrolled in the study (cases of emergency use) will not contribute to the final sample size of 266 transplanted livers.

9.3 Additional Analyses and Deviation from the Statistical Analysis Plan

Additional analyses may be performed as appropriate. In the final report, such analyses will clearly be described as post-hoc or exploratory. Any deviation from the original statistical analysis plan will require justification in the final study report.

10. Data Collection and Management

10.1 Data Collection Methods

10.1.1 Source Data

Source documents are where data are first recorded, and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the eCRF), clinical and office charts, laboratory reports,

pharmacy records, subject diaries or logs, microfiches, radiographs, correspondence, device accountability records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, and at medico-technical departments involved in the clinical investigation.

All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

10.1.2 Data Recording

Data collection will be achieved using an electronic data capture (EDC) system. Data will be input by local study Investigators/coordinators trained in the use of the system prior to receiving log-in details.

All blood samples will be analyzed in local laboratories and results recorded in common units.

10.2 Data Management

10.2.1 Data Forms and Data Entry

As described in section 10.1.2, data will be entered into an electronic data capture (EDC) system. Validation rules will ensure that data are entered in the correct format, within valid ranges and minimize the chance of missing data. Data already entered will be retrievable for viewing. The extent of an individual user's activity in the database will be limited by privileges associated with his/her log-in and password.

All electronic data will be stored in a secure fashion on password protected servers, with data identified only by the unique participant study ID. Identification of study participants, if required for safety reasons, will be available at the study site.

10.2.2 Discrepancies and Missing Data

Visual and electronic data review will be performed to identify potential discrepancies and missing data. Manual and automatic queries will be created in the EDC system by NAMSA and will be issued to the study site to address. The Investigators/coordinators will be responsible for resolving the queries in the database.

10.2.3 Security and Backup of Data

The database will reside on a server hosted by the EDC provider. All changes made to the data will be captured in an electronic audit trail and available for review. The EDC software has been designed to meet regulatory compliance for deployment as part of a validated system compliant with laws and regulations applicable to the conduct of clinical studies pertaining to the use of electronic records and signatures. Database backups are performed regularly.

10.2.4 Data Access

Access to the database will be controlled by username and password. NAMSA will be responsible for assigning users access to the database.

10.2.5 Data Retention

The study sponsor will store all data captured in the database for at least 15 years following study close-out.

11. Quality Assurance, Audit and Training

11.1 Monitoring

The sponsor will be responsible for the local Investigator's compliance with the trial protocol and for performing source document verification.

At the site initiation visit the sponsor will review the study protocol and all associated study documentation and procedures with the Investigator and study personnel. Applicable local site personnel will receive training for device use and use of the data collection tool, as appropriate to their role, as described in section 11.3.

During the course of the study, the sponsor will maintain regular contact with the investigative sites and conduct central monitoring, on-site monitoring visits, and source data verification on a regular basis to ensure compliance with this study protocol. All subject consent forms will be monitored, and source data will be monitored as specified in the Monitoring Plan.

The Investigator and study personnel must set aside a reasonable amount of his/her time for these visits and the time of the relevant site personnel.

11.2 Quality Assurance Auditing and Inspection

During the course of the study, the sponsor may appoint quality assurance personnel to audit the administration and conduct of the study at the study site. These procedures are in accordance with Good Clinical Practice (GCP) to ensure that complete, accurate, and timely data are collected, that the trial protocol requirements are followed and that all complications and adverse events are reported in a timely manner. The FDA could potentially conduct audits/inspections.

The Investigator and the relevant site personnel must set aside a reasonable amount of his/her time for study related monitors, audits, and inspection by the authorized representatives of the sponsor, IRB, FDA, and institution compliance and quality assurance groups, and provide adequate access to all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data, etc.).

11.3 Local Investigator and Site Personnel Training

All key site personnel must undergo relevant training in advance of the site initiation in accordance with Good Clinical Practice (GCP) guidelines. Such training will be documented.

In addition, training on the investigational device will be provided in advance of recruitment of the first subject. A record of all device training will be maintained. All personnel involved in randomization and data entry will also be trained in the use of the online randomization and data collection tool by members of the clinical trials unit, and records of such training will be maintained.

11.4 Study Documentation

It is the responsibility of the local Investigator to maintain complete, accurate, and current study records. Each Investigator will be provided with an Investigator site file, access to the online case reporting system, and other associated study specific documentation by the trial management team. Such records will be maintained during the course of the study and for a period of 2 years after the latter of the following two dates: The date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application or notice of completion of a product development protocol.

12. Protocol Deviations

12.1 Definitions

The Investigators shall conduct this study in accordance with this protocol and any conditions of approval/notification imposed by the FDA. Failure to comply with and/or inability to meet these regulations may jeopardize further participation of the Investigator or investigative site in this clinical study.

A “protocol deviation” is a failure to adhere to the requirements specified in this study protocol. Examples may include, but are not limited to, the enrollment of a study subject who does not meet all of the inclusion/exclusion criteria specified in section 5.3, missed study procedures, or missed study visits.

12.2 Reporting of Protocol Deviations

Investigators should report protocol deviations to the sponsor as soon as possible and in accordance with 21 CFR 812.150.

13. Ethical and Regulatory Considerations

13.1 IRB and FDA Approval

This protocol and the template informed consent form will be submitted to the FDA for approval. This protocol, the site-specific informed consent form, and any materials supplied to the subject, will be submitted to the investigative site’s IRB for approval. Before the study can begin, each Investigator must have written evidence of IRB approval and the sponsor must have approval from the FDA.

Once approval has been granted, the Investigator is responsible for ensuring that he/she complies with the terms of the approval, namely with adverse event reporting, protocol deviation reporting, notification of amendments, interim, annual, and final reports on the progress of the study.

13.2 Protocol Amendments

Any change or addition to this study protocol which may impact the conduct of the study, potential benefit to the subject or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures or significant administrative aspects will require a formal written amendment to the study protocol.

All amendments to the protocol will be notified to the local regulatory authorities and IRBs for approval. Approved amendments will be circulated promptly to all Investigators by the trial management team. Amendments will be tracked by version number and date in Appendix A2 of this document.

13.3 Reporting

13.3.1 Sponsor Reports

The following reports are required by the sponsor under §812.150. All reports to FDA should be identified as IDE Reports.

- **Unanticipated Adverse Device Effects**

The sponsor must report the results of an evaluation of an unanticipated adverse device effect to FDA and all reviewing IRBs and investigators within 10 working days after the sponsor first receives notice of the adverse effect.

- **Withdrawal of IRB Approval**

The sponsor must notify FDA and all reviewing IRBs and participating investigators of the withdrawal of IRB approval of an investigation (or any part of an investigation) within 5 working days of receipt of the withdrawal of approval.

- **Withdrawal of FDA Approval**

The sponsor must notify all reviewing IRBs and participating investigators of any withdrawal of FDA approval within 5 working days after receipt of the notice.

- **Current List of Investigators**

Every six months the sponsor must submit to FDA a current list of the names and addresses of all investigators participating in a significant risk device investigation.

- **Progress Reports (or Annual Reports)**

At regular intervals and at least yearly, the sponsor must provide progress reports to all reviewing IRBs. For a significant risk device, the sponsor must also submit the progress report to FDA. A suggested format is provided below.

- **Recalls and Device Disposition**

The sponsor must notify FDA and all reviewing IRB's of any request that an investigator return, repair, or dispose of any unit of an investigational device. The notice must be made within 30 working days after the request is made and must state why the request was made.

- **Final Report**

The sponsor must notify FDA and all reviewing IRBs within 30 working days of the completion or termination of the investigation. The sponsor must also submit a final report to FDA and all reviewing IRBs and participating investigators within 6 months after the completion or termination of the investigation. A suggested format is provided by FDA.

- **Failure to Obtain Informed Consent**

Sponsors must submit a copy of any report by an investigator of the use of a device without first obtaining informed consent. The report must be made to FDA within 5 working days after receipt of the notice of such use.

- **Significant Risk Device Determination**

If an IRB determines that the device is a significant risk device and not a non-significant risk device as the sponsor had proposed to the IRB, a report must be submitted to FDA within 5 working days after the sponsor learns of the IRB's determination.

- **Other Reports**

The sponsor must provide accurate, complete, and current information about any aspect of the investigation upon request from the reviewing IRB or FDA.

13.3.2 Investigator Reports

The investigator must provide the following reports to the sponsor in a timely manner under §812.150.

- **Unanticipated Adverse Device Effects**

The investigator must submit to the sponsor and the reviewing IRB a report of any unanticipated adverse device effect as soon as possible but no later than 10 working days after the investigator first learns of the effect.

- **Withdrawal of IRB Approval**

The investigator must report to the sponsor a withdrawal of approval of the reviewing IRB within 5 working days.

- **Progress Reports**

The investigator must submit progress reports to the sponsor and the reviewing IRB at regular intervals but no less than on a yearly basis.

- **Deviations from the Investigational Plan**

The investigator must notify the sponsor and the reviewing IRB of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency. The notice must be provided as soon as possible but no later than 5 working days after the emergency occurred. If it is not an emergency, prior approval from the sponsor is required for changes in or deviations from the investigational plan. If the change or deviation may affect the scientific soundness of the investigational plan or the rights, safety, or welfare of the subject, the sponsor is required to obtain prior IRB approval and also to obtain FDA approval for a significant risk device investigation by submitting an IDE supplement.

  - If an investigator uses a device without obtaining informed consent, the investigator must report the use to the sponsor and to the reviewing IRB within 5 working days after the use occurs.
  - Final Report: The investigator must submit a final report to the sponsor and to the reviewing IRB within 3 months after termination or completion of the investigation.
  - Other Reports: The investigator must provide accurate, complete, and current information about any aspect of the investigation upon request from the reviewing IRB or FDA.

- **Investigator Annual Progress Reports and Final Reports**

The IDE regulations do not specify the content of the annual progress or final reports. Therefore, the contents of these reports may largely be dictated by the sponsor. With respect to reports to the IRB, the IRB itself may specify what information it wishes to be included in these reports. Because FDA does require the information listed below, it is suggested that, at a minimum, the annual progress and final reports to the sponsor and the IRB include the following items:

- IDE number
- Device name
- Indications for use
- Brief summary of study progress in relation to investigational plan
- Number of subjects enrolled
- Number of devices received, used, and, in the final report, the final disposition of unused devices
- Brief summary of results and, in the final report, conclusions
- Summary of anticipated and unanticipated adverse device effects
- Description of any deviations from investigational plan
- Reprints of any articles published by the investigator in relation to the study

13.4 Participant Confidentiality

All study-related information will be stored securely both at the study sites and with the trial management team. Written information will be stored in locked filing cabinets in areas with limited access. All documentation and specimens will be identified by a unique study ID number to maintain participant confidentiality.

Participant's information will not be released outside of the study without the written consent of the participant, except as necessary by regulatory authorities.

13.5 Expenses and Benefits

Where possible, study visits and investigations will be conducted during routine hospital attendances.

14. Dissemination Policy

14.1 Data Analysis and Release of Results

By conducting the study, the local Investigators agree that all information provided by the sponsor and trial management team will be maintained by the local Investigators and the site personnel in strict confidence. It is understood that the confidential information provided to local Investigators will not be disclosed to others without authorization from the sponsor and/or trial management team.

The scientific integrity of the study requires that all data must be analyzed study-wide and reported as such.

14.2 Primary Outcome Publications

At the conclusion of the study, a multi-center manuscript led by the national study Principal Investigator will be prepared with the assistance of OrganOx and NAMSA for publication in a reputable scientific journal. The publication of the principal results from any single center experience within the trial is not allowed until the preparation and publication of the multi-center results as indicated in the Clinical Trial Agreement. Exceptions to this rule require the prior approval of OrganOx. For the purposes of timely abstract presentation and publication, secondary publications will be delegated to the appropriate principal authors, and final analyses and manuscript review for all multi-center data and/or single-center experience reports will require review from OrganOx.

APPENDIX A1: TIMELINE FOR INTERVENTIONS AND ASSESSMENTS DURING THE STUDY

Activity	Pre-study Screening	Pre-study Baseline	Pre-storage	Pre-reperfusion	Post-reperfusion	Postoperative										
						D1	D2	D3	D4	D5	D6	D7	D10	D30	M3	M6	M12
Visit Windows						12-24h	24-48h	48-72h	72-96h	96-120h	120-144h	144-168h	216-240h	+/-7d	+/-14d	+/-14d	+/- 30d
Informed consent	X																
Inclusion/ exclusion criteria	X																
Randomization		X															
Donor & recipient demographics		X															
Perfusion parameters/samples				X													
Lactate values					X												
Surgical variables					X												
Graft biopsy			X	X	X												
Serum AST						X	X	X	X	X	X	X		X	X	X	
Serum ALT						X	X	X	X	X	X	X		X	X	X	
Serum ALP						X	X	X	X	X	X	X		X	X	X	
Total Serum Bilirubin						X	X	X	X	X	X	X		X	X	X	
Serum GGT**						X	X	X	X	X	X	X		X	X	X	
PT/INR						X	X	X	X	X	X	X		X	X	X	
Serum Albumin**						X	X	X	X	X	X	X		X	X	X	
Serum Creatinine**						X	X	X	X	X	X	X		X	X	X	
Serum lactate(*)(**)						X	X	X	X	X	X	X					
Medication Log												X		X	X	X	
Primary non-function													X				
Graft survival						X	X	X	X	X	X	X	X	X	X	X	X
Subject survival						X	X	X	X	X	X	X	X	X	X	X	X

Quality of life measure (EQ-5D-5L)		X														X	
Resource use														X	X	X	X
Safety outcomes					X	X	X	X	X	X	X	X	X	X	X	X	
Readmissions														X	X	X	X
RRT Requirement						X	X	X	X	X	X	X	X	X	X	X	X

* Serum lactate will be recorded daily while the recipient is admitted to high level (ICU) care.

** Only collected if Standard of Care

APPENDIX A2: PROTOCOL AMENDMENTS

Version	Date	Amendments
1.0	26/11/2014	Original Version
2.0	10/07/2015	Amended primary endpoint and other modifications
3.0	01/10/2015	Amended post-transplant follow up visits
4.0	07/09/2015	FDA review questions
5.0	02/11/2015	FDA A001
6.0	20/11/2015	Fixing grammatical errors
7.0	23/11/2015	Updated interventions and assessments table
8.0	16/12/2015	IDE number added to first page
9.0	22/03/2016	FDA A003
10.0	24/06/2016	FDA A004

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