INVESTIGATIONAL PLAN/PROTOCOL

International Randomized Trial to Evaluate the Effectiveness of the Portable Organ Care System (OCS™) Liver for Preserving and Assessing Donor Livers for Transplantation (OCS Liver PROTECT Trial)

Number OCS-LVR-092014

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Sponsor:
TransMedics, Inc.
200 Minuteman Road, Suite 302
Andover, MA 01810

Sponsor Primary Contact:
Waleed Hassanein, MD
President & CEO
Phone: (978) 552-0901
Fax: (781) 998-0354
Email: whassanein@transmedics.com

Sponsor Secondary Contact:
Tamer Khayal, MD
Chief Medical Officer
Phone: (978) 552-0902
Fax: (978) 552-0978
Email: tkhayal@transmedics.com

CONFIDENTIAL – PROPRIETARY INFORMATION

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OCS™ LIVER PROTECT TRIAL SYNOPSIS

Protocol Title	International Randomized Trial to Evaluate The Effectiveness of The Portable Organ Care System (OCS™) Liver For Preserving and Assessing Donor Livers for Transplantation (OCS™ Liver PROTECT Trial)
Objectives	To evaluate the effectiveness of the OCS™ Liver to preserve and assess donor livers intended for transplantation
Trial Design	A prospective, "Phased Pivotal," international randomized trial. The trial will have two parts, (Part A rolling into large Part B, assuming stopping rule is not triggered): • Part A: Include the first 20 randomized transplanted liver recipients with pre-specified stopping rule to ensure safety; • Part B: assuming no stopping rule was triggered, the trial enrollment will continue beyond the initial 20 transplanted recipients in Part A into Part B, while the data from the first 20 subjects is being reviewed. All of the final analyses of the study will be based on the pooled data from Parts A and B.
Trial Size	A maximum of 25 participating sites worldwide to enroll 300 transplanted liver recipients
Screening and Treatment	Primary Liver transplant candidates will be screened for trial eligibility. Every eligible candidate will be asked to participate. Eligible liver transplant candidates will be randomized to receive a donor liver preserved either on OCS™ Liver (OCS) or using cold preservation solution (Control) Donor livers will be screened for trial eligibility. Eligible donor livers will be preserved either using the OCS™ Liver system (OCS) or cold flush and storage using cold preservation solution (Control)
Donor Liver Eligibility Criteria	**Inclusion** Donor meets at least one of the following: • Donor age ≥40 years old; or • Expected total cross clamp/cold ischemic time ≥6 hours; or • Donor after circulatory death (DCD) with age ≤55 years old; or • Steatotic liver >0% and ≤40% macrosteatosis at time of retrieval (based on retrieval biopsy readout (only if the donor liver was clinically suspected to be fatty by the retrieval surgeon at time of liver retrieval) **Exclusion** Donor Livers will be excluded if they meet any of the following criteria: • Living donors • Liver intended for split transplants • Positive serology (HIV, Hepatitis B surface antigen & C) • Presence of moderate or severe traumatic liver injury, or anatomical liver abnormalities that would compromise ex-vivo perfusion of the donor liver (i.e., accessory blood vessels or other abnormal anatomy that require surgical repair) and livers with active bleeding (e.g., hematomas) • Donor livers with macrosteatosis of >40% based on retrieval biopsy readout
Recipient Eligibility Criteria	**Inclusion** Recipients are required to meet all the following criteria on the day of transplant: • Registered primary Liver transplant candidate, male or female

	• Age ≥18 years old • Signed: 1) written informed consent document and 2) authorization to use and disclose protected health information **Exclusion** Recipients will be excluded if they meet any of the following criteria on the day of transplant: • Acute, fulminant liver failure • Prior solid organ or bone marrow transplant • Chronic use of hemodialysis or diagnosis of chronic renal failure, defined as chronic serum creatinine of >3 mg/dl for >2 weeks and/or requiring hemodialysis • Multi-organ transplant • Ventilator dependent • Dependent on >1 IV inotrope to maintain hemodynamics
Primary Effectiveness Endpoint	Incidence of Early liver Allograft Dysfunction (EAD) or primary non-function, defined as presence of one or more of the following criteria: • AST level >2000 IU/ml within the first 7 postoperative days; • Bilirubin≥10 mg/dl on postoperative day 7; • INR ≥1.6 on postoperative day 7; or • Primary non-functioning graft within the first 7 days (defined as irreversible graft dysfunction requiring emergency liver re-transplantation or death, in the absence of immunologic or surgical causes)
Secondary Effectiveness and OCS Donor Liver Assessment Endpoints	• OCS donor liver assessment during perfusion, defined as, among donor livers preserved using OCS for the entire preservation period, the proportion of livers on which measurements of all of the following during perfusion will be available on OCS device before transplant o Lactate level (every two hours) o Average bile production rate (based on total bile production volume and duration of OCS perfusion) o Hepatic Artery Pressure (continuously) o Portal Vein Pressure (continuously) • Patient survival at day-30 post-transplantation • Patient survival at initial hospital discharge post liver transplantation
Other Endpoints	• Length of initial post-transplant ICU stay • Length of initial post-transplant hospital stay • Evidence of ischemic biliary complications diagnosed at 6 and at 12 months • Extent of reperfusion syndrome as assessed based on the rate of decrease of lactate over the following time points: o During anhepatic phase immediately before reperfusion of the transplanted liver o 30-40 minutes after hepatic artery and portal vein reperfusion of the transplanted liver o 90-120 minutes after reperfusion of the transplanted liver • Pathology sample score for liver tissue samples taken at the following time points (applies to both OCS and Control arms): o Donor liver pre-retrieval o Post-OCS and Control preservation at the end of back preparation and immediately before the start of re-implantation o Post reperfusion 90-120 after reperfusion of the transplanted liver

	(prior to abdominal closure)
Safety Endpoints	Safety will be analyzed principally by examination of the frequency of liver graft-related serious adverse events (SAEs) up to the 30-day follow-up after transplantation. This endpoint is defined as the average number of liver graft-related serious adverse events through the 30-days post-liver transplantation per subject, consisting of the following serious adverse events (at most one per type per person): • Primary non-function (defined as irreversible graft dysfunction requiring emergency liver re-transplantation or death with the first 10 days, in the absence of immunologic or surgical causes); • Ischemic biliary complications (ischemic biliary strictures, and non-anastomotic bile duct leaks); • Vascular complications (Liver graft-related coagulopathy, hepatic artery stenosis, hepatic artery thrombosis and portal vein thrombosis); • Liver allograft infections (liver abscess, cholangitis, etc.)
Follow-up	All patients will be followed for a minimum of 30 days post liver transplant. Patients will be followed for a maximum of (24 months) 2 years post-transplant. The following data will be collected at 6 and 12 months: • Patient and graft survival • Incidence of ischemic biliary complications and method of diagnosis • Liver graft related SAEs at 6 months only • Liver graft related re-hospitalized after initial discharge, and, if yes, the primary reason/diagnosis for the hospitalization and the length of stay The following data will be collected at 24 months: • Patient and graft survival
Analysis Populations	• The Per Protocol (PP) Population will consist of all randomized subjects who are transplanted and have no major protocol violations and for whom the donor liver received the complete preservation procedure as per the randomization assignment. In analyses based on the PP Population, subjects will be analyzed as randomized. The primary analysis of effectiveness will be based on the PP Population. • The Modified Intent-to-Treat Population (mITT) will consist of all randomized subjects who are transplanted. In analyses based on the mITT Population, subjects will be analyzed as randomized. The mITT analyses will be considered secondary analyses of effectiveness. • The As Treated Population (AT) will consist of all treated subjects, i.e., all subjects who are transplanted in the study with a donor liver preserved with either OCS or Control. In analyses based on this population, subjects will be analyzed as treated. A subject who receives a liver with some preservation with OCS and some with standard of care will be analyzed according to the initial preservation. After the initiation of preservation, any switchover in preservation method should be recorded as a protocol deviation and those subjects will not be considered part of the AT population. Analyses of safety endpoints will be performed based on the AT Population. • The Donor Liver Population will consist of all donor livers for which the

	potential recipient was randomized and which have preservation initiated using OCS or Control. In some of the analyses based on this population, livers will be analyzed as randomized; in others, they will be analyzed as treated. For the analyses based on the actual treatment of the liver, a liver with some preservation with OCS and some with standard of care will be analyzed according to the initial preservation.
Effectiveness Analysis	The primary effectiveness endpoint for this study is Early Liver Allograft Dysfunction (EAD), defined as presentation of one or more of the following criteria: • AST level >2000 IU/ml within the first 7 postoperative days; • Bilirubin ≥10 mg/dl on postoperative day 7; • INR ≥1.6 on postoperative day 7; or • Primary non-functioning graft within the first 7 days The primary hypothesis for this study is that the OCS treatment is non-inferior to the standard of care treatment with respect to this endpoint. The non-inferiority margin δ is taken to be 0.075. If non-inferiority is demonstrated using a significance level of 0.05, a two-sided test of superiority will be performed. The primary effectiveness endpoint will be analyzed by calculating, for each treatment group, the sample proportion of subjects meeting the primary effectiveness endpoint, as well as an exact (Clopper-Pearson) 95% confidence interval for the corresponding population proportion. The 95% upper bound of the exact unconditional one-sided confidence interval based on Farrington and Manning score statistic will be calculated for the difference between the two population proportions (OCS – Control). An upper confidence limit less than $\delta = 0.075$ will result in rejection of the null hypothesis in favor of the alternative hypothesis and the demonstration of non-inferiority of OCS to Control for the primary effectiveness endpoint. In the event non-inferiority is demonstrated, Fisher's exact test (two-sided) will be used to test for superiority. This endpoint will be analyzed using the Per Protocol and mITT Populations. The Per Protocol analysis will be considered the primary analysis. Multiple imputation methods will be used for data imputation for any patients with missing values for this endpoint. The secondary effectiveness and OCS donor liver assessment endpoints for this trial, listed in the order in which they will be tested using the fixed sequence testing procedure, are as follows: • OCS Measurements during organ perfusion • Patient survival at day-30 post-transplantation • Patient survival at initial hospital discharge post liver transplantation The hypothesis for the endpoint of OCS donor liver assessment during perfusion is that, among donor livers preserved using OCS for the entire preservation period, the proportion of livers on which measurements of lactate level, average bile production rate, Hepatic Artery and Portal Vein Pressure during perfusion are available on OCS device before transplant is at least 85%. This endpoint will be analyzed by calculating the sample proportion of donor livers meeting the endpoint, as well as an exact 95% one-sided lower confidence bound for the corresponding population proportion. A lower confidence bound greater than 0.85 will result in the demonstration that the true proportion is greater than 0.85 for the OCS donor liver

	assessment endpoint Each secondary effectiveness endpoint will be summarized using counts and percentages and an exact 95% confidence interval for the true percentage based on the binomial distribution. The secondary effectiveness endpoints will be analyzed using the PP and the mITT Populations. The Per Protocol analysis will be considered the primary analysis. The primary hypothesis for the first secondary effectiveness endpoint is that the OCS treatment is non-inferior to the standard of care treatment. The non-inferiority margin is taken to be 0.075. This endpoint will be analyzed by calculating the 95% upper confidence limit based on the normal approximation for the difference between the two population proportions (Control - OCS). An upper confidence limit less than $\delta = 0.075$ will result in rejection of the null hypothesis in favor of the alternative hypothesis and demonstration of non-inferiority of OCS to Control. In the event non-inferiority is demonstrated, Fisher's exact test (two-sided) will be used to test for superiority. The second secondary effectiveness endpoint, patient survival at initial hospital discharge post liver transplantation, will be analyzed in a manner analogous to the first secondary effectiveness endpoint with the same non-inferiority margin of 0.075. Because fixed sequence testing will be used for the secondary endpoints, no adjustment for the multiplicity of these endpoints needs to be made. The endpoints will be tested in the order listed above. The test for non-inferiority for the first secondary effectiveness endpoint will be performed only if the null hypothesis has been rejected for the OCS donor liver assessment endpoint. The test for non-inferiority for the second secondary effectiveness endpoint will be performed only if the null hypothesis has been rejected in favor of the alternative hypothesis of non-inferiority of the OCS treatment to the Control treatment for the first secondary effectiveness endpoint. Similarly, the test for superiority for the second secondary effectiveness endpoint will be performed only if the null hypothesis of equality has been rejected in favor of superiority of the OCS treatment to the Control treatment for the first secondary effectiveness endpoint (and non-inferiority has been demonstrated for the given secondary effectiveness endpoint). These endpoints will be analyzed using the Per Protocol and mITT Populations, with the Per Protocol analysis being considered the primary analysis. Multiple imputation methods will be used for data imputation for patients with missing values for these endpoints.
Safety Analysis	Safety will be analyzed principally by examination of the frequency of liver graft-related serious adverse events (SAEs) up to the 30-day follow-up after transplantation. This endpoint is defined as the average number of liver graft-related serious adverse events through the 30 days post-liver transplantation per subject, consisting of the following serious adverse events (at most one per type per person): • Primary non-function (defined as irreversible graft dysfunction requiring emergency liver re-transplantation or death with the first 10 days, in the absence of immunologic or surgical causes); • Ischemic biliary complications (ischemic biliary strictures, and non-

	anastomotic bile duct leaks); • Vascular complications (Liver graft-related coagulopathy, hepatic artery stenosis, hepatic artery thrombosis and portal vein thrombosis); • Liver allograft infections (liver abscess, cholangitis, etc.) This endpoint will be summarized by treatment group using descriptive statistics. For each treatment group, a 95% confidence interval for the mean based on the t-distribution will be presented. Also, a 95% confidence interval based on the t-distribution will be presented for the difference in means between the two treatments. For the number of liver graft-related SAEs, the hypothesis is that the OCS treatment is non-inferior to the standard of care treatment. The non-inferiority margin is taken to be 1.00. The safety endpoint will be analyzed using a one-sided, two-sample t-test with an alpha level of 0.05. If non-inferiority is demonstrated, a corresponding (two-sided) test of superiority will be performed. This endpoint will be analyzed based on the As Treated Population. In addition, the numbers and percentages of subjects experiencing at least one liver graft-related AE, at least one (definitely or probably-related) device-related AE, at least one unanticipated AE, and at least one serious AE, and the number and percentage of deaths will all be tabulated by treatment group. Also, the number of liver graft-related adverse events and the number and percentage of subjects experiencing liver graft-related adverse events will be tabulated by system organ class and preferred term using MedDRA. A similar analysis will be performed for liver graft-related SAEs. AEs will also be tabulated at the event level by system organ class and preferred term and the relationship of the adverse event to the device using counts and percentages. Similar analyses will be performed by the severity of the adverse event. The numbers and percentages of donor livers in the Donor Liver Population that are treated as randomized and transplanted, treated not as randomized and transplanted, treated as randomized and not transplanted, and treated not as randomized and not transplanted will be presented. For livers that are treated not as randomized, the reason will also be summarized using counts and percentages. Both of these analyses will be done based on the randomized treatment for the liver, but the following analyses will be based on the actual treatment of the liver. For livers that are not transplanted, the reason(s) for non-transplantation (including device failure) will be summarized using counts and percentages. The number and percentage of donor livers in the Donor Liver Population for which these was a device failure will also be presented.
Randomization	After confirmation of eligibility, obtaining informed consent, and a matching donor liver is identified, potential liver transplant recipients will be randomized 1:1 to have their donor livers preserved using either the OCS Liver perfusion or the standard cold static preservation technique (Control) using cold flush and storage. Randomization will be performed through the Interactive Web Response System (IWRS). Subjects who are not transplanted with the matching donor liver will be re-randomized and treated as a new subject.
Sample Size Determination	The sample size for this trial was determined based on the effectiveness endpoint, Early Liver Allograft Dysfunction (EAD) in the first 7 days post liver

	transplantation. The sample size calculation assumed a non-inferiority, test based on the upper bound of the Exact unconditional one-sided confidence interval for the difference in proportions using Farrington and Manning score statistic. An alpha level of 0.05, a non-inferiority margin of 0.075, a 1:1 allocation, true proportions for the primary effectiveness endpoint of 0.2 for the OCS treatment and 0.25 for the Control treatment, and power of 80%. Based on these specifications, the required sample size was determined to be 144 transplanted recipients per treatment group, or 288 total transplanted subjects. To ensure an adequate number of subjects in the Per Protocol Population, the sample size was increased to a total of 300 transplanted subjects. Subjects will be enrolled until there are either 290 subjects in the Per Protocol Population or a total of 300 transplanted subjects, whichever comes first.
Sample Size Re-estimation	A sample size re-estimation will be performed for the purpose of confirming the current sample size is sufficient for testing for non-inferiority for the primary effectiveness endpoint and to assess the feasibility of demonstrating superiority. It will be conducted after there are 200 transplanted subjects to allow for a possible upwards adjustment in sample size. The sample size will not be increased by more than 150 subjects.
Trial Sponsor	TransMedics, Inc. 200 Minuteman Road, Suite 302 Andover, MA, USA 01810

1. INTRODUCTION AND BACKGROUND INFORMATION

1.1. Liver Transplantation and Current Clinical Challenges

Over the last two decades liver transplantation has evolved as the gold standard for treating end-stage liver failure disease and those with tumors of hepatic origin in the setting of liver dysfunction. The success of liver transplantation is now its primary obstacle, as the pool of donor livers fails to keep pace with the growing number of patients added to the national liver transplant waiting list. As of August 2014, there are nearly 16,000 patients listed on the U.S. national waiting list for liver transplantation, yet only 6,200 patients were transplanted in 2013 depriving thousands of patients the gift of new Livers to treat their end-stage Liver disease, according to the Organ Procurement and Transplant Network (OPTN)[1] database. In addition, this significant discrepancy between supply and demand results in approximately 20% of patients either dying or becoming too sick to receive a liver transplantation (see Figure 1 below).

Figure 1: U.S. National Liver Transplant Waiting List Dynamics 2009-2011. Source: OPTN 2011/Scientific Registry of Transplant Recipients (SRTR) 2011 Annual Report: Transplant data 2011.

	2010	2011	2012	
Patients at start of year	14,956	15,360	15,428	
Patients added during year	10,349	10,359	10,143	
Patients removed during year	9,925	10,272	10,281	
Patients at end of year	15,380	15,447	15,290	
Removal reason				
Deceased donor transplant	5,450	5,539	5,468	
Living donor transplant	209	187	192	
Patient died	2,458	2,506	2,187	14%
Patient refused transplant	53	60	73	
Improved, tx not needed	552	541	644	
Too sick to transplant	362	482	815	5%
Other	841	957	902	

The current technique for liver preservation using cold flush and storage of donor livers plays a major role in the above large and growing clinical problem in liver transplantation due to the following severe limitations:

- It subjects the donor livers to significant time-dependent ischemic injury[2,3] and subsequent reperfusion injury that may impair liver function post-transplant. This causes transplanting physicians to only select for procurement those Livers most likely to withstand the potential damage associated with cold storage preservation. It also imposes significant time and geographical limitations on the liver retrieval process, adversely impacting the utilization of available donor livers. In addition, this time-dependent ischemic injury has been directly correlated to post- transplant complications.

- It lacks any perfusion capabilities to maintain the liver in a near-physiologic[4] *(in-vivo-like)* environment after the donor liver is retrieved from the body of the donor. This limitation results in significant underutilization of the donor livers with fatty cells (macrosteatotic livers). This further limits the use of these donor livers that otherwise are functioning normally in the body of the donor.
- It lacks any ability to evaluate organ metabolic state and function after procurement and preservation to determine the suitability of the donor Livers for transplantation[4].. This significantly limits the utilization of donor livers that are subjected to the negative, non-physiologic conditions of brain death in the donor. More importantly, it severely limits the utilization of donor livers classified as donors after circulatory death (DCDs).

1.2. Potential Clinical Solutions to Overcome Limitation in Donor Liver Utilization

Over the past 10+ years, there has been a global focus on *ex-vivo* organ perfusion in a near physiologic condition as a promising technique to overcome the current challenges in organ preservation and to potentially increase utilization of donor organs (liver, lung, heart and kidney) that are currently not used due to shortcomings of cold storage.[13]

Isolated liver perfusion has been extensively studied for over 25+ years using a variety of different perfusates and perfusion temperatures. More recently, there has been an international focus in the liver transplant clinical and scientific community on normothermic liver perfusion using blood based perfusate to minimize the negative impacts of cold ischemic preservation on donor livers for transplantation. In addition, by maintaining livers in an active metabolic and functioning state, donor livers from heart beating or DCD donors can be assessed ex-vivo for suitability for transplantation.

The TransMedics' Organ Care System (OCS™) Liver technology is a portable system for ex-vivo perfusion and assessment of the donor Livers for transplantation. The OCS™ Liver maintains the donor liver in a metabolically active and functioning state (producing bile), by perfusing the liver with warm oxygenated and nutrient enriched blood based perfusion solution. The OCS™ Liver is intended to significantly reduce ischemia and reperfusion injuries on the donor Livers and enable metabolic and functional assessment of donor livers to assess their suitability for transplantation. The OCS™ Liver may enable the following clinical advantages:

- Reduction of the ischemia and reperfusion injuries on the donor Livers during preservation, thus, eliminating the significant logistical and geographical barriers to Liver transplantation that currently exist with cold storage preservation.
- Optimization of donor Liver ex-vivo environment by optimizing oxygen and substrate delivery, while also maintaining near normothermic condition to avoid the negative impact of cold temperature on fatty livers.
- Ex-vivo assessment of donor liver metabolic and functional state utilizing standard clinical tests of liver enzymes, bile production and lactate metabolism. This could enable the transplanting clinical team to judge the suitability of the donor liver for transplantation and substantially minimizing the risk of transplanting questionable donor livers into recipients.

1.3. Subject enrollment and health insurance

Approximately 30-40% of liver transplant recipients have their care paid for by Medicare due to their age or disability status. Therefore, it is expected that Medicare patients will consent to participate in this study. Since Medicare patients in need of transplantation do not differ from transplant candidates receiving coverage from other payors, Medicare patients will be treated in the same manner as all other study Subjects and will realize the same potential benefits and risks experienced by all other Subjects in the study.

1.4. Summary of Prior Testing and Investigations

1.4.1. OCS™ Liver Engineering Testing

The OCS™ Liver device components have been CE marked for Heart and lung perfusion and TransMedics is currently in the process of expanding our CE mark indication for liver perfusion. The OCS™ system and perfusion modules have undergone extensive preclinical testing to demonstrate its safety, effectiveness, and readiness for clinical use. The Liver Perfusion Set has also been evaluated and tested in accordance with ISO-10993 "Biological Evaluation of Medical Devices," including evaluations for acute toxicity, irritation, sensitization, cytotoxicity, hemolysis, genotoxicity, and pyrogenicity. These test results demonstrated that the device and its materials are biocompatible and suitable for their intended use. The Liver Perfusion Set will be provided sterile using validated methods, and is appropriately packaged to maintain sterility. The OCS has also undergone extensive preclinical bench testing for: electrical safety; electromagnetic compatibility; and validation and verification testing (including validation of the device software). All tests and results have demonstrated that the OCS meets its expected performance specifications and is safe and suitable for clinical use.

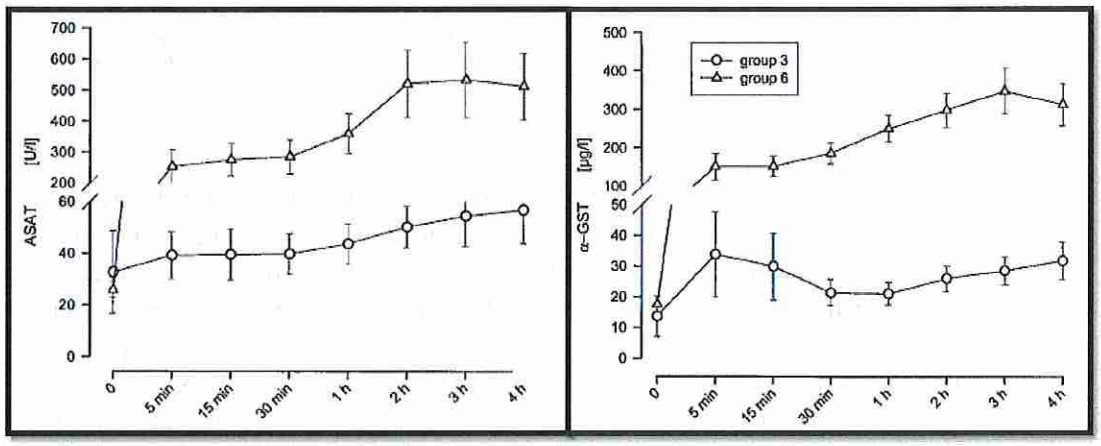
1.4.2. Independent Pre-Clinical Testing of Normothermic Machine Perfusion for Donor Livers

Isolated liver perfusion has been extensively studied for over 25+ years using a variety of different perfusates and perfusion temperatures. More recently, there has been an international focus in the liver transplant clinical and scientific community on normothermic liver perfusion using blood based perfusate to minimize the negative impacts of cold ischemic preservation on donor livers for transplantation. In addition, by maintaining livers in an active metabolic and functioning state, donor livers from DCD donors can be assessed ex-vivo for suitability for transplantation. The following is a summary of relevant clinical and scientific published studies supporting the concept of ex-vivo near-normothermic machine perfusion using blood-based perfusate for donor livers:

- In 2001, Schön MR et al.[17] reported successful swine liver transplantation after 4 hours of normothermic ex-vivo blood based perfusion of both the hepatic artery and portal vein. This report demonstrated that normothermic perfusion groups had better metabolic function and histological picture for livers that were immediately perfused after harvest as well as livers that were subjected to 1 hour of warm ischemia (to simulate DCD donors), prior to normothermic machine perfusion. The metabolic function of the liver was assessed ex-vivo by measuring serial AST, GGT levels (see Figure 2 below) as well as bile production. The perfusate mix used for this study consisted of:
  - Swine blood

- Buffered electrolyte solution
- Heparin
- NaHCO3 for buffers

Figure 2: Shön et al, AST and GGT levels during 4 hours of ex-vivo machine perfusion for group 3= heart beating swine livers and group 6= DCD swine livers.



- In 2002, Butler AJ, Friend PJ, et al.[6] reported successful extracorporeal porcine liver perfusion for up 72 hours using swine blood as perfusate. The swine livers were perfused by cannulating the hepatic artery and the portal vein. Liver metabolic function was well preserved as evidence of stable pH of the perfusate, stable ALT and alkaline phosphatase levels (see Figure 3 below). In addition, bile production was well maintained up to 10 hours of perfusion, then significantly dropped due to lack of bile salts/acids in the circuit and bile sludge noticed/reported by the author. The perfusate mix used for this study consisted of:
  - Swine whole blood
  - Colloid solution
  - Antibiotics
  - Methylprednisolone steroid
  - $NaHCO_3$. for buffer
  - Heparin
  - Vitamins and trace elements
  - Prostacyclin for vasodilatation
  - Calcium chloride
  - Taurocholic acid for bile salts
  - Parenteral nutrition solution supplemented with amino acids
  - Insulin

Figure 3:

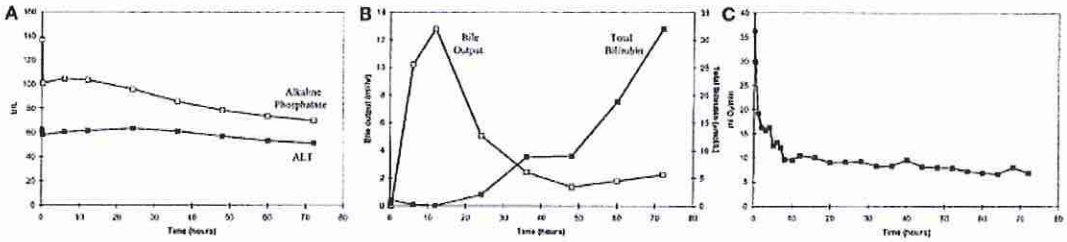


Fig. 3 . **Markers of liver injury and function (n=5) during extracorporeal liver perfusion. (A) ALT (serum glutamic pyruvic transaminase) levels (normal porcine range 31–58 U/L) and alkaline phosphatase levels (normal porcine range 180–813 U/L). (B) Bile output measured as ml of bile produced per hour and total bilirubin expressed in μmol/L (normal porcine range up to 0–17.1 μmol/L). (C) Oxygen consumption calculated by the Fick equation and expressed as ml O_2/min/liver.**

- In 2009, Brochman J., et al.[7] reported the successful preservation of heart beating and DCD swine livers using blood based, normothermic ex-vivo machine perfusion for up-to 20 hours and subsequent transplantation. This study demonstrated a clear trend towards better liver function in the normothermic perfusion groups as compared to cold storage for both heart beating and DCD livers. In addition, this report suggested that bile production; AST levels and portal vein pressure as potential ex-vivo perfusion predictors for post-transplant viability in pigs. The perfusate mix used for this study consisted of:
  - Swine whole blood
  - Colloid solution
  - Antibiotics
  - Methylprednisolone steroid
  - $NaHCO_3$. for buffer
  - Heparin
  - Vitamins and trace elements
  - Prostacyclin for vasodilatation
  - Calcium chloride
  - Taurocholic acid for bile salts
  - Parenteral nutrition solution supplemented with amino acids
  - Insulin

- In 2011, Fondevila, et al.[8] reported successful transplantation of swine DCD livers after 90 minutes of cardiac arrest by using ex-vivo normothermic blood based perfusion (NMP) of donor swine livers. This report demonstrated that when normothermic ex-vivo perfusion was used it resulted in 100% survival of the swine post-transplantation as compared to 83% when in-vivo NMP was used and 0% when cold storage was used. This report demonstrated good histological pictures of all

livers perfused using ex-vivo normothermic blood based perfusion. The perfusate mix used for this study consisted of:

- Swine whole blood
- NaHCO3 for buffer
- Heparin

- In 2014, Liu Q, et al.[9-11] reported that normothermic machine perfusion (NMP) is superior to cold storage (CS) for preservation of swine DCD livers after 10 hours of preservation followed by simulated transplantation. This study evaluated ex-vivo liver enzymes during NMP as well as perfusion parameters; hepatic artery and portal vein flow and pressure as signs of liver metabolic activities (see Figure 4 below). During simulated transplantation phase, liver function was assessed using liver enzyme levels as markers to hepatocellular injury and showed clear advantage of NMP vs. CS (see Figure 5 below). The perfusate mix used for this study consisted of:
  - Swine whole blood
  - Colloid solution
  - Antibiotics
  - Methylprednisolone steroid
  - $NaHCO_3$ for buffer
  - Heparin
  - Prostacyclin for vasodilatation
  - Calcium Gluconate
  - Taurocholic acid for bile salts
  - Parenteral nutrition solution supplemented with amino acids
  - Insulin

Figure 4:

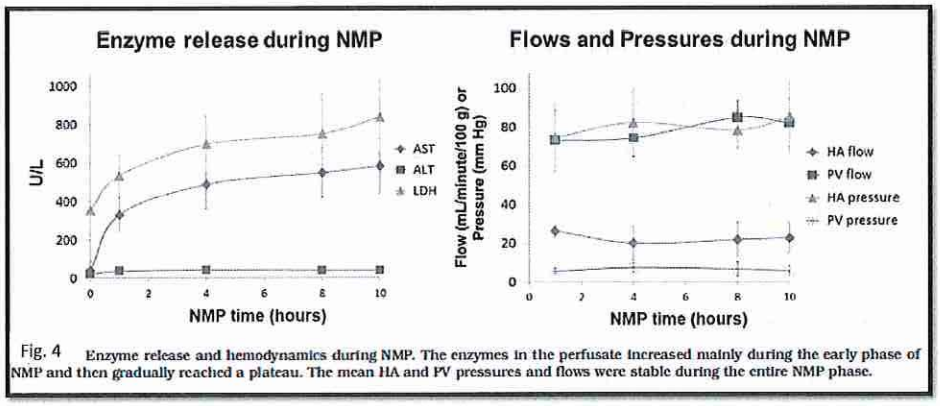


Fig. 4 Enzyme release and hemodynamics during NMP. The enzymes in the perfusate increased mainly during the early phase of NMP and then gradually reached a plateau. The mean HA and PV pressures and flows were stable during the entire NMP phase.

Figure 5:

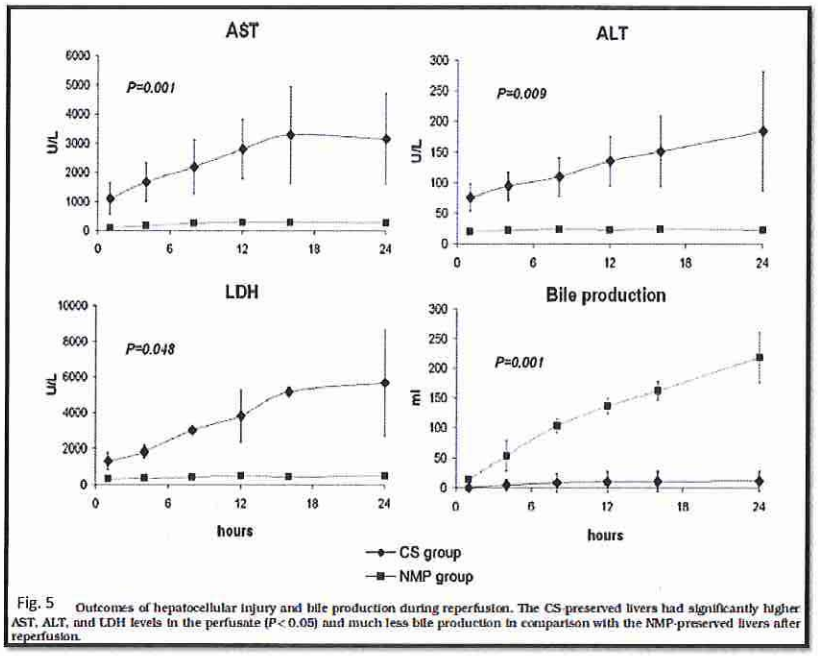


Fig. 5 Outcomes of hepatocellular injury and bile production during reperfusion. The CS-preserved livers had significantly higher AST, ALT, and LDH levels in the perfusate ($P < 0.05$) and much less bile production in comparison with the NMP-preserved livers after reperfusion.

1.4.3. OCS™ Liver Pre-Clinical Testing

The OCS™ Liver System has been extensively tested in the large swine animal model and has demonstrated excellent results in maintaining liver function ex-vivo. The OCS liver preclinical testing was conducted in two distinct phases/steps.

1.4.3.1. Phase 1 Ex-vivo OCS Liver Perfusion for up-to 12 hours

- 28 consecutive large swine studies were conducted using ex-vivo OCS Liver perfusion to maintain livers for up to 12 hours. The perfusate used to perfuse these 28 livers was identical to the one described in the published scientific and clinical literature above. The OCS perfusate consisted of:
  - Swine washed blood cells
  - Colloid solution using 25% albumen
  - Buffered crystalloid solution (PlasmaLyte™)
  - Antibiotics (gram negative and gram positive coverage)
  - Steroids – methylprednisolone and dexamethasone
  - $NaHCO_3$. for buffer
  - Heparin
  - Calcium Gluconate
  - Vitamins and trace elements
  - Parenteral nutrition solution supplemented with amino acids
  - Insulin

- Prostacyclin for vasodilatation – as needed
- Taurocholic acid for bile salts – as needed

- Liver function on OCS was evaluated using the identical parameters that were described in the above published independent scientific and clinical literature:
  - Perfusion parameters – Hepatic Artery Pressure (HAP); Portal Vein Pressure (PVP)
  - Markers of liver injury: Liver enzymes
  - Marker of liver metabolism: Lactate level
  - Marker for liver function: Bile production
  - Histopathology to evaluate parenchymal and bile duct injury

Figure 6: OCS Liver perfusion parameters over 12 hours of ex-vivo perfusion demonstrating stable and near physiologic HA and PV pressures

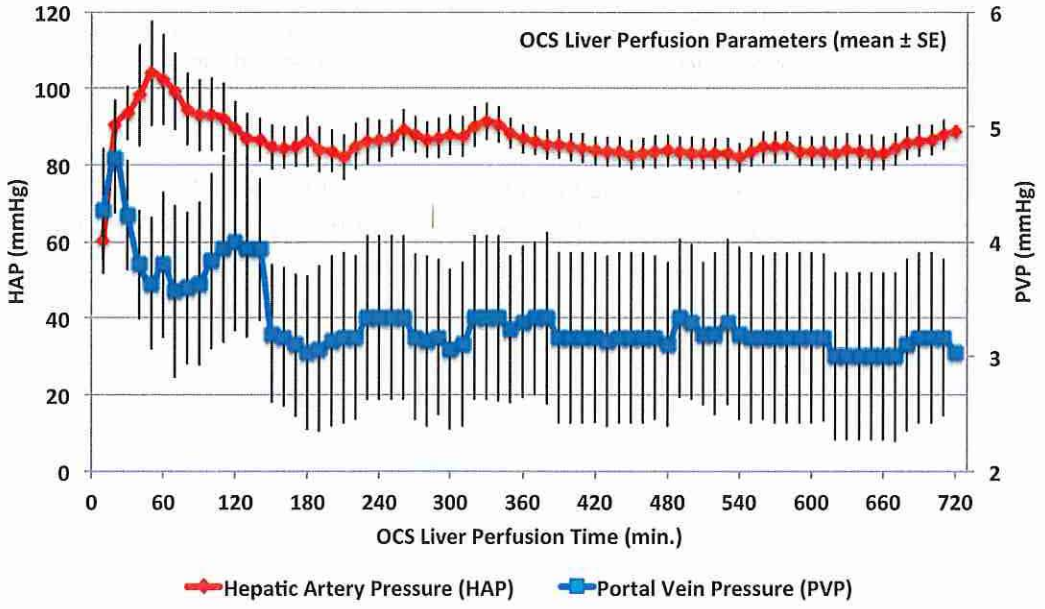


Figure 7: **Donor liver AST levels during OCS Liver perfusion demonstrating a positive trend towards base line (BL) levels indicating normal liver function and no evidence of injury. Lactate levels trending down indicating normal liver metabolic function and a sign of adequate perfusion**

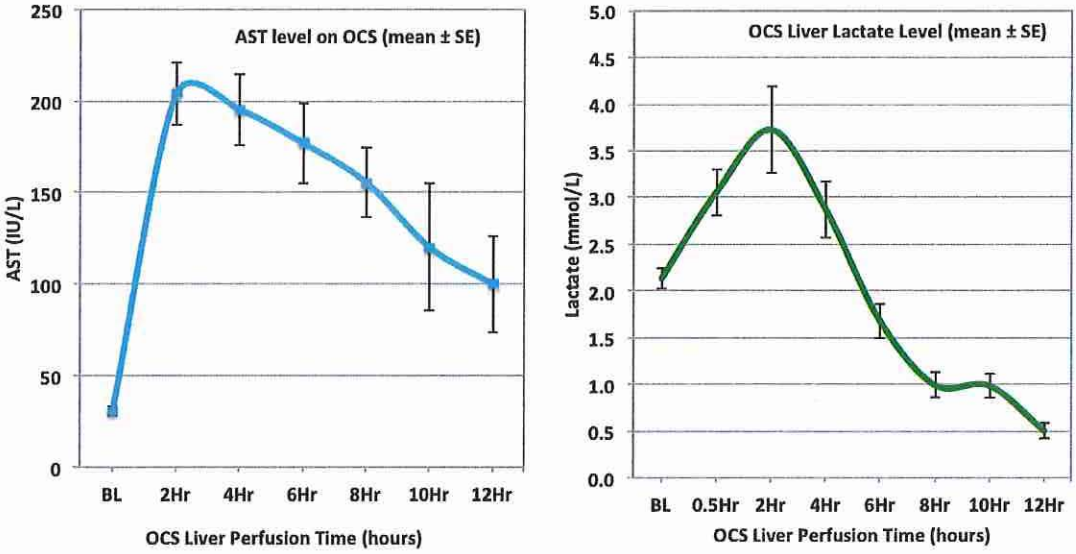


Figure 8: **Total bile production during OCS Liver perfusion measured hourly showing steady and consistent bile production by the liver indicating that liver function is well maintained**

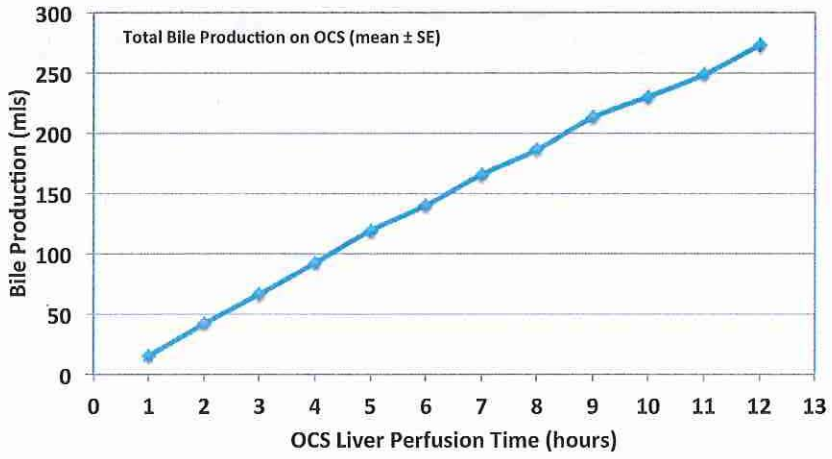
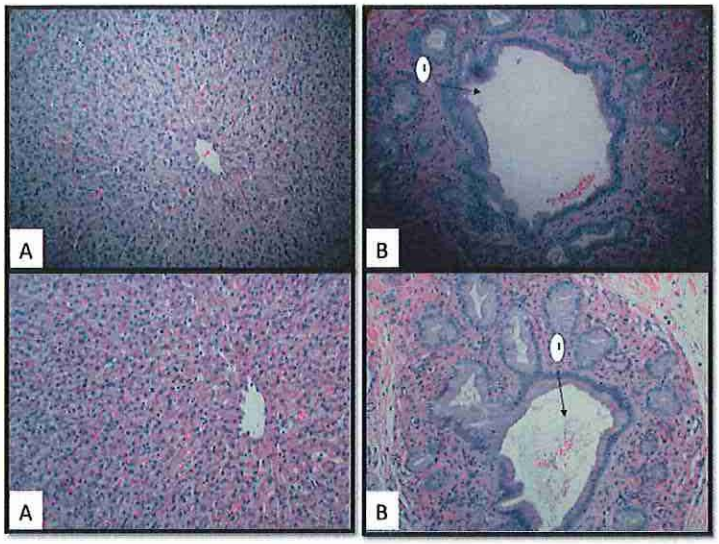


Figure 9: Histological examination of A) Parenchymal tissue and B) Bile duct tissue shows normal liver sinusoidal structure with no evidence of necrosis or ischemia and normal bile duct epithelial cells indicating adequate perfusion and lack of ischemic injury after 12 hours of OCS Liver machine perfusion



1.4.3.2. Phase 2 Simulated Transplants After OCS Liver Perfusion:

This group of studies focused on evaluating donor swine liver in a simulated transplant model after 8 hours of ex-vivo OCS Liver perfusion using identical perfusate to the one used in Phase 1 OCS pre-clinical studies above. The simulated transplant procedure used whole blood from a different animal without any modifications and livers were studied for 4 hours in the simulated transplant condition. Liver function was assessed using identical parameters to the ones measured in Phase 1. Post-transplant histological samples were evaluated in an identical fashion to the phase 1 animal studies, looking at both liver parenchyma and bile duct samples. Below are the summary results of these simulated transplanted studies.

Figure 10: OCS Liver preservation and post-transplant perfusion parameters demonstrating stable and near physiologic HA and PV pressures and stability of perfusion

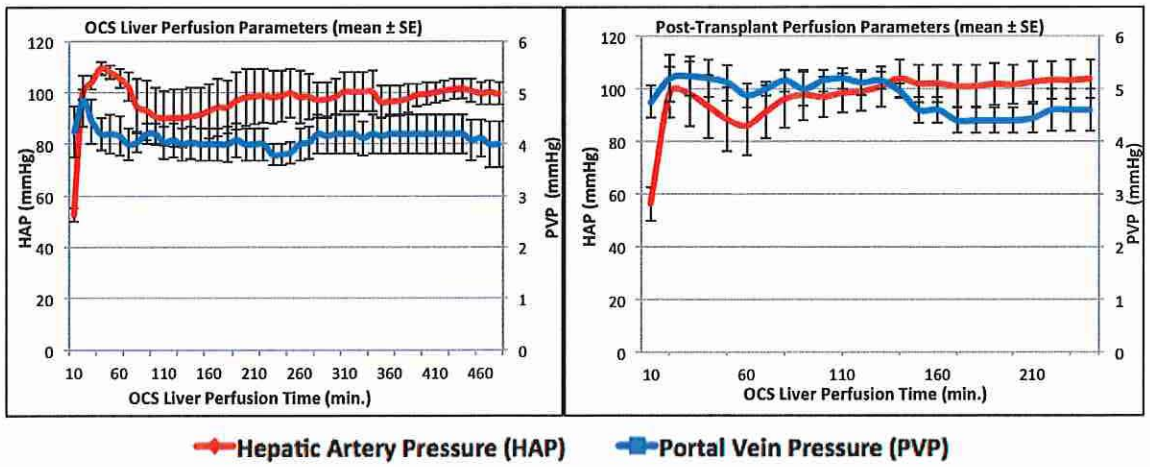


Figure 11: OCS Liver preservation and post-transplant AST liver enzyme levels showing a positive trend towards base line (BL) levels indicating normal liver function and no evidence of injury.

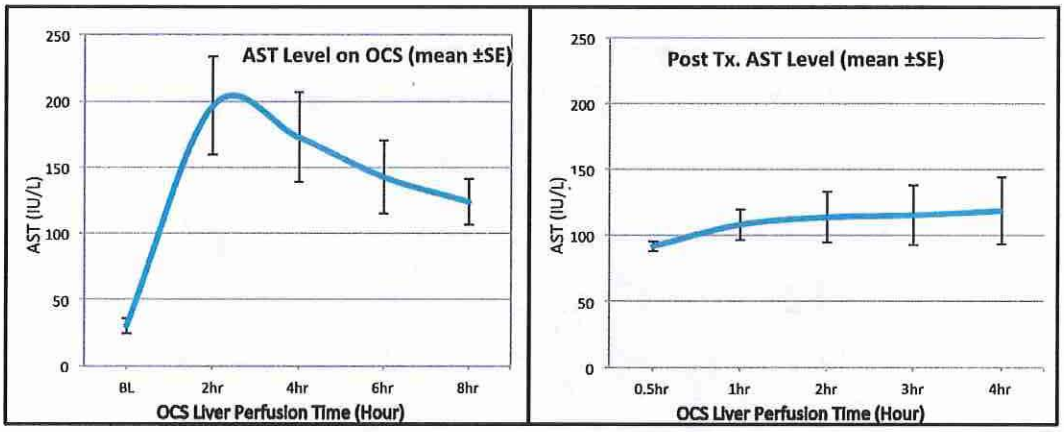


Figure 12: OCS Liver preservation and post-transplant Lactate levels showing a Lactate levels trending down indicating normal liver metabolic function and a sign of adequate perfusion.

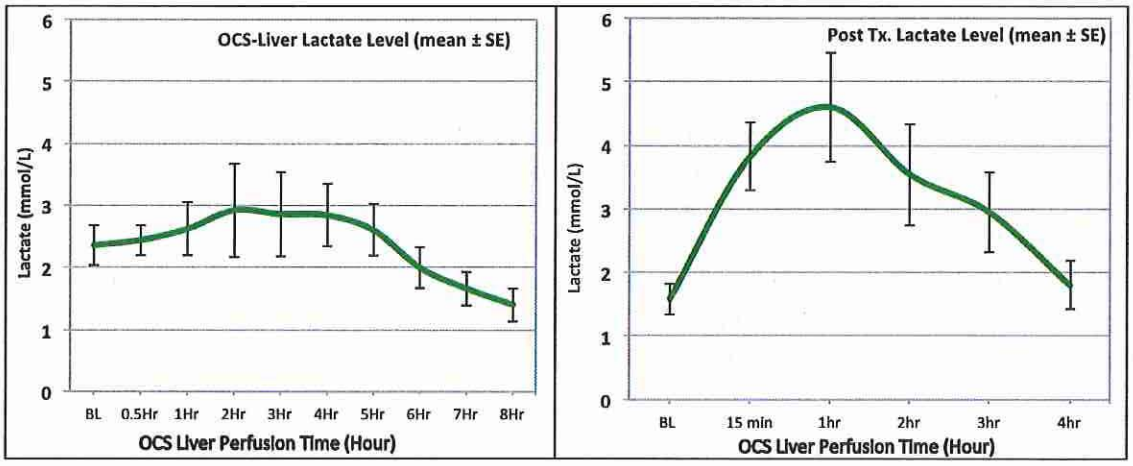


Figure 13: **Total bile production during simulated transplant measured hourly showing steady and consistent bile production by the liver indicating that liver function is well maintained**

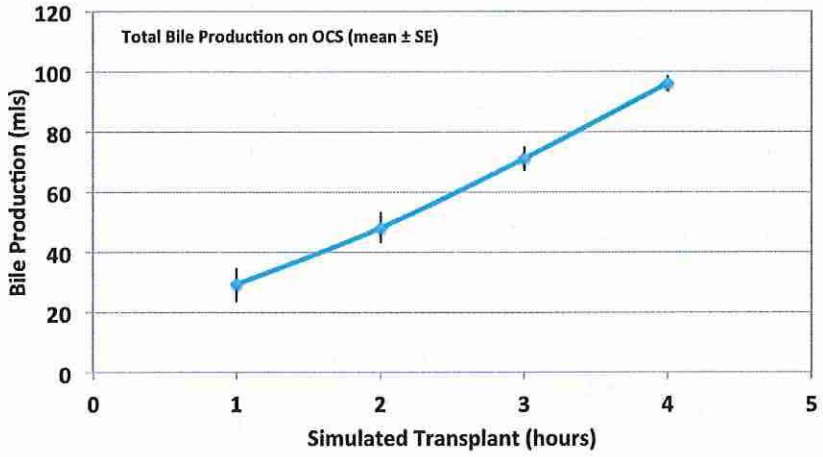
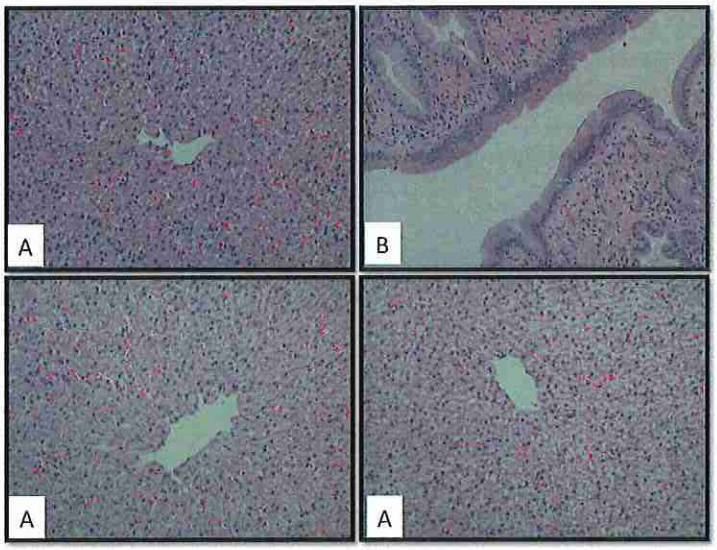
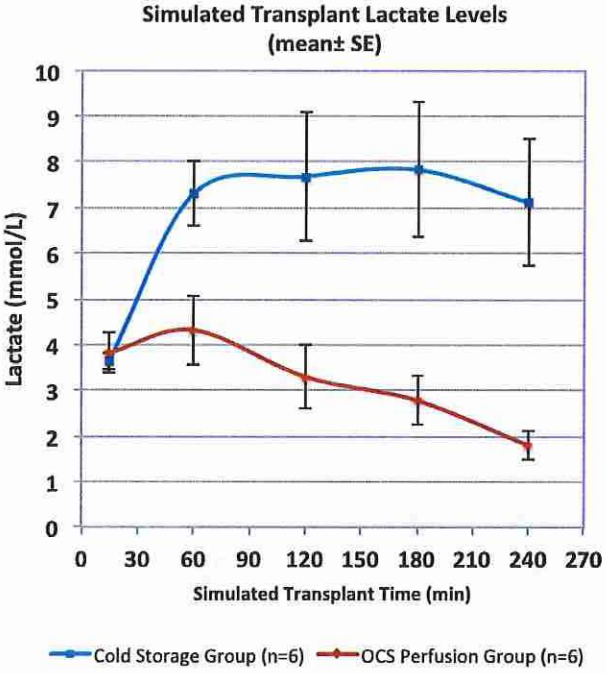


Figure 14: **Histological examination of A) Parenchymal tissue and B) Bile duct tissue shows normal liver sinusoidal structure with no evidence of necrosis or ischemia and normal bile duct epithelial cells indicating adequate perfusion and lack of ischemic injury after 8 hours of preservation on OCS liver system, 45 minutes of cold storage and 4 hours of simulated transplantation**



In addition, we have compared the OCS arm to a control arm of 8 hours cold stored swine livers using standard of care cold preservation solution. The control arm was also assessed in a simulated animal transplant model identical to the one described above. Liver function was assessed using identical parameters to the ones measured in Phase 1. Below are the summary results of the OCS vs. Control group during simulated transplant phase.

Figure 15: **Lactate and AST levels for OCS vs. Control arms during 4 hours of simulated transplantation. The data demonstrates that OCS perfused swine livers had a significantly better metabolic function, as evidenced by their ability to metabolize Lactate to baseline levels as compared to cold stored livers where lactate continued to rise to significantly high levels. In addition, the OCS perfused swine livers had a significantly lower AST levels throughout the 4 hour transplant phase as compared to rising level of AST in the cold storage group. This indicates significantly less liver injury to the graft in the OCS group as compared to the cold stored group.**



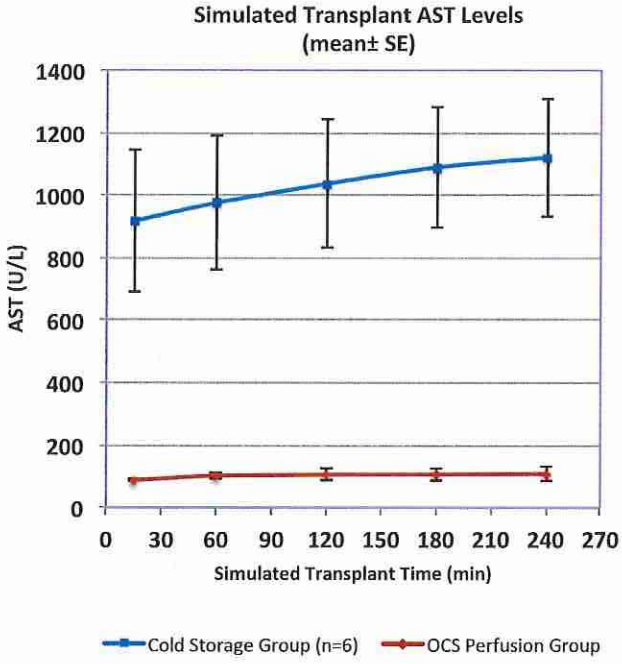
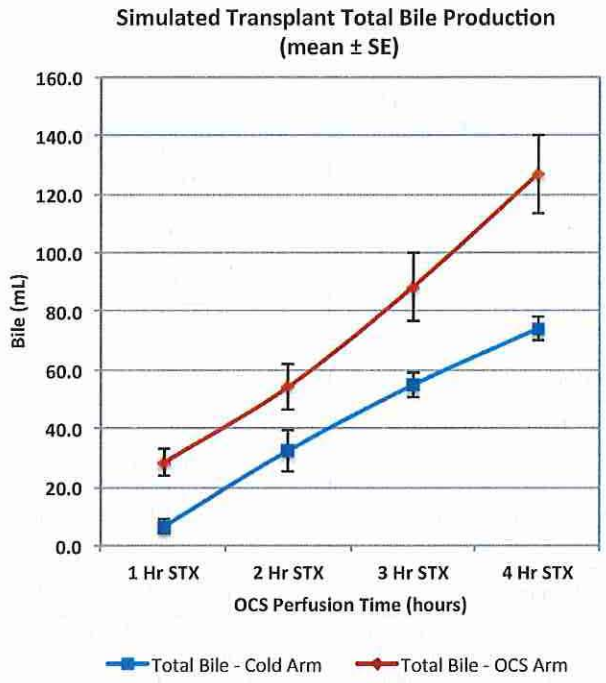


Figure 16: **Total Bile Production for OCS vs. Control arms during 4 hours of simulated transplantation. The above graph demonstrates that OCS perfused swine livers had a higher bile production rate as compared to Cold stored livers. This indicates better liver graft function in the OCS group vs. Cold group**



1.4.4. Clinical Feasibility Testing of Ex-vivo normothermic Liver Perfusion:

In 2014, Friend P, et al. reported the successful clinical feasibility of normothermic machine perfusion in 20 clinical liver transplants in a UK trial using the Metra liver perfusion device. The Metra device shares identical characteristics to the OCS Liver perfusion device. In addition, the donor inclusion criteria represented a mix of routine, DCD and steatotic donor livers which is a true reflection of current realities in clinical liver transplantation. More importantly, this study used an identical cellular perfusate and additives to the one described in the scientific/clinical literature and the OCS Liver perfusate. It included:

- Type specific packed red blood cells (pRBCs)
- Colloid solution
- Buffered crystalloid solution
- Antibiotics (gram negative and gram positive coverage)
- Methylprednisolone steroid
- $NaHCO_3$ for buffer
- Heparin
- Vitamins and trace elements
- Prostacyclin for vasodilatation

- Calcium chloride
- Taurocholic acid for bile salts
- Parenteral nutrition solution supplemented with amino acids
- Insulin

The summary clinical results are presented in the table below.

Friend, et al. Reported Clinical Outcomes	Normothermic Machine Perfusion	Cold Storage Matched Control
30-Day graft survival, n (%)	20 (100%)	37 (97.5%)
30-day patient survival, n (%)	20 (100%)	1 (97.5%)
3-month patient survival, n (%)	20 (100%)	39 (97.5%)
Primary non-function rate	0 (0)	0 (0)
Early Allograft Dysfunction, n (%)	3 (15%)	9 (22.5%)
Peak AST within 7 days, (IU/L), median (range)	417 (84-4681)	902 (218-8786)
Initial Post-Tx. ICU stay (days), median (range)	3 (1-8)	3 (1-41)
Hospital Stay (days), median (range)	12 (6-34)	14 (8-88)
Source: Rising Star Symposium, International Liver Transplant Society, June 5, 2014, London, England; Abstract O-1, Human Liver Transplantation Using Normothermic Machine Preservation, Friend P, et al. Sunrise Symposia; New Dimensions in Liver Transplantation, World Transplant Congress, July 31, 2014, San Francisco, CA, U.S.A.; Normothermic Machine Preservation of the Liver, Friend P, et al.		

Based on the above overwhelming supportive pre-clinical, scientific and clinical evidence of safety, feasibility and potential benefits of warm machine perfusion for liver transplantation using identical perfusion protocols/perfusates to the OCS liver technology, we are proposing to proceed with a pivotal prospective clinical investigation of the OCS liver technology to compare its outcomes to cold static storage in liver transplant recipients.

2. DEVICE DESCRIPTION

The OCS™ Liver System is an integrated portable platform designed to maintain donor Livers in a near physiologic, normothermic perfusion state. The OCS™ Liver System consists of:

- The Portable Console & Monitor: This is a compact electromechanical device that contains an integrated pulsatile perfusion pump, batteries, blood warmer, pressure, flow and saturation meters. In addition, it has an integrated wireless monitor that allows the clinical operator to control and display critical perfusion parameters of the preserved donor Livers.
- Single Use Sterile Perfusion Set: At the core of the OCS™ Liver is a sterile, biocompatible perfusion module that maintains the organ's physiologic environment and has embedded sensors to optimize and monitor the Liver perfusion parameters and bile production. In addition, the perfusion module enables perfusate sampling in order to monitor the Liver's metabolic condition.

Figure 17: System Components

3. TRIAL OBJECTIVES

To compare the safety and effectiveness of the OCS™ Liver (OCS) vs. standard cold storage (Control) to preserve and assess donor Livers having one or more of the following characteristics:

- Donor age ≥40 years old; or
- Expected cross clamp time ≥6 hours; or
- Donor after circulatory death (DCD) with age ≤55 years old; or
- Steatotic liver >0% and ≤40% macrosteatosis at time of retrieval (on pre-retrieval histology)

3.1. Type of the Trial

A prospective, multi-center, randomized, controlled Phased Pivotal trial with transplanted recipients assigned to either the OCS Liver arm (OCS - treatment) or standard of care cold storage arm (SOC - control). The trial will have two parts. (Part A rolling into large Part B, assuming stopping rule is not triggered):

- Part A: Include the first 20 randomized transplanted liver recipients with pre-specified stopping rule to ensure safety;
- Part B: assuming no stopping rule was triggered, the trial enrollment will continue beyond the initial 20 transplanted recipients in Part A into Part B, while the data from the first 20 subjects is being reviewed. All of the final analyses of the study will be based on the pooled data from Parts A and B.

3.2. Trial Size and Subject Follow-up

This trial will be conducted at no more than 25 institutions, in the United States and worldwide (Europe, Australia and Canada) and will include up to 300 transplanted Liver recipients. The number of subjects was determined as described in the statistical analysis section of this Investigational Plan/Protocol. All subjects will be followed for a minimum of 30 days post-transplant. Patients will be followed for a maximum of 24 months from the date of transplantation. The summary of the follow-up is in Appendix 2 as follows:

- All subjects will be followed from transplant to discharge
- 30-day patient and graft survival will be documented on day-30 post-transplant eCRF
- 6, 12, and 24 months patient follow-up

4. TRIAL ENDPOINTS

4.1. Primary Effectiveness Endpoint

Incidence of Early liver Allograft Dysfunction (EAD) or primary non-function, defined as presence of one or more of the following criteria:

- AST level>2000 IU/ml within the first 7 postoperative days;
- Bilirubin ≥10 mg/dl on postoperative day 7;
- INR ≥1.6 on postoperative day 7; or
- Primary non-functioning graft within the first 7 days

4.2. Secondary Effectiveness and OCS Donor Liver Assessment Endpoints

- OCS Measurements during organ perfusion
- Patient survival at Day 30 post-transplantation
- Patient survival at initial hospital discharge post liver transplantation

4.3. Other Endpoints

- Length of initial post-transplant ICU stay
- Length of initial post-transplant hospital stay
- Evidence of ischemic biliary complications diagnosed at 6 and at 12 months post-transplant
- Extent of reperfusion syndrome as assessed based on the rate of decrease of lactate over the following time points:
  - During anhepatic phase immediately before reperfusion of the transplanted liver
  - 30-40 minutes after hepatic artery and portal vein reperfusion of the transplanted liver
  - 90-120 minutes after reperfusion of the transplanted liver
- Pathology sample score for liver tissue samples taken at the following time points (applies to both OCS and Control arms):
  - Donor liver pre-retrieval
  - Post-OCS and Control preservation at the end of back preparation and immediately before the start of re-implantation
  - 90-120 minutes after reperfusion of the transplanted liver

4.4. Safety Endpoint

Safety will be analyzed principally by examination of the frequency of liver graft-related serious adverse events (SAEs) up to the 30-day follow-up after transplantation. This endpoint is defined as the average number of liver graft-related serious adverse events through the 30 days post-liver transplantation per subject, consisting of the following serious adverse events (at most one per type per person):

- Primary non-function (defined as irreversible graft dysfunction requiring emergency liver re-transplantation or death with the first 10 days, in the absence of immunologic or surgical causes)
- Ischemic biliary complications (ischemic biliary strictures, and non-anastomotic bile duct leaks)
- Vascular complications (Liver graft-related coagulopathy, hepatic artery stenosis, hepatic artery thrombosis and portal vein thrombosis)
- Liver allograft infections (liver abscess, cholangitis, etc.)

5. TRIAL POPULATION

The trial will include 300 Liver transplant recipients, at up to 25 investigational sites in the USA and worldwide (Europe, Australia and Canada).

5.1. Donor Eligibility Criteria

5.1.1. Donor Inclusion Criteria

Donor liver meets at least one of the following:

- Donor age ≥40 years old; or
- Expected total cross clamp/cold ischemic time ≥6 hours; or
- Donor after circulatory death (DCD) with age ≤55 years old; or
- Steatotic liver >0% and ≤40% macrosteatosis at time of retrieval (based on retrieval biopsy readout only if the donor liver was clinically suspected to be fatty by the retrieval surgeon at time of liver retrieval)

5.1.2. Donor Exclusion Criteria

Donor Livers will be excluded if they meet any of the following criteria:

- Living donors
- Liver intended for split transplants
- Positive serology (HIV, Hepatitis B surface antigen & C)
- Presence of moderate or severe traumatic liver injury, or anatomical liver abnormalities that would compromise ex- vivo preservation of the donor liver (i.e., accessory blood vessels or other abnormal anatomy that require surgical repair) and livers with active bleeding (e.g., hematomas)

- Donor livers with macrosteatosis of > 40% based on retrieval biopsy readout

5.2. Recipient Eligibility Criteria

5.2.1. Recipient Inclusion Criteria

Recipients are required to meet all the following criteria on the day of transplant:

- Registered primary Liver transplant candidate, male or female
- Age ≥18 years old
- Signed: 1) written informed consent document and 2) authorization to use and disclose protected health information

5.2.2. Recipient Exclusion Criteria

Recipients will be excluded if they meet any of the following criteria on the day of transplant:

- Acute, fulminant liver failure
- Prior solid organ or bone marrow transplant
- Chronic use of hemodialysis or diagnosis of chronic renal failure, defined as chronic serum creatinine of >3 mg/dl for >2 weeks and/or requiring hemodialysis
- Multi-organ transplant
- Ventilator dependent
- Dependent on >1 IV inotropic support to maintain hemodynamics

6. PRE-OPERATIVE TRIAL PROCEDURES

6.1. Subject Identification

All patients on the liver transplant waiting list who are being treated by trial investigators will be identified. Those patients who initially appear eligible for the trial will have the trial thoroughly explained to them, be invited to participate, and will be asked to sign an informed consent for participation in the trial prior to treatment. When a matching donor Liver becomes available, the inclusion and exclusion criteria will be re-verified. When a final decision is made at the recipient site to transplant the Liver, the recipient will be assigned a subject identification number.

6.2. Recipient Day of Transplant Assessment

The purpose is to conduct a final assessment of whether the potential consented recipient still meets the eligibility criteria. The following information will be verified and recorded on the day of transplant:

- **Eligibility:** Investigator will review and confirm that the potential consented recipient continues to meet all inclusion criteria and no exclusion criteria.
- **Demographics/Characteristics:**
  - Date of birth or Age

- Gender, body mass index
- Race and Ethnicity
- Blood type and RH factor
- Recipient Model for End-stage Liver Disease (MELD) Score
- Recipient ID

- **Baseline Liver Function Tests:**
  - Bilirubin levels
  - Aspartate aminotransferase (AST)
  - Alanine aminotransferase (ALT)
  - Gamma-glutamyl transpeptidase (GGT)
  - Alkaline phosphatase
  - International normalized ratio (INR)
  - Serum Lactate level
- **Recipient Risk Factors & Medical History:**
  - Indication for transplantation: The primary etiology of Liver failure will be recorded
  - History of Hepatitis C
  - History of liver cancer
  - History of diabetes

6.3. Donor Screening and Acceptance

Using the inclusion and exclusion criteria, the investigator or a member of her/his transplant team will evaluate the donor and the quality and suitability of the Liver for the trial. The following evaluations will be conducted and recorded:

- **Organ Donor Identification Number and Type of Registry** (e.g. UNOS ID, Eurotransplant ID, etc.)
- **Demographics:** Age, Date of birth (if available), gender, race, and ethnicity
- **Donor Characteristics:** Blood type & RH factor, body mass index
- **Donor's Cause of Death:**
  - Cause of death and date and time of pronouncement of brain death
  - If the donor is a DCD donor, total time in minutes from discontinuation of support to cardiac stand still
- **Medical History:**
  - Active infection, positive serology for CMV, HIV, Hepatitis B or C, malignant tumors, and Liver disease

- History of diabetes (if available)

- **Donor Liver Assessment:** The donor Liver will be assessed prior to procurement and acceptance using the following methods:
  - **Liver Enzymes :** the following tests will be collected if available:
    - Bilirubin levels;
    - Aspartate aminotransferase (AST);
    - Alanine aminotransferase (ALT);
    - Gamma-glutamyl transpeptidase (GGT);
    - Alkaline phosphatase;
    - International normalized ratio (INR);
    - Lactate level;
  - **Liver Biopsy (see Appendix 1.):** A needle biopsy will be collected from all donor livers at time of retrieval at time of cross clamp in the donor. Only donor livers that are clinically suspected to be fatty by retrieval team visualization, will require a pre-retrieval biopsy readout to estimate the degree of macrosteatosis and confirm eligibility (>0 and ≤40% macrosteatosis).

- **Eligibility:** The donor will be evaluated to document whether the eligibility criteria are met. All eligibility criteria for not accepting a donor Liver at final assessment before retrieval will be recorded. Any other clinical reasons for not accepting donor liver at final assessment before retrieval will be recorded.

6.4. Donor Liver Retrieval and OCS Preservation and Assessment

After final evaluation of the donor Liver in donor's abdomen and upon acceptance into the trial, the investigators will retrieve and preserve the donor Liver according to the following protocol:

- **Initial Liver Flush in Donor Body:** all donor Livers will be flushed using cold Belzer UW® solution or Custodiol® HTK preservation solutions according to the institution's standard of care practice and the manufacturer instruction for use.

- **OCS Liver Back Table Flush:** All donor livers randomized to be perfused on OCS Liver system will be flushed on the back table using cold PlasmaLyte® solution supplemented with sodium bicarbonate (NHCO3) 10 mml/L, Epoprostenol Sodium 2 mics/L, and Methylprednisolone ~160 mg/L flush below, according to the following protocol:
  - **Hepatic Artery:** 1 liter flush at ~50-70 mmHg pressure bag
  - **Portal Vein:** 2 liters gravity drain

- **OCS™ Liver Perfusion:** The OCS™ Liver system will be primed using the following:

OCS Liver Perfusate Composition	Recommended Dose
Packed Red Blood Cells (pRBCs)*	4-8 units
Albumin 25%	400 mls
PlasmaLyte® solution	700-800 mls
Methylprednisolone	500 mgs

OCS Liver Perfusate Composition	Recommended Dose
Dexamethasone	20 mgs
Sodium Bicarbonate ($NaHCO_3$) 8.4%	70 mmol
Adult Multivitamins for infusion e.g., INFUVITE®	1 unit
Calcium Gluconate 10% (100 mg/ml)	10 mls
Antimicrobials:	
Gram positive antibiotic e.g., Cefazolin	1 gm
Gram negative antibiotic e.g., Cirprofloxacin	100 mg
* type specific (or O negative blood group), pathogen free, leukocyte and platelet reduced	

- **OCS™ Liver Perfusion Additives:** In addition to the above OCS Liver perfusate, the following will be infused to the perfusate mix using an ex-vivo solution pump:

OCS Liver Perfusate Additives	
Continuous Infusion Mix	**Dose**
Total parenteral nutrition (TPN) Mix: *CLINIMIX E TPN (4.25% Amino Acid / 10% Dextrose); PLUS* *Insulin 30 IU* *Glucose 25 gms* *Heparin 40,000 units*	30 ml/hr
As Needed Additives	**Dose**
Prostacyclin infusion as needed to control Hepatic artery pressure e.g., Epoprostenol Sodium 0.5 mg	0-20 mics/hr
Bile Salts e.g., Taurocholic acid sodium (1 gm/50 ml)	3 ml/hr
$NaHCO_3$. 8.4% to correct metabolic acidosis	1.5 meq/1 base excess

- **Bile Salt Preparation:** All study sites will be provided sealed Gamma-sterilized vials of 1 gm of Taurocholic acid sodium in salt form to be mixed prior to retrieval according to the following steps::
  - Add 50 ml of sterile water for injection using aseptic technique
  - Mix well to ensure all salt is dissolved

  At the time of use the OCS operator will spike the stopper of the vial using a sterile OCS infusion line and connect to the OCS infusion delivery system.
- **The OCS™ Liver Preservation Parameters:** Donor livers will be perfused on the OCS Liver device with OCS parameters maintained within the following ranges
  - Hepatic Artery Pressure (mean HAP): 75-100 mmHg

- Hepatic Artery Flow (HAF): 300-700 ml/min
- Portal Vein Pressure (mean PVP): 4-8 mmHg
- Portal Vein Flow (PVF): 700-1700 ml/min
- Perfusate Temperature (Temp): 34°C
- Oxygen gas flow 400-700 ml/min
- Circulating arterial Lactate (Lact) trend: trending down over time

- **Lactate and Liver Enzyme Levels During OCS Liver Perfusion Sampling Scheme**: The organ retrieval team will collect samples from the arterial port of the OCS perfusion circuit to measure lactate level using a standard blood analyzer according to the following protocol:
  - OCS Baseline arterial sample within the first 10-30 min of liver instrumentation.
    - Lactate
    - Liver enzymes: AST, ALT, GGT, total bilirubin, and ALP
  - Lactate samples will continue to be collected from the device at approximately hourly intervals until lactate level is trending down, at this time, lactate samples should be done every 2 hours or after any active HAF or HAP adjustments.
  - OCS Final arterial sample before cooling liver on OCS
    - Lactate
    - Liver enzymes: AST, ALT, GGT, total bilirubin, and ALP
- **Final Liver Cooling and Flush Arrest on OCS:** all donor Livers on OCS will be cooled and flushed on the OCS using cold PlasmaLyte® solution supplemented with sodium bicarb olone onate (NHCO3) 10 mml/L, Epoprostenol Sodium 2 mics/L, and Methylprednis~160 mg/L flush below, according to the following protocol:
  - **Hepatic Artery:** 1 liter flush at ~50-70 mmHg pressure bag
  - **Portal Vein:** 2 liters gravity drain
- **Liver Biopsy (see** Appendix 1.**):** a needle biopsy will be collected from donor livers preserved on OCS or control on the back table, post preservation, immediately before re-implantation.
- **OCS Preservation Information:** the following information will be collected
  - Device Information (serial number etc.)
  - Device Malfunction(s)
  - OCS system data collected by the device during the preservation run and uploaded into the EDC database
- **Donor Retrieval Details:** The following information will be collected at time of Liver retrieval
  - Date and time of cross clamp of donor aorta

- **OCS Details (Liver Instrumentation details):**
  - Total cold ischemia time between initial donor cross clamp and start of OCS™ Liver perfusion
- **OCS Parameters and Enabled Measurements:** The following OCS™ Liver perfusion parameters and Liver metabolic conditions will be recorded:
  - HAP
  - HAF
  - PVP
  - PVF
  - Total Bile volume
  - OCS Liver arterial lactate level

6.5. Donor Liver Acceptance for Transplantation

All donor livers that are preserved on OCS or Control are expected to be transplanted into a matching consented recipient. Any decision to turn down Livers after the Livers have been retrieved, preserved on either OCS™ Liver or cold storage should be done with notification to the Site PI and the donor Liver examined by the transplant center's qualified liver transplant pathologist and/or tissue slides/samples to be sent to core lab for the study for further examination. All reasons for turning down a donor liver after it has been preserved will be collected in the eCRFs.

7. TRANSPLANT, IMMEDIATE POST-OPERATIVE AND LONG TERM FOLLOW-UP

7.1. Transplant Details

The following information concerning the transplant procedure will be collected:

- The organ recipient unique post-transplant patient identifier
- Total cross clamp duration in minutes (from donor cross-clamp application to removal of cross-clamp in the recipient)
- Total cold ischemia time will be collected (Control= total cross clamp time; OCS = cold ischemic time pre and post OCS Liver perfusion)
- Any surgical complications encountered during surgery
- Use of circulatory bypass will be recorded
- Type of liver anastomosis
  - Bicaval anastomosis; or
  - Piggyback anastomosis
- The total amount of any blood product and clotting factors transfusion will be collected (pRBCs, Fresh Frozen Plasma (FFP), platelets, etc.)

- Any inotropic support needed to maintain hemodynamics will be collected for both arms
- A liver tissue biopsy will be collected from Right lobe of the transplanted liver immediately before abdominal closure in the recipient. The biopsy will be collected according to Liver Biopsy protocol in Appendix 1.

7.2. Post-transplant Functional Assessments Day 0 – Day 30:

- **Early Liver Allograft Dysfunction (EAD) Surveillance in the first 7 days:**
  - AST level >2000 IU/ml within the first 7 postoperative days;
  - Bilirubin ≥10 mg/dl on postoperative day 7;
  - INR ≥1.6 on postoperative day 7; or
  - Primary non-functioning graft within 7days
- **Inotropic Support to Maintain Hemodynamics at T0, T24 and 48 and 72 hours after ICU admission (if applicable) will be collected**
- **Initial use of Mechanical Respiratory Support:** duration of initial post-transplant invasive ventilator support will be recorded from the time of initial admission to ICU post-Liver transplant until extubation.
- **Initial Post-Transplant ICU Stay:** Intensive care unit (ICU) admission time, and date and time when clinical order for ICU discharge is written.
- **Renal Replacement Therapy:** Need for dialysis treatments in the first 10 days post liver transplantation for patients that did not have renal replacement prior to transplantation
- **Immunosuppression Medications:** The type of immunosuppression medication and dose will be recorded at day 7 and at time of discharge from the hospital. Immunosuppression induction will be recorded if applicable.
- **Patient and Graft Survival at Day 30 and At Initial Hospital Discharge:** Patient and graft survival will be assessed on day 30 post-transplant and at initial hospital discharge post-liver transplantation.
- **Adverse Events:** All Liver graft-related serious adverse events and any Liver graft-related adverse events will be followed and documented until the investigator designates the event to be either resolved or its effect on the patient's condition stabilized.
- **Medications:** Medications used to treat all serious Liver graft-related adverse event (SAE)-related will be recorded in the trial electronic database until the SAE is resolved.

These follow-ups will be attempted within ± 3 days of the designated periods except for the Early Liver Allograft Dysfunction (EAD) Surveillance, in the first 7 days which must be collected at the designated times. The evaluations may be conducted over several days.

7.3. Long Term Follow-up: 6 and 12 & 24 months

Follow-up data collection will be conducted at approximately 6, 12, and at 24 months post-transplant.

7.3.1. 6 and 12-Month Follow-up

At approximately 6 and 12 months post-transplant, the patient will be evaluated at an office visit if this is the institution's standard of care, and, if not, by phone contact by the site. The patient's medical record may be reviewed to confirm patient's answers. This follow up will collect information on:

- Patient and graft survival;
- Liver graft related SAEs (6 months only);
- Liver graft related re-hospitalized after initial discharge, and, if yes, the primary reason/diagnosis for the hospitalization and the length of stay;
- Information will also be collected on any diagnosis of ischemic biliary complications and, if so, the method of diagnosis and treatment.

The 6 and 12-month follow-up will be collected within ± 1 month of the designated period and will be recorded on the 6 and 12 month follow-up electronic form.

7.3.1.1. 24-Month Follow-up

At approximately 24 months post-transplant, the patient will be evaluated at an office visit if this is the institution's standard of care, and, if not, by phone contact by the site. The patient's medical record may be reviewed to confirm patient's answers. This follow up will collect information on:

- Patient and graft survival;
- Results of standard clinical practice or for cause liver biopsy will be collected (if applicable).

The 24 months follow-up will be collected within ± 1 month of the designated period and will be recorded on the 24-month follow-up electronic form.

8. EVALUATION OF ADVERSE EVENTS

8.1. Evaluation of Liver Graft-Related Adverse Events

Liver Graft-Related Adverse Events are those, which have any untoward effect on the health, or safety of the patient and that are related to the transplanted Liver function (except for acute rejection). Liver graft-related adverse events will be collected from the time a subject is transplanted with a Liver preserved on OCS or Control until the completion of the 30-day follow-up evaluation. A Liver graft-related adverse event will be followed until resolution or stabilization of the event.

8.2. Serious Adverse Events (SAEs)

An adverse event will be classified as serious if it meets any of the following criteria:

- Results in, leads to, or contributes to, a death;
- Is life-threatening;
- Results in permanent disability or incapacity (i.e., permanent impairment of a body function or permanent damage to a body structure);
- Requires in-patient hospitalization or prolongs hospitalization;
- Necessitates medical or surgical intervention to preclude a permanent disability or incapacity;
- Results in fetal distress, fetal death or a congenital anomaly/birth defect

All Liver graft-related SAEs will be followed until their resolution.

8.3. Anticipated and Unanticipated Adverse Events

The investigator will assess each adverse event for whether it is anticipated or unanticipated. An unanticipated adverse event is defined as any adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device if that effect, problem or death was not previously identified in nature, severity or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the safety, or welfare of subjects.

Adverse events are associated with Liver transplant procedures and have been documented within the first 30 days after Liver transplant, and are therefore anticipated. The list of events includes, but is not limited to:

- Acute rejection
- Atrial and ventricular arrhythmias
- Bleeding
- Hemodynamic instability
- Death
- Fever
- Early liver allograft dysfunction (EAD)
- Respiratory failure
- Liver primary non-function
- Bile leaks
- Hepatic artery thrombosis
- Portal vein thrombosis
- Cholangitis
- Liver abscess
- Diaphragmatic injury
- Phrenic nerve injury
- Sepsis
- Renal dysfunction and/or failure
- Hyperammonaemia

- Malignancy (post-transplant lymphoproliferative disorder (PTLD)
- Multiple organ failure
- Myocardial infarction
- Neurological dysfunction
- Hepatic dysfunction
- Diabetes due to steroid and anti-rejection medications
- Pancreatitis
- Peptic ulceration
- Gastritis
- Gastro esophageal reflux disease (GERD)
- Aspiration
- Cardiac tamponade
- Pneumo-mediastinum
- Pneumothorax
- Hemothorax
- Ascites
- Pleural effusion
- Venous thromboembolism (deep venous thrombosis [DVT])
- Pulmonary embolism (PE)
- Abdominal wound dehiscence
- Organ deemed not transplantable after retrieval
- Stroke
- Psychosis
- Ileus
- Bowel obstruction
- GI Bleeding (upper or lower)
- Cerebrovascular accident
- Peripheral vascular clotting or occlusion due insertion of mechanical support or equivalent
- Delirium, confusion and neurological complications
- Hepatic coma
- Retransplantation
- Limb gangrene due to vascular occlusion due insertion of mechanical support
- Use of mechanical circulatory support
- Coagulopathy
- Blood product transfusion
- Transfusion reaction
- Hyperacute rejection
- Anastomotic site complications; narrowing, bleeding or occlusion
- Bowel thromboembolic complications and gangrene
- Protamine and other anti-heparin medication reaction
- Heparin induced thrombocytopenia

8.4. Unanticipated Adverse Device Effect (UADE)

An UADE means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified or encountered before at least once in standard clinical practice, in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.5. Recording and Reporting of Adverse Event

All Liver graft-related adverse events and serious adverse events are to be recorded on the electronic case report forms until post-transplant day 30 or until initial hospital discharge, whichever comes first. The description of the adverse event will include: the date of onset, duration, severity, seriousness, the relationship of the event to the trial treatment, anticipated or not, and any treatment required. All serious adverse events occurring during the course of the first 30 days post-transplant will be reported to TransMedics, Inc., as well as documented on the appropriate electronic case report form(s). For all SAEs, the investigator is required to supply any additional data that may be deemed necessary by the Sponsor. Additionally any serious adverse events (SAE) and unanticipated adverse device effects (UADE) should be reported to TransMedics, Inc., preferably within 48 hours of the time the investigator learns of the event, but in no case later than 5 working days. Liver graft-related AEs will be recorded up to the 30-day follow-up or through hospital discharge only if longer than 30 days. For any particular patient, the Independent Medical Monitor if required to protect patient safety may specify a different follow-up period. The Sponsor is responsible for the classification and reporting of Liver graft-related adverse events to the appropriate regulatory authorities, and for the on-going safety evaluation of the trial in accordance with ISO 14155 and governing regulatory requirements.

8.6. Relationship of an Adverse Events to OCS™ Liver (OCS) or Standard of Care (Control)

The investigator will assess the relationship of the AE to the OCS or Control methods of preservation. The relationship will be assessed using the following categories:

- ***Definitely Related:*** There is a reasonable causal and temporal relationship between preservation with the OCS™ Liver and the adverse event.
- ***Probably Related:*** It is more likely than not that there is a reasonable causal relationship between preservation with the OCS™ Liver the adverse event.
- ***Possibly Related:*** There is a reasonable relationship with preservation with the OCS™ Liver and the adverse event, but the causal relationship is unclear or lacking.
- ***Unlikely Related:*** There is a temporal relationship with preservation with the OCS™ Liver and the adverse event, but there is not a reasonable causal relationship between the trial device and the event.
- ***Unrelated:*** There is no relationship between preservation with the OCS™ Liver and the adverse event.

8.7. Severity

The investigator will rate the severity of the adverse event using the following categories:

- ***Mild:*** The adverse event is transient and/or easily tolerated by the subject.
- ***Moderate:*** The adverse event causes the subject discomfort and interrupts the subject's usual activities.
- ***Severe:*** The adverse event causes considerable interference with the subject's usual activities.

8.8. Pre-Existing Conditions

Pre-existing diseases or conditions will not be reported as adverse events.

9. STATISTICAL METHODS

9.1. General

Continuous variables will be summarized using descriptive statistics, specifically the mean, median, standard deviation, minimum, and maximum. Categorical variables will be summarized using frequencies and percentages.

9.2. Analysis Populations

9.2.1. Per Protocol Population

The Per Protocol (PP) Population will consist of all randomized subjects who are transplanted and have no major protocol violations and for whom the donor liver received the complete preservation procedure as per the randomization assignment. The major protocol violations that will exclude a subject from this population are the following:

- Ineligible for the study according to the recipient inclusion and exclusion criteria
- Ineligible for the study according to the donor organ inclusion and exclusion criteria
- Subject is transplanted with a liver with preservation other than that to which the subject was randomized
- Failure to complete adequate post-transplant assessments to support the primary, secondary or safety endpoints
- Other major protocol violations

In analyses based on the PP Population, subjects will be analyzed as randomized. The primary analysis of effectiveness and of secondary and other endpoints will be based on the PP Population.

9.2.2. Modified Intent-to-Treat Population

The Modified Intent-to-Treat Population (mITT) will consist of all randomize subjects who are transplanted. In analyses based on mITT Population, subjects will be analyzed as randomized. The mITT analyses will be considered secondary analyses of effectiveness.

9.2.3. As Treated Population

The As Treated Population (AT) will consist of all treated subjects, i.e., all subjects who are transplanted in the study with a donor liver preserved with either OCS or Control. In analyses based on this population, subjects will be analyzed as treated. A subject who receives a liver with some preservation with OCS and some with standard of care will be analyzed according to the initial preservation. After the initiation of preservation, any switchover in preservation method should be recorded as a protocol deviation and those subjects will not be considered part of the AT population. Analyses of safety endpoints will be performed based on the AT Population

9.2.4. Donor Liver Population

The Donor Liver Population will consist of all donor livers for which the potential recipient was randomized and which have preservation initiated using OCS or Control. In some of the analyses based on this population, livers will be analyzed as randomized; in others, they will be analyzed as treated. For the analyses based on the actual treatment of the liver, a liver with some preservation with OCS and some with standard of care will be analyzed according to the initial preservation.

9.3. Analysis of Effectiveness Endpoints

9.3.1. Primary Effectiveness Endpoint

The primary effectiveness endpoint for this study is Early Liver Allograft Dysfunction (EAD), defined as presentation of one or more of the following criteria:

- AST level >2000 IU/ml within the first 7 postoperative days;
- Bilirubin ≥10 mg/dl on postoperative day 7;
- INR ≥1.6 on postoperative day 7; or
- Primary non-functioning graft within the first 7 days

The primary hypothesis for this study is that the OCS treatment is non-inferior to the standard of care treatment with respect to this endpoint. The primary statistical hypotheses are as follows:

H_{10}: $\pi_{1,OCS} \geq \pi_{1,CONTROL} + \delta$ and
H_{11}: $\pi_{1,OCS} < \pi_{1,CONTROL} + \delta$,

where $\pi_{1,OCS}$ and $\pi_{1,CONTROL}$ are the true proportions of subjects with Early Liver Allograft Dysfunction for the OCS and standard of care treatments, respectively, and δ is the non-inferiority margin which is here taken to be 0.075. If non-inferiority is demonstrated using a significance level of 0.05, a two-sided test of superiority will be performed.

The primary effectiveness endpoint will be analyzed by calculating, for each treatment group, the sample proportion of subjects meeting the primary effectiveness endpoint, as well as an exact (Clopper-Pearson) 95% confidence interval for the corresponding population proportion. The 95% upper bound of the exact unconditional one-sided confidence interval based on the Farrington and Manning score statistic[18] will be calculated for the difference between the two population proportions ($\pi_{1,OCS} - \pi_{1,CONTROL}$). An upper confidence limit less than $\delta = 0.075$ will result in rejection of the null hypothesis (H_{10}) in favor of the alternative hypothesis (H_{11}) and the

demonstration of non-inferiority of OCS to control for the primary effectiveness endpoint. In the event non-inferiority is demonstrated, Fisher's exact test (two-sided) will be used to test for superiority.

This endpoint will be analyzed using the Per Protocol and mITT Populations. The Per Protocol analysis will be considered the primary analysis. Multiple imputation methods will be used for data imputation for any patients with missing values for this endpoint.

Subgroup analyses of the primary effectiveness endpoint will be performed for the following subgroups of patients:

- DCD (donation after circulatory death) patients
- Fatty liver patients
- Other liver patients

No data imputation or statistical tests will be performed for these subgroup analyses.

9.3.2. Secondary Effectiveness and OCS Donor Liver Assessment Endpoints

The secondary effectiveness endpoints for this trial are as follows:

- OCS Measurements during organ perfusion
- Patient survival at day-30 post-transplantation
- Patient survival at time of initial hospital discharge post-transplantation

The null and alternative hypotheses for the endpoint of OCS donor liver assessment during perfusion are:

H_0: $\pi_3 \leq 0.85$ and

H_1: $\pi_3 > 0.85$,

respectively, where π_3 is the true proportion of livers, among donor livers preserved using OCS for the entire preservation period, on which measurements of lactate level, average bile production rate, Hepatic Artery and Portal Vein Pressure during perfusion will be available on OCS device before transplant.

This secondary endpoint on OCS liver assessment during organ perfusion will be analyzed by calculating the sample proportion of donor livers placed on OCS meeting this secondary endpoint, as well as an exact (Clopper-Pearson) 95% one-sided lower confidence bound for the corresponding population proportion. A lower confidence bound greater than 0.85 will result in rejection of the null hypothesis in favor of the alternative hypothesis and the demonstration that the true proportion is greater than 0.85 for the OCS donor liver assessment endpoint.

Each secondary effectiveness endpoint will be summarized using counts and percentages and an exact 95% confidence interval for the true percentage based on the binomial distribution. The secondary effectiveness endpoints will be analyzed using the PP Population.

The statistical hypotheses for the first secondary effectiveness endpoint, patient survival at day-30 post-transplantation, are as follows:

H_{20}: $\pi_{2,OCS} \leq \pi_{2,CONTROL} - \delta$ and

H_{21}: $\pi_{2,OCS} > \pi_{2,CONTROL} - \delta$,

where $\pi_{2,OCS}$ and $\pi_{2,CONTROL}$ are the true proportions of the subjects surviving to day-30 post-transplantation for the OCS and standard of care treatments, respectively, and δ is the non-inferiority margin, which is here taken to be 0.075. This endpoint will be analyzed by calculating the 95% upper confidence limit based on the normal approximation for the difference between the two population proportions ($\pi_{2,CONTROL} - \pi_{2,OCS}$). An upper confidence limit less than $\delta = 0.075$ will result in rejection of the null hypothesis (H_{20}) in favor of the alternative hypothesis (H_{21}) and demonstration of non-inferiority of OCS to Control. In the event non-inferiority is demonstrated, Fisher's exact test (two-sided) will be used to test for superiority.

The second secondary effectiveness endpoint, patient survival at initial hospital discharge post liver transplantation, will be analyzed in a manner analogous to the first secondary effectiveness endpoint with the same non-inferiority margin of 0.075.

Because fixed sequence testing will be used for the secondary endpoints, no adjustment for the multiplicity of these endpoints needs to be made. The endpoints will be tested in the order listed above. The test for non-inferiority for the first secondary effectiveness endpoint will be performed only if the null hypothesis has been rejected for the OCS donor liver assessment endpoint. The test for non-inferiority for the second secondary effectiveness endpoint will be performed only if the null hypothesis has been rejected in favor of the alternative hypothesis of non-inferiority of the OCS treatment to the Control treatment for the first secondary effectiveness endpoints. Similarly, the test for superiority for the second secondary effectiveness endpoint will be performed only if the null hypothesis of equality has been rejected in favor of superiority of the OCS treatment to the Control treatment for the first secondary effectiveness endpoints (and non-inferiority has been demonstrated for the given secondary effectiveness endpoint). Due to statistical power limitations, it is not expected that non-inferiority will be demonstrated for patient survival at day 30 or at initial hospital discharge.

These endpoints will be analyzed using the Per Protocol and mITT Populations, with the Per Protocol analysis being considered the primary analysis. Multiple imputation methods will be used for data imputation for patients with missing values for these endpoints.

Subgroup analyses of the secondary endpoints will be performed for the same subgroups of patients as for the primary effectiveness endpoint. No data imputation or statistical tests will be performed for these subgroup analyses.

9.4. Analysis of Safety

Safety will be analyzed principally by examination of the frequency of liver graft-related serious adverse events (SAEs) up to the 30-day follow-up after transplantation. This endpoint is defined as the average number of liver graft-related serious adverse events through the 30 days post-liver transplantation per subject, consisting of the following serious adverse events (at most one per type per person):

- Primary non-function (defined as irreversible graft dysfunction requiring emergency liver re-transplantation or death with the first 10 days, in the absence of immunologic or surgical causes);
- Ischemic biliary complications (ischemic biliary strictures, and non-anastomotic bile duct leaks);

- Vascular complications (Liver graft-related coagulopathy, hepatic artery stenosis, hepatic artery thrombosis and portal vein thrombosis);
- Liver allograft infections (liver abscess, cholangitis, etc.)

This endpoint will be summarized by treatment group using descriptive statistics. For each treatment group, a 95% confidence interval for the mean based on the t-distribution will be presented. Also, a 95% confidence interval based on the t-distribution will be presented for the difference in means between the two treatments.

For the number of liver graft-related SAEs, the statistical hypotheses are as follows:

H_{30}: $\mu_{OCS} \geq \mu_{CONTROL} + \delta$ and

H_{31}: $\mu_{OCS} < \mu_{CONTROL} + \delta$,

where μ_{OCS} and $\mu_{CONTROL}$ are the true mean numbers of liver graft-related SAEs up to the 30-day follow-up after transplantation per subject with the OCS and standard of care treatments, respectively, and δ is the non-inferiority margin, which is here taken to be 1.00. The safety endpoint will be analyzed using a one-sided, two-sample t-test with an alpha level of 0.05. If non-inferiority is demonstrated, a corresponding (two-sided) test of superiority will be performed.

This endpoint will be analyzed based on the As Treated Population.

In addition, the numbers and percentages of subjects experiencing at least one liver graft-related AE, at least one (definitely or probably-related) device-related AE, at least one unanticipated AE, and at least one serious AE, and the number and percentage of deaths will all be tabulated by treatment group. Also, the number of liver graft-related adverse events and the number and percentage of subjects experiencing liver graft-related adverse events will be tabulated by system organ class and preferred term using MedDRA. A similar analysis will be performed for liver graft-related SAEs. AEs will also be tabulated at the event level by system organ class and preferred term and the relationship of the adverse event to the device using counts and percentages. Similar analyses will be performed by the severity of the adverse event.

The numbers and percentages of donor livers in the Donor Liver Population that are treated as randomized and transplanted, treated not as randomized and transplanted, treated as randomized and not transplanted, and treated not as randomized and not transplanted will be presented. For livers that are treated not as randomized, the reason will also be summarized using counts and percentages. Both of these analyses will be done based on the randomized treatment for the liver, but the following analyses will be based on the actual treatment of the liver. For livers that are not transplanted, the reason(s) for non-transplantation (including device failure) will be summarized using counts and percentages. The number and percentage of donor livers in the Donor Liver Population for which these was a device failure will also be presented.

9.5. Randomization

After confirmation of eligibility, obtaining informed consent, and a matching donor liver is identified, potential liver transplant recipients will be randomized 1:1 to have their donor livers preserved using either the OCS Liver perfusion or the standard cold static preservation technique using cold flush and storage. Randomization will be performed through the Interactive Web Response System (IWRS). Subjects who are not transplanted with the matching donor liver will be re-randomized and treated as a new subject.

9.6. Sample Size Determination

The sample size for this trial was determined based on the primary effectiveness endpoint, Early Liver Allograft dysfunction (EAD) in the first 7 days post-transplantation. The sample size calculation assumed a one-sided, normal approximation test for non-inferiority, an alpha level of 0.05, a non-inferiority margin of 0.075, a 1:1 allocation, true proportions for the primary effectiveness endpoint of 0.2 for the OCS treatment and 0.25 for the Control treatment, and power of 80%. Based on these specifications, the required sample size was determined to be 144 transplanted recipients per treatment group, or 288 total transplanted subjects. To ensure an adequate number of subjects in the Per Protocol Population, the sample size was increased to a total of 300 transplanted subjects. Subjects will be enrolled until there are either 290 subjects in the Per Protocol Population or a total of 300 transplanted subjects, whichever comes first.

9.7. Sample Size Re-Estimation

A sample size re-estimation will be performed for the purpose of confirming the current sample size is sufficient for testing for non-inferiority for the primary effectiveness endpoint and to assess the feasibility of demonstrating superiority. It will be conducted after there are 200 transplanted subjects to allow for a possible upwards adjustment in sample size. The increase in sample size, if any, required in order to obtain 80% conditional power for a test for non-inferiority for the primary efficacy endpoint will be determined. The increase in sample size, if any, required to obtain 80% power for a two-sided test for superiority for the primary effectiveness endpoint will also be determined. In both cases, conditional power will be calculated based on the observed proportions for the primary effectiveness endpoint. The sample size will not be increased by more than 150 subjects. If the required sample size increase is less than or equal to 150 subjects for both the test for non-inferiority and the test for superiority, the increase in sample size will be based upon the result for the test for superiority, which will be the larger increase. However, if the required sample size increase is less than or equal to 150 subjects for the test for non-inferiority, but greater than 150 for the test for superiority, the increase in sample size will be based upon the result for the test for non-inferiority. If the conditional power based upon the planned sample size is at least 80% for both the test for non-inferiority and the test for superiority or the required increase in sample size is greater than 150 subjects for both cases, the sample size will not be increased.

9.8. Interim Analyses

For regulatory purposes, descriptive summaries and/or data listings of key data will be provided to the FDA based on the first 10 transplanted liver recipients in Part A of the study after they have completed 30 days post-transplantation and on the 20 transplanted liver recipients in Part A of the study after they have completed 30 days post-transplantation.

9.9. Statistical Analysis Plan

A formal statistical analysis plan will be prepared and finalized prior to data lock.

10. RISK ANALYSIS

This clinical trial has been designed to ensure that the benefits and knowledge gained from the trial outweigh the potential risks to the subjects. The subjects are adults undergoing primary Liver transplants.

10.1. Potential Risks

The potential risks to subjects from participation in this clinical trial include the following:

- Potential Risks Associated with Liver Transplant Procedures: These risks include post-operative complications, such as graft failure, primary graft dysfunction, rejection, infection and other organs/systems complications, graft vessel disease (an expression of chronic rejection), infection, abnormal kidney function, diabetes, high level of cholesterol, high blood pressure, cancer and neurological complications.
- The Potential Risks Associated with the Investigational Device: As with any medical device, there is always a risk of extremely rare or previously unknown side effects developing from the treatment.

10.2. Manner in Which the Potential Risks Have Been Minimized

The Sponsor has relied upon a number of different means, including the device design, risk analysis and management process, preclinical testing, and the clinical protocol itself, to minimize the risks to subjects and to protect their safety and welfare. The sponsor has designed the device and conducted a risk analysis in accordance with ISO 14971 to minimize and mitigate the risks to subjects and users.

The Sponsor has conducted extensive preclinical testing of the OCS™ Liver to demonstrate its safety, effectiveness and readiness for clinical use. The OCS has undergone extensive preclinical and animal studies to demonstrate that the device performs as intended. These studies strongly indicate that the OCS™ Liver maintains Liver viability by providing a controlled environment that simulates near-normal physiological conditions, and monitors its function. The Liver Perfusion Set has been tested for biocompatibility to minimize the risk of adverse tissue reactions. These test results demonstrate the device and its materials are biocompatible and suitable for their intended use. The Liver Perfusion Set are provided sterile and for single use to minimize the risk of infection. The OCS™ Liver has also undergone and continues to undergo extensive design verification and validation testing to optimize its performance and minimize the risks associated with its use. Preclinical studies demonstrate the device performs as intended and meets its performance specifications including the perfusion pump, the blood warmer, and other functions. The software has undergone and continues to undergo extensive testing to demonstrate it, and its safety functions and alarms, perform as intended.

This clinical protocol incorporates several procedures to minimize the risks to subjects and to ensure the benefits of the clinical trial outweigh its potential risks.

- Subjects will be monitored before, during and after the operative procedure to help ensure their safety. The investigators are members of transplant teams who have extensive experience with Liver transplants and who will be trained to use the OCS™ Liver to further minimize risk.
- Subjects in the trial will undergo frequent visits and routine monitoring to help detect any abnormal changes and to provide appropriate treatment as necessary.
- The trial will be monitored to ensure the identification, documentation, and analysis of adverse events; and to ensure compliance with the protocol and procedures that are in place for conducting research to protect the safety and well-being of all subjects.

10.3. Potential Benefits

The OCS™ Liver system's preservation and assessment capabilities could potentially increase the rate of utilization of donor Livers that are currently less utilized due to the limitations of cold storage techniques. This could dramatically improve the chances of waiting list recipients to receive a lifesaving Liver transplant and reduce waiting list time and mortality.

In addition, the OCS™ Liver's physiologic preservation of donor Livers could result in improved short and long-term post-transplant outcomes in the form of increased survival and lower graft dysfunction/rejection rates.

10.4. Risks Benefits Ratio

Based on the above, the benefits of using OCS™ Liver technology to preserve and assess donor Livers to ensure their suitability for Liver transplantation outweigh any potential risks to the trial subjects.

11. DEVICE/SITE MANAGEMENT

11.1. Packaging and Labeling

The OCS™ Liver Perfusion Set and accessories will be supplied sterile and are intended and labeled for single use.

The OCS™ and its components will be clearly labeled as an investigational device according to 21 CFR 812.5. The labeling provides instructions for use for the device. A copy of the Instructions for Use will be provided to each investigational site.

11.2. Storage

The investigational devices will be stored in a secure place. Access should be strictly limited to the investigators and their designees. Neither the investigators nor any designees may provide the investigational device to any subject not participating in this trial. Special storage instructions for the components are described below. The OCS™ Liver Perfusion Set should be stored at temperatures between -20°C and 50°C, and ambient humidity from 10-95%, no condensing.

Note: The OCS™ Liver Perfusion Set should be operated at ambient temperatures (10°C to 35°C), and ambient humidity (20%-90%).

11.3. Accountability

The investigator or designee will maintain an inventory record of investigational devices received, used for treatment, otherwise discarded, and returned to the Sponsor to assure FDA and the Sponsor that the investigational new device will not be dispensed to any person who is not a subject under the terms and conditions set forth in this protocol.

11.4. Device Complaints and Malfunctions

The investigator will inform the Sponsor of any complaints or malfunctions during the course of the trial. The Sponsor will investigate all device complaints and malfunctions.

12. REGULATORY / ETHICS

This clinical trial will be conducted in accordance with the requirements of the FDA Investigational Device Exemptions regulation (21 CFR Parts 50, 56, 812, and 45 CFR part 46), ISO Standard 14155, and in accordance with good clinical practices.

12.1. Institutional Review Boards (IRB) or Ethics Committee (EC)

In accordance with the conditions imposed by the reviewing Institutional Review Board (IRB) or Ethics Committee (EC) to applicable regulations from Federal agency (i.e. U.S. Food and Drug Administration (FDA), with local regulations, prior to initiation of any trial procedures, the protocol, informed consent template and device labeling (if requested) will be submitted to each site's IRB or EC for review and approval. In addition, any amendments to the protocol or informed consent form will be reviewed and approved (if necessary) by the IRB or EC. The Sponsor must receive a letter documenting the IRB's or EC's approval at the clinical site prior to the initiation of the trial at that particular site.

12.2. Informed Consent

Written informed consent will be obtained from all subjects before any trial-specific procedures are performed. Informed consent will be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of the research trial.

Investigators have both an ethical and legal responsibility to ensure that each patient being considered for inclusion in this trial is given a full explanation of the protocol. This will be documented via a written informed consent form approved as part of the full trial approval granted by the Institutional Review Board (IRB) or Ethics Committee (EC) for the site. Each informed consent form will include the elements required by 21 CFR Part 50. The investigator agrees to also obtain approval from the Sponsor and IRB/EC for any written informed consent form used in the trial.

The approved written informed consent form will be signed and dated by the subject and the individual obtaining the consent. The subject will be given a copy of the signed informed consent form. The original will be kept in the patient's file by the investigator.

A copy of the proposed draft Informed consent template is included as Appendix 3.

13. DATA COLLECTION/RECORDS/REPORTS

13.1. Investigator Records

Prior to participation in the investigation, the investigator will provide the following documentation to the Sponsor:

- Investigator Agreement, signed by the investigator and disclosure of any financial interest;
- A copy of the primary investigator's curriculum vitae (CV), as well as copies of CVs for any co-investigators;
- Written approval of the trial from the IRB or EC; and,
- A copy of the approved informed consent document.

During the trial, investigators will be responsible for complete and accurate entry of data into the trial's database, and will be required to maintain on file the following accurate, complete and current records relating to this trial:

- All relevant correspondences and required reports that pertain to the trial;
- Records of receipt, use or disposition of the investigational device, including the type and quantity of the device; the dates of receipt; the lot number; the names of all persons who received, used or disposed of each device; and why and how any units of the device have been returned to the Sponsor, repaired, or otherwise disposed;
- Records of each subject's case history and exposure to the device;
- Signed and dated consent forms;
- Relevant observations, including records concerning adverse events, condition of each subject upon entering and results of diagnostic tests;
- Protocol, and any amendments;
- Subject recruiting materials; and,
- Investigator curricula vitae.

The investigator will not dispose of any records relevant to this trial without (1) written permission from the Sponsor and (2) providing an opportunity for the Sponsor to collect such records. The investigator will take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this trial. Such documentation is subject to inspection by the Sponsor and regulatory authorities.

13.2. Investigator Reports

In accordance with the FDA reporting requirements, the investigators will be required to prepare and submit to the Sponsor the following complete, accurate, and timely reports on this investigation when necessary:

- The investigator will notify the Sponsor of a subject death occurring during the investigation as soon as possible, preferably within 24 hours of learning of the subject's death, but in no event later than 48 hours. The investigator will also notify the Sponsor immediately of a serious adverse event, preferably within 48 hours of learning of the serious adverse event, but in no event later than 5 working days.
- The investigator will notify the Sponsor of any unanticipated adverse device effects (UADE) preferably within 48 hours after the investigator first learns of the effect, but in no event later than 5 working days. The investigator will notify its IRB or EC of any unanticipated adverse device effects as soon as possible, but no later than 10 working days after the investigator first learns of the effect.
- The investigator will notify the Sponsor of the withdrawal of IRB or EC approval as soon as possible, but no later than 5 working days after the investigator first learns of the withdrawal
- The investigator will provide current progress reports to the Sponsor and reviewing IRB or EC at regular intervals but at least on an annual basis.

- The investigator will notify the Sponsor and the IRB or EC of any deviation from the investigational plan to protect the life and physical well-being of a subject in an emergency as soon as possible, but no later than 5 working days after the emergency occurred.
- The investigator will notify the Sponsor and IRB or EC that an informed consent was not obtained from a subject as soon as possible, but no later than 5 working days after such an occurrence.
- The investigator will provide a final summary report within 3 months after termination or completion of the trial to the IRB or EC. The site trial completion report may serve as the trial completion for the Sponsor
- The investigator will provide any other information upon the request of the IRB or EC, or the Sponsor.

13.3. Data Collection

During each subject assessment, an investigator participating in the trial will record progress notes to document all significant observations. In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes. For transmission to the Sponsor, information from the trial progress notes and other source documents will be promptly entered into the electronic database (eCRFs).

All data required by the trial protocol will be completely and accurately entered into the trial database by the investigator or his or her designate. A copy of draft eCRFs is provided in Appendix 4.

13.4. Source Documents

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents will include, but are not limited to, progress notes, electronic data, computer printouts, screening logs, and recorded data from automated instruments. All source documents pertaining to this trial will be maintained by the investigators and made available for inspection by authorized persons.

13.5. Archiving of Records

Essential trial documents must be maintained by the Investigator for at least 2 years after the last marketing approval by a regulatory body, as determined by the Sponsor. The documents should be retained for a longer period, however, if required by the applicable regulatory requirements. Records will be kept in a secure, dry location controlled by the institution.

13.6. Sponsor Records and Reports

The Sponsor will conform to all records and reports requirements imposed by FDAs regulations.

13.7. Final Study report

All study data will be reviewed and monitored throughout the trial. A formal statistical analysis will be conducted in which validated data will be produced to support the overall analysis of the trial endpoints. The formal statistical data analysis will be shared with the Steering Committee and Study Investigators.

The Sponsor will work collaboratively with the Steering Committee and Study Investigators to publish all positive and negative outcomes of the study in peer review journals and at national/international scientific conferences within 18 months of the completion of the study analysis. If the study is terminated early for any negative or positive findings, the above-described process will begin at the date of the early termination of the study.

14. CLINICAL MONITORING

14.1. Monitoring

The Sponsor has ethical, legal and scientific obligations to carefully follow this trial in a detailed and orderly manner in accordance with established research principles. As part of a concerted effort to fulfill these obligations (maintain current personal knowledge of the progress of the trial), the Sponsor's monitors will visit the center during the trial in addition to maintaining frequent telephone and written communications. The following guidelines are provided to describe the Sponsor's procedures for monitoring the clinical studies. If the investigator is not complying with the signed Investigator Agreement, the protocol, or any condition of the trial, (e.g., incomplete data forms) the Sponsor has the right to terminate the investigator's participation in the trial. The Sponsor is responsible for selecting trial monitors qualified by training and experience to conduct monitoring of the trial and for ensuring the quality of the trial monitoring visits by the monitor. The Sponsor's general monitoring procedures for investigational studies are described below.

14.2. Pre-Trial Monitoring Procedures

14.2.1. Selection of Monitors

All monitors will be qualified by education, training, and experience.

14.2.2. Initiation Visit

A monitor will be responsible for determining and documenting that each investigator clearly understands and accepts the responsibilities and obligations incurred in conducting a clinical trial. The monitor will ensure, prior to trial initiation, that the investigator:

- Understands the requirements for a well-controlled trial.
- Understands the nature of the clinical protocol.
- Understands his/her reporting obligations.
- Understands the requirements for device accountability.
- Understands and accepts the obligations to obtain informed consent.
- Understands and accepts the obligation to obtain IRB or EC review and approval of the clinical investigation before it is initiated and to ensure continuing review of the trial by the IRB or EC, and to keep the Sponsor informed of all IRB or EC actions concerning the trial.
- Has adequate facilities, support staff, and access to an adequate number of suitable subjects to conduct the investigation.

- Has the required documentation on file, including IRB or EC approval and a signed investigator agreement.
- Financial disclosure information will be obtained.

14.3. Periodic Monitoring Visits

Monitoring visits will be conducted as scheduled by the sponsor. The monitor should visit each site as needed to ensure the following:

- Facilities continue to be adequate and acceptable.
- Informed consent has been obtained.
- The protocol is being properly followed.
- The IRB or EC has approved or been notified of any protocol changes.
- Accurate, complete and current records are being maintained, and the information recorded and submitted to the Sponsor is representative of the subject's record and other supporting documentation.
- Accurate, complete and timely adverse event reports are being submitted to the Sponsor.
- The reason for a subject's withdrawal from the trial has been documented.
- Reports are being submitted to the IRB or EC and Sponsor.
- The appropriate staff is carrying out trial activities.
- SAEs identified and reported in a timely fashion

14.4. Frequency of Monitoring Visits

The frequency of monitoring visits will be determined on the basis of several factors, including the duration of the trial, number of subjects enrolled, number of investigators/sites, complexity of the trial, and number of outstanding issues from previous visits. All routine monitoring functions will be performed prior to the trial termination.

14.5. Trial Completion Visit

The trial termination visit may be combined with a monitoring visit. The following tasks will be completed at the last visit by the monitor.

- Ensure that all electronic forms have been completed.
- Ensure that any database queries have been resolved.
- Remind the investigator of the obligation to retain the records.

14.6. Reports of Monitoring Visits

Monitoring reports will be completed for all visits. Reports will include the date of the visit, list of trial personnel present, and a summary of the findings.

14.7. Additional Auditing

Regulatory authorities worldwide may also audit the investigator during or after the trial. The investigator will contact the Sponsor immediately if this occurs, and will fully cooperate with the audits conducted at a reasonable time in a reasonable manner.

14.8. Protocol Deviations

This trial will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of a subject requires a protocol deviation, based on the judgment of the investigator (or a responsible, appropriately trained professional designated by the investigator). If the deviation from the protocol is necessary to protect the life and physical well-being of a subject in an emergency, such protocol deviations will be reported to the Sponsor and the IRB or EC as soon as possible, but no later than 5 working days after the emergency occurred. In the event of a significant deviation from the protocol due to an accident or mistake, the investigator or designee will contact the Sponsor at the earliest possible time by telephone to discuss the deviation and its impact on the trial and subject continuation in the trial. These discussions will be documented by the investigator and the Sponsor, reviewed by the monitor, and documented on the Protocol Deviation eCRF.

14.9. Medical Monitor

The Sponsor will utilize an independent Medical Monitor to provide individual serious adverse event adjudication for the trial. It is anticipated that the Medical Monitor will meet with the Sponsor on a periodic basis, or as needed, depending on the rate of patient accrual. The primary responsibilities of the Medical Monitor are to:

- Review serious adverse events that occur over the course of the trial and the subsequent classification of these adverse events as related to the device
- Review donor and recipient treatment group eligibility issues.
- Evaluate possible protocol deviations.
- Provide oversight for issues affecting general patient welfare.
- Provide recommendations to extend the length of follow-up past 30 days post-transplant for a subject experiencing a serious adverse event.
- Review all site reported EAD diagnosis based on the pre-specified protocol criteria and actual clinical case of the trial patient.

14.10. Stopping Rule

The stopping rule will be confirmed every time a trigger event occurs (i.e., each time a subject in either arms has experienced an early liver dysfunction or death). In addition, Stopping rule analyses will be conducted twice, once after the first 10 transplanted liver recipients in Part A of the study have completed 30 days post-transplantation and a second time after the 20 transplanted liver recipients in Part A of the study have completed 30 days post-transplantation. The stopping rule will be based on the occurrence of either early liver graft dysfunction within 7 days or death within 30 days post-transplantation. The null and alternative hypotheses to be tested are as follows:

H_{40}: $\pi_{3,OCS} \leq \pi_{3,CONTROL}$ and

H_{41}: $\pi_{3,OCS} > \pi_{3,CONTROL}$,

where $\pi_{3,OCS}$ and $\pi_{3,CONTROL}$ are the true proportions of subjects with either early liver graft dysfunction within 7 days or death within 30 days post-transplantation for the OCS and Control treatments, respectively. A one-sided Fisher's Exact Test will be conducted to test the null hypothesis H_{40}, and the study will be stopped if the p-value is less than 0.15.

The table below provides an example indicating the circumstances under which we would stop the study and those under which we would continue the study for the analysis to be performed after the 20 transplanted liver recipients in Part A of the study have completed 30 days post-transplantation, assuming 10 recipients in each treatment group.

Conditions Under which the Study Would be Stopped									
Number of CONTROL Patients Experiencing an Event	**Number of OCS Patients Experiencing an Event**								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0	Cont.	Cont.	Cont.	Stop	Stop	Stop	Stop	Stop	Stop
1	Cont.	Cont.	Cont.	Cont.	Stop	Stop	Stop	Stop	Stop
2	Cont.	Cont.	Cont.	Cont.	Cont.	Stop	Stop	Stop	Stop
3	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Stop	Stop	Stop
4	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Stop	Stop
5	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Stop
6	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.
7	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.
8	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.
Key: Note: Cont. = Continue									

TransMedics will be responsible for implementing the stopping rule and will stop the trial if a stopping rule was triggered until the data is reviewed by the DMB.

14.11. Data Monitoring Board

An independent Data Safety Monitoring Board (DMB) will be established by the Sponsor to periodically assess the progress of the trial, the safety data and the primary effectiveness and safety endpoints. The DSMB will make recommendations to the Sponsor regarding continuation, modification or termination of the clinical trial. The DMB will review all data submitted to them by the Sponsor and may request additional information to assist in their decision process. They will attend scheduled meetings and issue written minutes of their meetings; furthermore, the appointed Chair will be responsible for issuing final written recommendations.

14.12. Investigator Training

Device, protocol and electronic database training will be provided to all the participant investigators and support staff prior to patient enrollment in the trial. Device training will be conducted at the TransMedics clinical training facility or equivalent training facility. Protocol training will include a thorough review of this protocol. Electronic database training will consist of an explanation of the structure of the database, the data elements to be collected, simulated use of the database, error handling, and instructions regarding the handling of queries.

15. CONFIDENTIALITY

Confidentiality agreements will be executed prior to the study related activities commencing. All information generated in this trial will be considered highly confidential and must not be disclosed to any persons not directly concerned with the trial without written prior permission from the Sponsor. Authorized regulatory officials and Sponsor personnel (or their representatives) will be allowed full access to inspect and copy the records. All investigational devices, subject bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor. Subjects will be identified only by initials and unique subject numbers on the case report forms. If necessary, their full names may be made known to the Sponsor, a regulatory agency, or other authorized officials.

16. AMENDMENT POLICY

The investigator will not make any changes to this protocol without prior written consent from the Sponsor and subsequent approval by the IRB or EC, except if the deviation from the protocol is necessary to protect the life and physical well-being of a subject in an emergency. Such protocol deviations will be reported to the Sponsor and the reviewing IRB or EC as soon as possible, but no later than 5 working days after the emergency occurred. Any permanent change to the protocol, whether it is an overall change or a change for specific trial center(s), will be handled as a protocol amendment. Any amendment to the protocol that appears indicated as the trial progresses will be fully discussed by the investigator(s) and the Sponsor. If agreement is reached regarding the need for an amendment, it will be written by the Sponsor. The written amendment will be submitted to the chairman of the IRB or EC responsible for reviewing amendments. Except for "administrative letters," investigators will await IRB or EC approval of protocol amendments before implementing the change(s). Administrative letters are defined to have no effect on the validity of the data or information resulting from the completion of the approved protocol, or the relationship of likely patient risk to benefit relied upon to approve the protocol; the scientific soundness of the investigational plan or protocol; and, the right, safety or welfare of the human subjects involved in the investigation. The Sponsor will notify the FDA of such changes in a 5-Day Notice. When, in the judgment of the IRB or EC, the investigators and/or the Sponsor, the amendment to the protocol substantially alters the trial design and/or increases the potential risk to the subject; such changes will be approved by the FDA and the IRB and EC.

17. REFERENCES

1. OPTN 2011/Scientific Registry of Transplant Recipients (SRTR) 2011 Annual Report: Transplant data 2011.
2. Yuan Zhai, Henrik Petrowsky, et al. Ischemia-Reperfusion Injury in Liver Transplantation-From Bench to Bedside. Nat Rev Gastroenterol Hepatol. 2013 February; 10 (2): 79-89.
3. Jason A. Wertheim, MD, PhD, Henrik Petrowsky, MD, Et al. major Challenges Limiting Liver Transplantation in the United States. Am J Transplant. 2011 September; 11 (9): 1773-1784.
4. Thomas Vogel, Jens G. Brockmann, et al. The Role of Normothermic Extracorporeal Perfusion in Minimizing Ischemia Reperfusion Injury. Transplantation Reviews 26 (2012) 156-162
5. Shawn D St Peter, Charles J Imber, Peter Friend. Liver and Kidney Preservation by Perfusion. The Lancet, vol 359, February 16, 2002
6. Jens Brockmann, MD, Srikanth Reddy , FRCS, et al. Normothermic perfusion a New Paradigm for Organ Preservation. Annals of Surgery. Volume 250, Number 1, July 2009; 250:1-6
7. Andrew J. Butler, Michael A. Rees, et al. Successful Extracorporeal Porcine Liver perfusion For 72 Hr. Transplantation. Vol. 73, 1212-1218, No. 8, April 27, 2002
8. Constantino Fondevila, MD, PhD, Amelia J. Hessheimer, MD, et al. Superior Preservation of DCD Livers With Continuous Normothermic Perfusion. Annals of surgery. Volume 254, Number 6, December 2011.
9. Ahmed Nassar, MD, Qiang Liu, MD, et al. Ex Vivo Normothermic Machine Perfusion Is Safe, Simple and Reliable: results From a Large Animal Model. Surgical Innovation 1-9, 2014
10. Ahmed Nassar, Qiang Liu, et al. Role of Vasodilation During Normothermic Machine Perfusion of DCD Porcine Livers. Int J Artif Organs 2014; 37 (2): 165-172
11. Qiang Liu, Ahmed Nassar, et al. Sanguineous Normothermic Machine Perfusion Improves Hemodynamics and Biliary Epithelial Regeneration in Donation after Cardiac death Porcine Livers. Liver Transplantation 20: 987-999, 2014
12. Erik Barthel, Falk Rauhfus , et al. The PRAISE Study: A Prospective, Multi-Center, Randomized, Double Blinded, Placebo-Controlled Study for the Evaluation of iloprost in the early Postoperative Period After liver Transplantation (ISRCTN 12622749). BMC Surgery 2013, 13:1
13. Kim M. Olthoff, Laura Kulik, et al. Validation of a Current Definition of Early Allograft Dysfunction in Liver Transplant Recipients and Analysis of Risk factors. Liver Transplantation 16(8):943-949, 2010
14. Hoyer D, et al. Donor Information Based Prediction for Early Allograft Dysfunction and Outcome in Liver Transplantation. Liver Int. 2014;1-8.
15. Deschênes, et al. EARLY ALLOGRAFT DYSFUNCTION AFTER LIVER TRANSPLANTATION: A Definition and Predictors of Outcome. Transplantation: 15 August 1998 - Volume 66 - Issue 3 - pp 302-310
16. Van Raemdonck D, Neyrinck A, et al. Machine perfusion in organ transplantation: a tool for ex-vivo graft conditioning with mesenchymal stem cells? Cur Opinion transplant. 2013 Feb; 18(1): 24-33.
17. Schön MR, Kollmar O, et al. Liver transplantation after organ preservation with normothermic extracorporeal perfusion. Ann Surg. 2001 Jan; 233(1):114-23.

18. Chan, I.S.F, Zhang, Z., 1999. Test-based confidence intervals for the difference of two binomial proportions. Biometrics 55, 1201-1209.

APPENDIX 1. LIVER TISSUE BIOPSY, BILE DUCT SPECIMEN, & SCORING PROTOCOL

Liver Parenchyma Tissue Sample:

- Each tissue specimen is a 2.0 cm long 16-gauge needle biopsy taken perpendicular to the liver capsule from the right lobe. It should be stressed that the needle NOT be inserted parallel to the capsule.
- The tissue sample can then be placed in 10% neutral-buffered formalin, which is standard at most institutions.
- The specimen container must be CLEARLY LABELED with an anonymous code and specimen information. Bar coding is preferable.
- The label should also contain the timing of the biopsy: a) donor pre-retrieval; b) post-preservation (before implant); and c) post-reperfusion.

Timing of Liver Biopsy Samples:

- Liver tissue samples taken at the following time points:
  - Donor liver pre-retrieval. Only donor livers that are clinically suspected to be fatty by retrieval team visualization, will require a pre-retrieval biopsy readout to estimate the degree of macrosteatosis and confirm eligibility (≤40% macrosteatosis)
  - Post-OCS and Control preservation at the end of back preparation and immediately before the start of re-implantation
  - 90-120 minutes after reperfusion of the transplanted liver (prior to abdominal closure)

Bile Duct Specimens (if applicable):

- Any tissue trimmed from the bile duct or cystic duct should be placed into 10% neutral buffered formalin, as above, and CLEARLY LABELED, as to the center and participant and location of the sample. For example, 001-001 "distal common bile duct trimming."

Timing of Bile Duct Specimen:

- Pathology sample of common bile duct taken post preservation (applies to both OCS and control arms).
  - Post preservation at the end of back preparation and immediately before the start of re-implantation OR
  - Post reperfusion-Prior to biliary anastomosis

Refer to core lab collection manual in the PROTECT Study Manual

Score

PARENCHYMAL CHANGES:

Total biopsy length:_____ mm Total number of portal tracts: _____ Biopsy Adequate: Yes No

Overall architectural integrity: normal/intact mild moderate severe distortion

1. Lobular necrosis:
   - 1.1 Overall severity: ( ) NONE ( ) MILD ( ) MOD ( ) SEV ( ) N/A
   - 1.2 Primary location: ( ) Periportal ( ) Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
   - 1.3 Secondary location ( ) Periportal ( ) Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
   - 1.4 Tertiary location: ( ) Periportal ( ) Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
2. Lobular inflammation:
   - 2.1 Overall severity: ( ) NONE ( ) MILD ( ) MOD ( ) SEV ( ) N/A
   - 2.2 Primary location: ( ) Periportal ( ) Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
   - 2.3 Secondary location: ( ) Periportal ( ) Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
3. Lobular inflammation type:
   - 3.1 Primary type: ( ) Neutrophils ( ) Eosinophils ( ) Macrophage/monocyte ( ) Lymphocytes ( ) Plasma cells
   - 3.2 Secondary type: ( ) Neutrophils ( ) Eosinophils ( ) Macrophage/monocyte ( ) Lymphocytes ( ) Plasma cells
   - 3.3 Tertiary type: ( ) Neutrophils ( ) Eosinophils ( ) Macrophages/monocytes ( ) Lymphocytes ( ) Plasma cells
4. Lobular Steatosis:
   - 4.1 Overall severity: ( ) NONE ( ) MILD ( ) MOD ( ) SEV ( ) N/A
   - 4.2 Predominant distribution: ( ) Periportal ( )Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
5. Liver Sinusoidal Endothelial Cell (LSEC) integrity/coverage (based on CD31 staining)
   - 5.1 Overall percentage of sinusoids covered _____ (nearest 10%)
   - 5.2 Primary LSEC loss: ( ) Periportal ( )Midzonal ( ) Perivenular ( ) Panlobular ( ) NA
   - 5.3 Secondary LSEC loss: ( ) Periportal ( )Midzonal ( ) Perivenular ( ) Panlobular ( ) NA

EXTRA-HEPATIC BILE DUCT CHANGES:

(adapted from Hansen et al Virchows Arch 2012;461:41-48; and op den Dries ..J HepatoL. 2014 Jun;60(6):1172-9):

1. Surface epithelial loss: ( ) 0: no loss ( ) 1: ≤ 50% ( ) > 50%
2. Bleeding: ( ) 0: no bleeding ( ) 1: ≤ 50% ( ) > 50%
3. Thrombi: ( ) 0: no thrombi ( ) 1: thrombi present
4. Vascular lesions: ( ) 0: no lesions ( ) 1: ≤ 50% vessels ( ) > 50% vessels
5. Arteriolonecrosis: ( ) 0: no lesions ( ) 1: ≤ 50% vessels ( ) > 50% vessels
6. Duct necrosis: ( ) 0: no lesions ( ) 1: <25% ( ) 2: 25-50% ( ) 3: 50-75% ( ) > 75% necrosis
7. Inflammation: ( ) 0: none; ( ) 1: at least > 10 leukocytes/HPF; ( ) 2: >50/HPF
8. Subluminal gland injury: ( ) 0: no injury ( ) 1: ≤ 50% ( ) > 50%
9. Deep gland injury: ( ) 0: no loss ( ) 1: ≤ 50% ( ) > 50%

APPENDIX 2. SCHEDULE OF CLINICAL ASSESSMENTS

Evaluations	Donor & Liver Assessments	
	OCS Preservation	Cold Flush and Storage (Control)
Eligibility & ID	X	X
Demographics/Characteristics	X	X
Donor Cause of Death	X	X
Donor Medical & Social History	X	X
Donor Liver Assessment	X	X
Donor Cross Clamp Time and Flush Detail	X	X
Post-Preservation Liver Biopsy and if applicable Bile duct specimen	X	X
OCS Preservation Parameters	X	
OCS Liver Enzymes & Lactate Levels	X	
Device Malfunction (if applicable)	X	
Non-transplant Reasons (if applicable)	X	X

Evaluations	Recipient Schedule of Assessments					
	Tx. Day -Day 7 Post-Tx.	Discharge	Day 30	Month 6	Month 12	Month 24
Eligibility & Informed Consent	X					
Randomization	X					
Demographic/Characteristics	X					
Medical & Risk Factors	X					
Transplant Details	X					
Early Liver Allograft Dysfunction (EAD) Surveillance	X					
Mechanical Ventilator Support	X					
Patient Survival	X	X	X	X	X	X
Graft Survival	X	X	X	X	X	X
Immunosuppressive Meds & Induction (if applicable)	X	X				
Initial ICU & Hospital Stay	X	X				
Liver Graft-Related (AE's & SAE's)	X	X	X			
Liver Biopsy *	X				X	X
* ONLY Tests regularly scheduled per center standard of care or performed due to a clinical cause at these time-points will be collected.						

APPENDIX 3. DRAFT PATIENT INFORMED CONSENT FORM TEMPLATE

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APPENDIX 4. DRAFT ELECTRONIC CASE REPORT FORMS

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