Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: van Rijn R, Schurink IJ, de Vries Y, et al. Hypothermic machine perfusion in liver transplantation — a randomized trial. N Engl J Med 2021;384:1391-401. DOI: 10.1056/NEJMoa2031532

This supplement contains the following items:

1. Original Version of Study Protocol

A Multicenter Randomized Controlled Trial to Compare the Efficacy of End-ischemic Dual Hypothermic Oxygenated Perfusion with Standard Static Cold Storage of Liver Grafts Donated after Circulatory Death in Preventing Non-anastomotic Biliary Strictures after Transplantation

DHOPE-DCD Trial

A Multicenter Randomized Controlled Clinical Trial
Version 1.0 - 21 September 2015

University Medical Center Groningen

LUMC
Leids Universitair Medisch Centrum

Erasmus MC
University Medical Center Rotterdam

PROTOCOL TITLE:

A Multicenter Randomized Controlled Trial to Compare the Efficacy of End-ischemic Dual Hypothermic Oxygenated Perfusion with Standard Static Cold Storage of Liver Grafts Donated after Circulatory Death in Preventing Non-anastomotic Biliary Strictures after Transplantation

Title in Dutch: Een gerandomiseerd, vergelijkend onderzoek om de effectiviteit van hypotherme geoxygeneerde perfusie van de lever te vergelijken met de standaard koude bewaarmethode in het voorkomen van vernauwingen in de galwegen van de lever na transplantatie wanneer de donor lever afkomstig is van een donor overleden aan een hartstilstand

Protocol ID	DHOPE-DCD Trial / ISRCTNxxx
Short title	End-ischemic DHOPE in DCD liver transplantation
EudraCT number	N/A
Version	Version 1.0 (21 September 2015)
Coordinating investigator/ project leader	R.J. Porte, r.j.porte@umcg.nl, +31503619027 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Sponsor	R.J. Porte, r.j.porte@umcg.nl, +31 50 3619027, University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Trial Coordinator	R. van Rijn, r.van.rijn@umcg.nl, +31652724616 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Principal investigator(s) per site	R.J. Porte, r.j.porte@umcg.nl, +31503619027 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands J. de Jonge, j.dejonge.1@erasmusmc.nl, +31633330449 Erasmus Medical Center, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands J. Ringers, j.ringers@lumc.nl, +31715269111 Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands
Subsidising party	Participating centers Foundation NutsOhra Bridge to Life
Independent expert (s)	Philip Dutkowski, MD, PhD, philipp.dutkowski@usz.ch, +41442554236, UniversitätsSpital Zürich, Klinik für Viszeral- und Transplantationschirurgie, Rämistrasse 100, 8091 Zürich, Zwitserland
Company name	Organ Assist B.V., Aarhusweg 4-7, 9723 JJ, Groningen, the Netherlands, +31503131905

	Bidge to Life Ltd., 128 Suber Rd., Columbia, SC 29210, USA, +18035450080
Laboratory sites	Surgical Research Laboratory, CMC V, Y2144, BA44, Hanzeplein 1, 9713 GZ Groningen, the Netherlands LETIS Erasmus MC Rotterdam, Nb1018, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands
Pharmacy	Pharmacy, Clinical Pharmacology and Pharmacy UMCG, Groningen, P.O. Box 30.001, 9700 RB Groningen, the Netherlands, Tel: +31503614071

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor / Coordinating investigator		
Prof.dr. R.J. Porte, MD HPB and Liver Transplant Surgeon University Medical Center Groningen		
Principal investigator per site:		
Prof.dr. R.J. Porte, MD HPB and Liver Transplant Surgeon University Medical Center Groningen		
Dr. J. de Jonge, MD HPB and Transplant Surgeon Erasmus Medical Center, Rotterdam		
Drs. J. Ringers, MD HPB and Transplant Surgeon Leiden University Medical Center, Leiden		
Local Investigators per site:		
Dr. W.J. Polak, MD Erasmus Medical Center		
Prof. dr. J.N.M. Ijzermans, MD Erasmus Medical Center		
Trial Coordinator:		
R. van Rijn, MD University Medical Center Groningen		
Independent Experts:		
Dr. P. Dutkowski, MD HBP and Liver Transplant surgeon UniversitätsSpital Zürich		
Adjudication Committee:		
S.V.K. Mahesh, MD Radiologist University Medical Center Groningen		
Dr. J.P. Pennings, MD Radiologist University Medical Center Groningen		
Nanda Krak, MD Radiologist Erasmus Medical Center, Rotterdam		
Data Monitoring Committee:		
Member of Trial Coordinating Center UMCG		
Member of Trial Coordinating Center UMCG		

Company:		
M. van Voorden Organ Assist B.V., Groningen		
A. Gilchrist Bridge to Life Ltd, USA		

INVESTIGATOR SIGNATURE PAGE

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

I understand that I may terminate or suspend enrolment of the study at any time if it becomes necessary to protect the best interests of the study subjects as advised by the DMC. This study may be terminated by the University of Oxford, with or without cause.

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

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

I will ensure that the requirements relating to the Ethics Committee (EC) review and approval are met. I will provide the University of Oxford with any material that is provided to the EC for ethical approval.

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

I agree to promptly report to the DMC, EC, and sponsor any changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without EC approval, except where necessary to ensure the safety of study participants.

Name…… **Signature……**

Date……

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AlkP	Alkaline Phosphatase
AKIN	Acute Kidney Injury Network
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EQ6D	European Quality of Life Instrument 6D
CCI	Comprehensive Complication Index
CV	Curriculum Vitae
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
ECD	Extended Criteria Donor
ERCP	Endoscopic Retrograde Cholangiopancreaticography
EU	European Union
GCP	Good Clinical Practice
γGT	Gamma-glutamyl Transferase
HMGB	High Mobility Group Box-1
DHOPE	Dual Hypothermic Oxygenated Perfusion
ICU	Intensive Care Unit
INR	International Normalized Ratio
IPF	Initial Poor Function
MDRD	Modification of Diet in Renal Disease
MELD	Model for End-stage Liver Disease
miRNA	Micro Ribonucleic acid
MP	Machine Perfusion
MREC	Medical Research Ethics Committee (in Dutch: METc)
MRCP	Magnetic Resonance Cholangiopancreatography
NA	Not Applicable
NAS	Non-anastomotic Biliary Strictures
NODAT	New Onset Diabetes After Transplantation
OLT	Orthotopic Liver Transplantation
PNF	Primary Non-Function
PT	Prothrombin Time
RIFLE	Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
(S)ADE	(Serious) Adverse Device Effects
(S)AE	(Serious) Adverse Event
SCS	Static Cold Storage
SOP	Standard Operation Procedure
UMCG	University Medical Center Groningen
USADE	Unanticipated Serious Adverse Device Effects
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Recent publications report good results of controlled donation after circulatory death (DCD) Maastricht category III liver transplantation when strict donor-recipient matching is applied and ischemia times are kept to a minimum. However a major concern remains the high rate of biliary complications after transplantation of DCD livers. Non-anastomotic biliary strictures (NAS) occur in 29% of patients receiving a DCD graft whereas the incidence of NAS in recipients of donation after brain death (DBD) liver grafts is 11%. NAS are associated with higher morbidity and increased cost of liver transplantation. Injury to the biliary epithelium and the peribiliary vascular plexus occurring during donor warm ischemia and static cold storage (SCS) has been identified as a major risk factor for development of NAS. Machine perfusion has been proposed as an alternative strategy for organ preservation, offering the opportunity to improve the quality of the organ by providing oxygen to the graft. Experimental studies have shown that end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) helps liver grafts to recover from ischemia by restoring mitochondrial function. Moreover, DHOPE has been shown to provide better preservation of peribiliary vascular plexus of the bile ducts, which could be an important step forward in reducing the incidence of NAS after transplantation.
Objective: To study the efficacy of end-ischemic DHOPE in reducing the incidence of NAS within six months after controlled DCD (Maastricht category III) liver transplantation.
Study design: An international, multicenter, prospective, randomized, controlled, interventional, clinical trial with a two parallel arm approach (treatment/control).
Study population: Adult patients (≥18 yrs old) undergoing a liver transplantation with a liver graft procured from a controlled DCD donor (Maastricht category III) with a body weight ≥40 kg.
Intervention: In the intervention group liver grafts will be subjected to two hours of hypothermic, oxygenated perfusion at the end of SCS and before implantation. In the control group donor liver grafts will be preserved in accordance to standard practice by SCS only.
Main study parameters/endpoints: The incidence and severity of NAS as diagnosed by an Adjudication committee (who are blinded for the group assignment) by means of magnetic resonance cholangiopancreatography (MRCP) at six months after DCD liver transplantation.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients participating in this trial will experience minimal burden. There are only three difference in follow-up compared with the routine practice: livers undergo DHOPE and patients fill in a questionnaire and undergo a MRCP. The intervention, DHOPE, is associated with a non-significant risk of injury of the isolated liver due to perfusion pressure or perfusion failure. The perfusion pressures in this protocol are very low and are reported to cause no harm to the organ. In case of perfusion failure, the liver can easily and quickly (within minutes) be brought to the same conditions as in the control group. In these bridging minutes the organ has a metabolism of 19% instead of the 11%. This is non-significant especially because the organ is saturated with oxygen before the possible event. There is a minimal burden but there are no risks related to the questionnaire or the MRCP, which is planned during a routine hospital visit. When the intervention is effective in reducing

the incidence of NAS, the patients participating in this trial benefit substantially when they are randomized to the intervention group. This study can only be performed in these patients because they undergo a DCD liver transplantation.

ADMINISTRATIVE INFORMATION

Investigators

Coordinating Investigator / Project Leader

The Coordinating Investigator / Project Leader is the Sponsor and will have oversight of:
- Design and conduct of the trial
- Preparation of protocols and revisions
- Preparation of standard operating procedures (SOPs)
- Preparation of electronic case report forms (eCRF)
- Organizing Investigator Meetings
- Publication of study reports
- Appoint members of the Data Monitoring Committee
- Review adverse events for immediate risk to patients

Principal Investigators

In each center a Principal Investigator will be identified, to be responsible for follow-up of recruitment, data collection and completeness of eCRFs in his/her center. The Principal Investigator is the liaison between the Local Investigators and the Trial Coordinator. Responsibilities will include:
- Obtaining local Ethics Committee and Research Governance approval (aided by the Trial Coordinator)
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants
- Publication of study reports of the own work package

Local Investigators

In each participating center Local Investigator(s) (transplant surgeon and/or hepatologist) will be identified. Responsibilities include:
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants

Trial Coordinator

The Trial Coordinator is responsible for tasks delegated to him/her including:
- Preparation of randomization tool
- Identification and recruitment of patients to the study
- Oversee data entry and monthly report on missing data

- Review adverse events for immediate risk to patients
- Report adverse events according to section 9.6

Data Monitoring Committee

The Data Monitoring Committee (DMC) is responsible for:
- Agreeing a charter for the conduct of the DMC (see Appendix 4)
- Reviewing data from the study according to the schedule set out in the charter
- Reviewing serious adverse events (device related or not) and any device deficiencies

Adjudication Committee

The Adjudication Committee is responsible for timely assessment of Magnetic Resonance Cholangiopancreatography (MRCP) images obtained in all participants. The Committee consists of clinical experts in the specific clinical area: two independent radiologists from the Sponsor site and one from the Erasmus MC. In order to allow for an unbiased endpoint assessment the members are blinded to treatment assignment when assessing the images. If there is disparity between the members, they will discuss the case to reach an unequivocal decision.

Company

Organ Assist is responsible for the production and delivery of the Liver Assist machine perfusion devices. They are responsible for the training of all individuals that operate the device according to its intended use. They are responsible for any malfunction, for repairs and are obligated to deliver a replacement device within reasonable time (24 hours) after a defect device has been reported to Organ Assist.

1. INTRODUCTION AND RATIONALE

Limited organ availability for orthotopic liver transplantation (OLT) remains to be a major concern (1). Utilization of livers with marginal quality or so called “extended criteria” donors (ECD) including older donors, donors with fatty livers and donation after circulatory death (DCD) donors have reduced organ deficit in recent years. In fact, the percentage of DCD donors in the United States of America has increased from 1.1% in 1995 to 11.2% in 2010 (2). However, poor post-transplant outcomes of these grafts have concurrently limited the utilization of these livers. The percentage of unused grafts, mainly attributed to the increasing number of DCD donors, increased from 9% in 2004 to 28% in 2010 (2). Longer hospital stays and increased costs have been associated with DCD transplants (3-5). Non-anastomotic biliary strictures (NAS) are a major complication after OLT and occur in 29% of patients receiving a DCD donor graft, compared to 11% incidence among recipients of donation after brain death (DBD) liver grafts in the University Medical Center Groningen (UMCG) (unpublished data).

Among the variety of risk factors described to be associated with NAS, ischemia/reperfusion related injury is one of the most important concerns. Donor warm ischemia, as well as cold ischemia injury caused during static cold storage (SCS), has been associated with the development of NAS after DCD (4, 6). Also, injury of the peribiliary vascular plexus is thought to play an important role in development of NAS (7, 8).

The need for an increased number of usable donor livers and a better use of ECD liver grafts necessitates the development of more qualified preservation methods. Machine perfusion (MP) is an alternative strategy that offers a more dynamic preservation and provides opportunities to improve the quality of organs derived from ECD (9). One of the most important benefits of MP compared to conventional SCS is the ability to provide oxygen to the graft. Even at very low temperatures of 12°C liver metabolism still requires 0.467 µmol oxygen/min/g liver tissue (10), which can be supplied by oxygenated MP. Other benefits are that nutrients are supplied to the graft and that toxic waste products are diluted and removed. A relatively simple technique to revitalize grafts after a time period of cold ischemia is end-ischemic hypothermic oxygenated perfusion (DHOPE) at the hospital of the recipient (11, 12).

Experimental animal studies have demonstrated that oxygenated machine perfusion can help organ recovery by improving cellular energy homeostasis. The restoration of mitochondrial function by oxygenated MP, resulting in an increased adenosine triphosphate (ATP) tissue concentration, leads to less cellular death after warm reperfusion (11-13), a better hepatocyte function, and enhanced energy dependent bile production (14). It has been shown that hypothermic oxygenated reconditioning of grafts leads to better preserved autophagic pathways (15) and thus a significant decrease of hepatocellular necrosis. To summarize, experimental animal studies have shown that 1-2 hours of hypothermic oxygenated perfusion of liver grafts after SCS not only improves organ integrity and function after reperfusion but also significantly increases survival after transplantation (16).

After these experimental studies, the method was investigated in the clinical setting of human liver transplantation in centers in New York and Zurich. These first clinical experiences have shown that hypothermic machine perfusion is safe, may improve graft function, and attenuates classical biochemical markers of liver preservation injury. Early results suggest there is a reduction in preservation injury that may be responsible for better early allograft function and fewer complications post transplantation, like less biliary complications, and shorter hospital stay in comparison to patients receiving a liver preserved with SCS alone (17, 18). The Zurich group has reported extensively on the feasibility and safety of DHOPE of the portal vein and they have reported excellent early and long-term liver graft function with low serum parameters for liver injury and cholestasis, short ICU stay, and no NAS after six months after transplantation (19). Feasibility and safety of DHOPE of both the portal vein and hepatic artery was recently demonstrated in a pilot study in Groningen (NTR4493; van Rijn et al. unpublished data)

Considering these promising results along with the relative simplicity and safety of this technique (20), end-ischemic DHOPE seems to be a suitable approach to be applied in the clinical setting. However, it is not routinely applied and the efficacy of DHOPE in reducing postoperative biliary complications has not yet been evaluated. Preliminary results have indicated that DHOPE may ameliorate vascular endothelial injury (12) and provide a better preservation of peribiliary vascular plexus (16). Since these injuries are associated with development of NAS, it is hypothesized that DHOPE reduces the incidence of NAS after liver transplantation. Especially in DCD liver transplantation, NAS is a major concern. Therefore, this graft type may have the greatest benefit from DHOPE. The present clinical trial is designed to establish the efficacy of two hours of end-ischemic DHOPE prior to implantation of a DCD (Maastricht category III) liver graft in reducing the postoperative incidence of NAS.

2. OBJECTIVES

Hypothesis:
End-ischemic DHOPE after SCS is a better method for preservation of the biliary tree resulting in a lower incidence of symptomatic NAS after DCD liver transplantation than SCS alone.

Primary Objective:

To study the efficacy of end-ischemic DHOPE in reducing the incidence of NAS after DCD Maastricht category III liver transplantation at 6 months after transplantation.

Secondary Objective(s):
To study the effect of the intervention (end-ischemic DHOPE after SCS), in comparison to the control group (SCS only), concerning:

1. The overall incidence of symptomatic and asymptomatic NAS
2. The severity of NAS after transplantation
3. The graft and recipient survival
4. The incidence of primary non-function (PNF)
5. The incidence of initial poor function (IPF)
6. The biochemical analysis of graft function and ischemia-reperfusion injury
7. The hemodynamic status of the recipient after graft reperfusion
8. Length of stay in the ICU and hospital
9. The incidence of postoperative complications, including infections and use of antibiotics
10. The renal function
11. The perfusion characteristics during DHOPE (in the intervention group only)
12. The perfusate analysis during DHOPE (in the intervention group only)
13. Prognostication of NAS, based on micro ribonucleic acid (miRNA) profiles in perfusion fluid (in the intervention group only)
14. Pathobiology of liver and bile duct parenchyma
15. Metabolic function, including new onset diabetes after transplantation (NODAT)
16. Overall cost of treatment within 6 months (in/excluding return to work)
17. Overall health related quality of life after transplantation

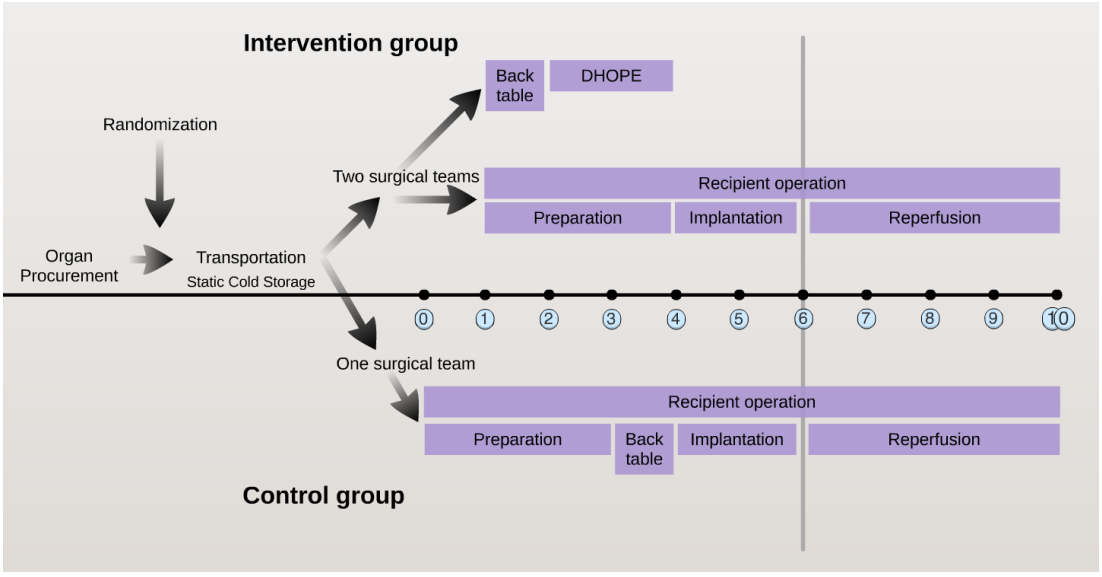
3. STUDY DESIGN

The trial is designed as a prospective, randomized, controlled, multicenter, superiority, clinical trial with two parallel groups. Primary endpoint is the incidence of NAS at six months after liver transplantation. Liver grafts in the treatment group will be preserved with SCS followed by DHOPE. Liver grafts in the control group will be preserved by SCS alone without any further intervention. **Figure-1** provides an overview of the study design.
The study setting is standard practice of OLT. The surgical procedure, post-operative care and follow-up are identical to the routine OLT-practice in each participating center. The surgical procedure will start a little earlier in the intervention group compared to the control group in an attempt to keep total preservation time equivalent.

Patients are followed during the first six months post-transplantation, during hospital stay and after discharge, via their routine hospital visits at 1 month, 3 months and 6 months after transplantation. Whenever patients included in this trial are suspected of NAS based on clinical parameters as judged by their physician, they will undergo routine examinations which include MRCP imaging of the biliary tree. The study duration is 6 months for each participant. This time interval is chosen because diagnosis of NAS is reported at a median of 3 to 4 months after transplantation (21, 22). Some studies have reported an occurrence of 100% of the cases of NAS within 4 months (22-25).

In case a patient in this trial is not diagnosed with NAS within 6 months, a MRCP is performed at six months after transplantation to assess the biliary tree for asymptomatic injury. All MRCP images will be evaluated by an Adjudication Committee in order to harmonise and standardise endpoint assessment. The Adjudication Committee consists of three independent radiologists. In order to allow for an unbiased endpoint assessment the members are blinded to study group assignment.

Figure 1.



4. STUDY POPULATION

4.1 Population (base)

Adult patients (≥ 18 years old) with end-stage chronic liver disease awaiting liver transplantation are screened for participation in this trial.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adult patients (≥ 18 years old)
- Signed informed consent
- Willing and able to attend follow-up examinations
- Donor liver graft from a controlled donation after circulatory death (Maastricht category III)
- Donors with a body weight ≥40 kg

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Simultaneous participation in another clinical trial that might possibly influence this trial
- Mental conditions rendering the subject incapable to understand the nature, scope and consequences of the trial
- Listed for liver transplantation due to fulminant liver failure or retransplantation because of PNF
- Recipient positive test for HIV
- Donor positive for HIV, Hepatitis B or C
- Expected cold ischemia time of far more than ≧ 8 hours, which would result in a total preservation time (SCS and DHOPE) of far more than ≧ 10 hours (26)
- Patients with contra-indications for MRCP (i.e. pacemaker)

4.4 Sample size calculation

The study is powered to detect a clinically relevant difference in incidence of NAS between the two study groups. The incidence of NAS is 29% after DCD liver transplantation and is 11% after DBD liver transplantation in patients transplanted in the UMCG from 2008 to 2013 (unpublished data). This is similar to incidence reported by Abt *et. al.* (27% in DCD versus 2% in DBD transplantation), Dubbeld *et. al* (24% versus 8%), Croome *et. al.* (22% versus 4%, and Meurisse *et. al.* (33% versus 12%) (4, 24, 27, 28). With the intervention (DHOPE) we aim to reduce the incidence of NAS after DCD liver transplantation to the level observed after DBD liver transplantation (absolute difference of 29-11=18%). We base this presumed reduction on our results in the pilot study in which 1 of 10 (10%) of the patients with a DHOPE treated liver developed NAS. The other previously reported pilot studies observed no NAS in any of the patients receiving a liver treated with end-ischemic hypothermic machine perfusion. For a power of 80% (β=0.80)

and a 5% significance level (1-sided test) in two independent cohorts, using a Chi-squared test, 61 livers are needed to be included in each arm, calculated with nQuery + nInterim 3.0. After consulting a statistician, Dr. K.M. Vermeulen, at the UMCG, a 1-sided test is deemed appropriate due to the results from the pilot studies indicating a reduction in NAS. Also, it seems ethically more just because fewer patients need to be included and inclusion will be achieved more quickly and results will be known earlier in time meaning that patients will be able to benefit earlier in time. Given that 21% of patients suffer from graft loss and or die before the primary end-point is reached, a total of 78 patients are required to be enrolled to achieve 61 patients in each arm. Therefore, the total number of patients to be included in this study will be 156.

4.5 Study duration

Based on expected numbers to be enrolled in the participating centers per country per year we estimate an inclusion period of 24 months, depending on participating centers. With a follow-up period of 6 months, the study duration total to 30 months.

An estimate for the number of inclusions per country per year is based on 2013:
- The Netherlands: 48 donors per year, 96 in two years

Potential partners outside the Netherlands (to be confirmed):
- Birmingham and Edinburgh, UK: 40 in two years
- Montreal and Halifax, Canada: 20 in two years
- Cordoba, Spain:
- Paris, France:

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The patients randomized to the control group will receive a liver graft preserved by conventional SCS without any further intervention. In the treatment group donor livers will be subjected to two hours of DHOPE applied by a medical device named the Liver Assist® (see section 6). During transportation from the donor hospital to the recipient hospital, the liver is preserved via SCS. The intervention is restricted to the liver graft after arrival in the transplant center and before implantation. A detailed description of DHOPE is given in section 6.5.

5.2 Use of co-intervention and concomitant care

Donors will be managed by local standard of care and protocols formulated by the local organ procurement organizations. No changes in donor management will be made for the sake of this trial. *In situ* flushing of the liver during organ retrieval will be done according to local standard of care and protocols. Concomitant care of the recipient, including the implantation procedure, postoperative care, immunosuppression and other medications will

reflect the current standard of care at the recipient hospital. Local immunosuppression protocols will not be altered for the sake of this trial. There is no restriction in behaviour, in use of co-interventions or medication for patients in this trial.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

The Liver Assist is a dedicated machine for *ex-vivo* liver perfusion during storage. It is a CE marked (European Union Certification of Safety, Health and Environmental Requirements) device that is designed, produced, and delivered by Organ Assist (Groningen, The Netherlands) and has been used in a pilot study (NTR 4493, van Rijn et al. unpublished data). Organ Assit will also be responsible for training of the relevant individuals on how to operate the device for its intended use as well as for maintenance and repairs to the machine as required. Organ Assist will deliver a replacement device within 24 hours in the event of a defect being reported to them. Training on the use of the Liver Assist machine will be provided in advance of recruitment of the first patient. A record of all device training will be maintained.

The Liver Assist enables dual perfusion via the portal vein and the hepatic artery using two centrifugal pumps to provide a continuous venous flow and a pulsatile arterial flow at 60 bpm. The system is pressure controlled which allows autoregulation of the flow through the liver, with constant pressure at variable flow rates. The perfusion fluid can be oxygenated by two hollow fiber membrane oxygenators and carbon dioxide van be removed. The temperature of the preservation fluid can be adjusted between 10 and 38°C. The system can be filled with any preferred perfusion fluid.

6.2 Description and justification of method of use in this trial

The instruction manual of the Liver Assist will be submitted as a separate document, but a summary of how the machine will be used in the trial is described below.

Preparation of the liver in both study groups
After circulatory death of the donor, the stand-by surgical team performs a median laparotomy and aortic cannulation to perfuse the abdominal organs with at least 4000 ml of cold (0-4°C) Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL) with 50.000 IU of heparin. The liver is procured with a segment of 5 cm circular supratruncal aorta left attached to the coelic trunk if possible. The portal vein and common bile duct is kept as long as possible. After procurement the liver is flushed via the portal vein with at least 1 liter of Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL) without applying pressure. The cystic duct is ligated and the bile ducts are flushed with Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL) preferably at the donor hospital. The gallbladder is left in situ. The liver is transported to the recipient hospital, where the conventional back table procedure is performed by the surgeon.

Preparation in intervention group only
Subsequently, the portal vein and the supratruncal aorta are cannulated. The supratruncal aorta is cannulated so that the hepatic artery is not damaged due to cannulation. The side branches of the hepatic artery are ligated or clipped during the back table preparation. Short before connection to the Liver Assist, the liver is flushed via the portal vein cannula with 1000 mL cold (0-4°C) Belzer machine perfusion solution (Bridge-to-Life, Ltd., Northbrook, IL) until the caval effluent is clear. Normally the back table preparation takes one hour on average.

Preparation of the Liver Assist
Simultaneously with the back table procedure, the Liver Assist is prepared for use. The disposable is connected to the machine and is filled with 4000 mL ice-cold machine perfusion solution Belzer UW ® (Bridge-to-Life, Ltd., Northbrook, Ireland), with additional 3 mmol/L glutathione (Biomedica, Foscama Group, Roma, Italy). The gluthatione is a component of the machine perfusion solution – Belzer UW, which is added at recommendation of Bridge-to-Life because it may have become inactive during shelf-time. The system will be pressure controlled with the pressure limited to a mean of 25 mmHg for the hepatic artery and 5 mm Hg for the portal vein. These pressure settings are based on previous studies and are lower than physiological pressures to avoid shear stress of the cold endothelium of the hepatic vasculature (12, 16, 29). The temperature of the perfusion fluid will be 12°C, when the temperature is set to 10°C. The thermoregulator of the Liver Assist is filled with crushed ice in the reservoir of the cooling unit to achieve the desired temperature. The oxygen flow is set at 500 mL/min of 100% oxygen on each of the two membrane oxygenators. This flow is adequate to obtain a pO2 which has been reported to be effective in increasing ATP and not harmful to the graft (10, 30). The Liver Assist is ready for perfusion once the temperature is reached and the solution is oxygenated for at least 15 minutes with oxygen. The preparation of the Liver Assist takes 30 minutes on average.

Perfusion of liver with Liver Assist
The surgeon connects the cannulas to the disposable tubings of the Liver Assist after which the pumps of the device are started. During the connection of the liver to the machine, the perfusion pressure will be adjusted manually in the first five minutes after connection so that a minimum of 100 ml/min flow via the portal vein is maintained, but without exceeding a portal vein pressure of 7 mm Hg. Perfusion fluid and liver will be cooled to 12°C by the thermoregulator. The reservoir of the cooling unit of the Liver Assist must be filled with crushed ice that is regularly replaced. The Liver Assist continuously registers flow rates and temperature and gives alarms in case of high flow or temperature. A surgeon supervises this procedure and is in the vicinity.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary endpoint of this trial is the incidence of symptomatic NAS at six months after DCD liver transplantation. The diagnosis of symptomatic NAS is defined as all of the following criteria (21):

- any irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
- which are diagnosed by cholangiogram (preferably by MRCP)
- in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
- and as assessed by the Adjudication Committee
- when imaging is indicated by clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up

This endpoint is selected as it is considered to reflect a clinically relevant sign of biliary injury caused by ischemia-reperfusion (21). Also, it is reproducibly attainable at all study sites and therefore can be objectified by blinded assessment by the Adjudication Committee including three independent radiologists. Moreover, the imaging modality is minimally invasive and is part of the routine diagnostic work-up in case of clinical suspicion of NAS.

8.1.2 Secondary study parameters/endpoints

1. The overall incidence of NAS is based on symptomatic NAS (see primary study parametersendpoint) and asymptomatic NAS. Asymptomatic NAS is defined as all of the following:
    a. irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
    b. which are diagnosed by cholangiogram (preferably by MRCP)
    c. in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
    d. in the absence of clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up
2. The severity and location of NAS will be determined by the Adjudication Committee, based on:
    a. Assessment of the images of the MRCP obtained in all patients at six months after transplantation (time window of 15 days) which will be performed based on a scoring system described by Buis et. al. (21)
    b. Required treatment for NAS (i.e. ursodeoxycholic acid, ERCP, retransplantation)
3. Graft (censored and uncensored for patient death) and patient survival at 7 days, 1, 3, 6 months after transplantation

4. PNF is defined as liver failure requiring retransplantation or leading to death within seven days after transplantation without any identifiable cause such as surgical problems, hepatic artery thrombosis, portal vein thrombosis and acute rejection (31).
5. IPF is defined based on a modification of the Olthoff criteria: Prothrombin time/INR >1.6 and or serum total bilirubin >10 mg/dL on postoperative day 7 (32).
6. Biochemical analysis of graft function and ischemia-reperfusion injury is determined with serum levels of alanine aminotransferase (ALT), AST, alkaline phosphatase (AlkP), gamma-glutamyl transferase (γGT), and total bilirubin at postoperative day 0 – 7 and 1, 3, 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day.
7. Hemodynamic status (blood pressure, heart rate and vasopressor dosage) will be recorded 5 min before reperfusion, as well as 10 and 20 minutes after reperfusion
8. Length of initial ICU and initial hospital stay is determined in days of admission following liver transplantation. Duration of follow-up hospital stay is determined in days of hospital admission after discharge and up to six months after liver transplantation.
9. Postoperative complications are graded according to the comprehensive complication index (CCI) (33). Special interest will be given to predefined infectious complications and the total length of use and cumulative doses of antibiotics.
10. Renal function is defined as estimated glomerular filtration rate (eGFR) according to the 4-variable Modification of Diet in Renal Disease (MDRD) equation (34) at day 7, and 1, 3, 6 months after transplantation. Kidney injury is scored according to acute kidney injury network (AKIN) and risk, injury, failure, loss of kidney function and end-stage kidney disease (RIFLE) criteria (35). In selected centers, urinary kidney injury markers (kidney injury molecule (15), tissue inhibitor of matrix metalloproteinases-2 [TIMP2], Insulin-like Growth Factor Binding Protein-7 [IGFBP7], and neutrophil gelatinase-associated lipocalin [NGAL]) are determined preoperatively, at arrival in the ICU and at day 1, 3, and 5 after transplantation.
11. Perfusion characteristics during DHOPE include flow, pressure and resistance at every fifteen minutes.
12. In selected centers, perfusate analyses will be performed to study the dynamics of experimental markers of tissue and mitochondrial injury. The perfusate at the start and end of DHOPE procedure, and every half hour in between will be analysed for pH, sodium, potassium, bicarbonate, lactate, ALT, AST, AlkP, γGT, urea, total bilirubin, thrombomodulin, high mobility group box-1 (HMBG) protein, cytochrome C.
13. In selected centers, prognostication of NAS is based on miRNA's: CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a determined in perfusate.
14. In selected centers, biopsies of liver parenchyma and bile duct, which are routinely taken during transplantation, are also taken in this trial at the time points: before DHOPE, after DHOPE, and after reperfusion at the time of bile duct anastomosis during anesthesia. The purpose is to underpin the histopathological status of the liver and bile ducts in both study groups. In addition, mechanistic research into molecular mechanisms of injury and repair during DHOPE will be done to identify pathophysiological pathways that might have potential to predict function and outcomes after transplantation.
15. Metabolic function, including new onset diabetes after transplantation (NODAT) in the first 90 days after transplantation. NODAT is defined according to the WHO criteria (36).

- Symptoms of diabetes and random plasma glucose ≥11.1 mmol/L. Symptoms include polyuria, polydipsia, and unexplained weight loss. OR

- Fasting plasma glucose ≥7.0 mmol/L. Fasting is defined as no caloric intake for at least eight hours. OR

- Two-hour plasma glucose ≥11.1 mmol/L during an oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

16. In selected centers, overall cost of treatment within 6 months (in/excluding return to work) is calculated according to the Cost and Outcome analysis of Liver Transplantation (COLT) study (5).

17. Health related quality of life will be determined using an EQ6D questionnaire obtained when the patient before transplantation and at 6 months after transplantation.

8.1.3 Other study parameters: Baseline values and parameters at inclusion

- Patient general demographics (age, gender, weight, height)
- Patient medical history including
    - Model for end-stage liver disease (MELD) score
    - Indication for OLT
    - Viral status
    - Current residence (home, hospital ward or ICU)
    - Kidney function
    - Medication
- Donor and liver graft demographics (age, gender, weight before and after DHOPE, height, cause of death, viral status, liver function, donor risk index, etc.)
- DCD characteristics such as time interval between withdrawal of life support and circulatory arrest, time interval between circulatory arrest and start cold perfusion *in situ,* etc.
- Surgical methods and technical difficulties or abnormalities
- Cold ischemia time, total preservation time (SCS and DHOPE) and warm ischemia time during implantation

8.2 Randomisation, blinding and treatment allocation

When a DCD donor liver becomes available, transplant centers involved in the trial will be informed by their local organ procurement organization. It is important to note that absolutely no changes will be made to national and international liver allocation rules. The standard local liver allocation rules will be followed. The study does not interfere or change the process of accepting or declining a liver offered to a certain patient in any way. Once a suitable recipient for the liver is identified, the recipient will be invited to the relevant transplant center for the surgical procedure as per routine procedure. Standard care at individual transplant centers is provided. After acceptance of the liver for a certain patient, the inclusion, exclusion criteria and informed consent are checked (see section 8.3).

The retrieval surgeon will assess the organ's suitability for transplantation. If the liver is suitable for transplantation and all inclusion and exclusion criteria are met, the liver will be randomized to either DHOPE after SCS or SCS only. The liver will be randomized by the local investigator or the trial coordinator using an online randomization tool. A computer-generated list of random assignments (block randomization per site) is prepared in advance by Clinical Trial Center Maastricht. All personnel involved in randomization will be trained in the use of the online randomization by the Trial Coordinator or the Principal Investigator of each site. When a patient is registered to the website, an inclusion number is generated consisting of study-center-inclusion; for example the first patient in UMCG will have inclusion number UMCG-DHOPE-01. The local investigator will register which graft the patient has received. This information will be stored, so in all cases, it will be possible to determine the study group the patient belongs to. The randomization website will send inclusion information to the mailbox of the Trial Coordinator, containing the inclusion number and the date and time.

Blinding

After randomization, the transplanting surgeon will be informed whether the patient is randomized to receive a graft after DHOPE or SCS only. Patients are blinded to study group. The study is blinded for assessment of the primary endpoint by the Adjudication Committee. When there is a breach of blinding, this is described in the eCRF and the Sponsor is notified.

8.3 Study procedures

Before transplantation

- Patient general demographics (age, gender, weight, height)
- Patient medical history including
    - MELD score
    - Indication for OLT
    - Viral status
    - Current residence (home, hospital ward or ICU)
    - Medication
    - Laboratory serum analyses:
        - Serum creatinine for the eGFR based on the MDRD-equation
- Donor and liver graft demographics (age, gender, weight, height, cause of death, viral status, donor risk index, latest serum values AST, ALT, total bilirubin, INR, APTT, etc.)
- DCD characteristics such as time interval between withdrawal of life support and circulatory arrest, time interval between circulatory arrest and start cold perfusion *in situ,* etc.

During transplantation

- Cold ischemia time, total preservation time (SCS + DHOPE) and warm ischemia time during implantation

- Surgical methods and technical difficulties or abnormalities
- Hemodynamic status is recorded routinely and continuously. Items recorded for this study are systolic and diastolic blood pressure, heart rate and vasopressor dosage. These will be recorded 5 min before portal reperfusion, as well as 10 and 20 minutes after reperfusion.
- Biopsies of the caudal part of the common bile duct (a ring of 1 mm) will be taken and stored in formaline. Biopsies are routinely taken during transplantation.
- Wedge biopsies of the liver parenchyma will be taken from the edge of segment III of the liver (1x1x1xcm) and divided into three pieces storing one of each piece in formaline for pathohistology and immunohistology, in RNA later for RNA analysis and in liquid nitrogen for ATP analysis. Biopsies are routinely taken during transplantation.

Intervention group only

- Perfusion characteristics are obtained during DHOPE by the personnel monitoring the perfusion. At the start of perfusion and every fifteen minutes thereafter the flow is noted. These data are noted directly in the eCRF.
- Perfusate will be sampled for a point of care analysis to determine the pH, sodium, potassium, bicarbonate, and lactate using standard biochemical methods. The time points will be before the start of DHOPE, at the end of DHOPE, and every half hour in between, equaling to 5 time points. Per time point 0.4 ml will be taken adding up to a total of 20 ml.
- Perfusate will also be sampled to determine ALT, AlkP, γGT, urea, total bilirubin, thrombomodulin, HMBG, and cytochrome c using standard biochemical methods. Also the amount of miRNA's CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a will be determined. The time points will include before the start of DHOPE, at the end of DHOPE, and every half hour in between, adding up to 5 time points. Per time point 20 ml will be taken adding up to a total of 100 ml.

After transplantation

- All patients are routinely followed during their admission postoperatively, and in the outpatient department at 1, 3, and 6 months after transplantation.
- Routine serum levels of ALT, AST, AlkP, γGT, total bilirubin, INR, PT, APTT, glucose, creatinine, leukocytes and CRP are assessed at day 0-7 and at 1, 3, and 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day.
- Renal function expressed as eGFR and the AKIN/RIFLE criteria will be determined by observation of oliguria and routine serum analyses including creatinine following transplantation.
- The urinary kidney injury markers will be determined in urine samples taken from the urinary catheter placed under anaesthesia routinely during OLT. For this end point, samples of 10 ml urine will be drawn from this catheter at time points: after induction of anesthesia, at arrival in the ICU and at day 1, 3, and 5 after transplantation. A total of 50 ml of urine will be taken and stored at -80ºC.

At six months

- The incidence of NAS will be calculated per group. The diagnosis is defined in section 8.1.1. To obtain this diagnosis, no additional procedures are necessary. Patients in this study will undergo a MRCP when there is a suspicion of NAS, which is part of the routine diagnostic work-up. The images of the MRCP will be reviewed by the Adjudication Committee in a later stage.
  - Severity of NAS will be determined by the type and frequency of interventions required.
- MRCP: In all patients who haven't been diagnosed with NAS within six months and haven't suffered from graft loss, a MRCP will be performed at six months after transplantation (time window of 15 days). The MRCP is an extra procedure for this study which is performed regardless of symptoms. A MRCP is a MRI scan of the bile ducts. It is a non-invasive imaging modality that does not use radiation. It is a painless and safe procedure which takes 30 to 60 minutes. It will be performed during a routine follow-up visit to the out-patient department.
- The images of the MRCP: the Adjudication Committee blinded for group treatment will assess all MRCP images of all patients (including those with diagnosed NAS) to objectively score the bile duct for strictures as defined by Buis et. al. (22).
  - the amount of lesions
  - the localization
  - the severity
- Graft and patient survival will be determined
- Incidence of PNF and IPF will be determined
- Incidence of postoperative complications graded according to CCI will be determined, including NODAT (see section 8.1.2)
- Length of hospital stay and stay in the ICU department directly following transplantation and in the follow-up period will be determined
- Cost-effectiveness will be determined based on data which are already obtained
- Health related quality of life will be determined based on EQ6D questionnaire obtained before transplantation and at six months after transplantation

Table 1. An overview of study endpoints per time point.

Endpoint / Time point	Baseline parameters	Perfusion characteristics	Hemodynamic status	Serum and urine analysis	Clinical follow-up	MRCP
Before OLT	X			x		
During DHOPE		x				
During OLT		x	x			
After reperfusion			x	x		
Day 0-7				x	x	
Month 1				x	x	
Month 3				x	x	
Month 6				x	x	x

A non-exhaustive list of data retrieved per eCRF can be found in Appendix 3: Data collection parameters.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. It can be possible that a patient has given informed consent, but does not fulfill the inclusion/exclusion criteria at the time of transplantation. In this case the patient is not be included in the trial and will not be randomized.

In case a participant wishes to withdraw consent before the procedure, they must contact the local investigator by e-mail, telephone or in person. In such an event, his/her liver will not be perfused and data will not be collected. In case the consent is withdrawn during follow-up and after the intervention, the participant will be asked if historical data and data from the patient file may be obtained. This case will be entered in the intention-to-treat analysis and an additional patient must be included.

8.5 Premature termination of the study

The study will be terminated prematurely if we find a higher rate of adverse events than expected (see section 9.4) that is possibly related to the study product.

9. SAFETY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited MREC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 Definition of AEs, SAEs, ADEs, SADEs, and USADEs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to DHOPE. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. This definition includes physical signs, symptoms and laboratory test values. At study enrolment, laboratory values that fall outside the relevant reference range will not be reported as AEs.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:
- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Is any other important medical event that may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above;
- Is a device deficiency that might have led to a serious adverse event if: a) suitable action had not been taken or b) intervention had not been made or c) circumstances had been less fortunate.
- Is not an anticipated (serious) adverse event defined in section 9.4

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

9.2.3 Adverse Device Effects (ADEs)

An adverse event related to the use of an investigational medical device. This definition includes any events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device. This definition also includes any event resulting from user error or from intentional misuse of the investigational device.

9.2.4 Serious Adverse Device Effects (SADEs)

Any untoward medical occurrence that can be attributed wholly or partly to the device, which resulted in any of the characteristics of a serious adverse event as described above.

9.2.5 Unanticipated Serious Adverse Device Effects (USADEs)

Any serious adverse device effect which, by its nature, incidence, severity or outcome, has not been identified in section 9.4.

9.2.6 Device Deficiency

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling. Device deficiencies resulting in SADEs will be managed as detailed in section 9.3. Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate will also be managed as detailed in section 9.5.

9.2.7 Use error

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

9.3 Causality of an AE related to the investigational medicinal device

- *Highly probable*: Apparent relationship in time between AE and intervention. Relationship between AE and intervention is already known or expected and there is an appropriate temporal relationship between therapy and AE.
- *Probable*: Known effect of the intervention with no possible other cause and appropriate temporal association.
- *Possible*: AE likely to be associated with the intervention and no other explanation for the AE, or known effect of intervention that could also be associated with another concomitant therapy, illness or external cause.
- *Unlikely*: Unlikely to be causally related; e.g. reaction occurred after intervention or is more likely to be due to another concomitant therapy, illness or external cause.
- *Definitely not*: AE known to be caused by another concomitant therapy, illness or external cause.
- *Not assessable*: Likelihood of AE not known, or relationship of AE to intervention, another concomitant therapy, illness or external cause is not clear. This category should be used very scarcely.

9.3.1 Grade of severity

- *Mild (grade 1):* patient is aware of symptoms but tolerates them easily. Symptoms do not interfere with daily activity.
- *Moderate (grade 2*): patient experiences discomfort that interferes with normal activity. No treatment is required except acetaminophen/paracetamol.
- *Severe (grade 3):* patient is unable to carry out normal activity. Treatment is required.
- *Life-threatening (grade 4):* emergency room visit, disabling or hospitalization.

9.4 Anticipated adverse events

All participants in this trial undergo liver transplantation, which is a surgical procedure with significant morbidity and mortality. This implies an intrinsic risk of AEs and SAEs anticipated after liver transplantation defined as:

Complications		**Incidence (%)**
Acute Rejection	Requiring biopsy or medication	7%
Primary Non Function	Requiring retransplantation	4%
Initial poor function		16%
Kidney dysfunction	Requiring alteration of medication up to dialysis	10%
Infectious complications		
	Wound Infection requiring opening	20%

	Infected ascites or intraabdominal abcess requiring drainage		30%
	Pneumonia requiring antibiotics		8%
	Urinary tract infection requiring antibiotics		20%
	Viral infection requiring virostatica		35%
	Blood stream infection requiring antibiotics		21%
Bleeding Complications	Surgical site bleeding requiring reoperation or transfusion		10%
Biliary Complications	Biliary leakage requiring drainage or endoscopy		22%
	NAS requiring intervention		29%
	Anastomotic biliary strictures requiring intervention		10%
Thrombotic and Ischemic Complications	Other venous thrombotic events (portal vein thrombosis or stenosis, deep venous thrombosis, pulmonary embolism)		3%
	Hepatic artery thrombosis requiring intervention or retransplantation		7%
	Cardiovascular events	Requiring medication or electroversion	6%
		Infarction	2%
		Arrhythmia	3%
		New congestive heart failure	2%
	Cerebrovascular events		3%
		Stroke	2%
		Hemorrhage	0,3-0,6%
Central nervous system	Including delirium requiring medication		10%
NODAT	Requiring insulin		7%
Death	3 months mortality		11%-15%

These events are to be reported as AE (section 9.5). However, if in the opinion of the investigator, the frequency or severity of the event is greater than would be expected based on the described incidence, the event must be reported to the Sponsor. The investigator will exercise his/her medical judgment in deciding whether an adverse event, a postoperative laboratory finding falling outside the relevant reference range or other abnormal assessment is clinically significant.

9.5 Recording of adverse events

It is the responsibility of the Principal Investigator to ensure that all adverse events (including ADEs) and device deficiencies occurring during the course of the study are collected. This will include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the device
- Whether the AE is serious or not
- Whether the AE arises from device deficiency

- Whether the AE arises from user error

Adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects. In addition each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms within the eCFR.

9.6 Reporting of adverse events

Reporting of all Serious Adverse Events will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010). It is the responsibility of the Principle Investigator to ensure that all adverse events which fall into the category of SAEs, SADEs, USADEs and device deficiencies are reported to the Coordinating Investigator as soon as possible after becoming aware of the event but no later than 24 hours. Details to be included in the report are described in Section 9.5.

All (serious) adverse events and (unanticipated) (serious) device effects or device deficiencies will be reported via the appropriate form in the eCRF, in which all but AEs and ADEs will be automatically forwarded to the clinical reviewers by e-mail. The clinical reviewers are the Coordinating Investigator and the Trial Coordinator. Reporting by Fax will provide a backup system (+31503619050) in the event that the online data collection tool is unavailable. The Fax machine is located at the Sponsor site and is manned during normal office hours only. Within the following 5 working days, the Principal Investigator should provide any additional information on the initial event in the appropriate electronic form using the same form submitted initially – do not create a new form for follow up information. This should include a copy of the completed form, and any other diagnostic or relevant information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the coordinating center and clinical reviewers using the same electronic form.

The clinical reviewers will review the events and, if they feel they pose an immediate risk to patient health or safety, they will report them to the DMC immediately and within 2 calendar days of becoming aware of the event to the device manufacturer and MREC via the webportal *ToetstingOnline*. The Coordinating Investigator will also inform all Principal Investigators concerned of relevant information about events that could adversely affect the safety of participants. All other events will be reported to the DMC within 7 calendar days of notification, if appropriate, except for events defined in section 9.4. These will be reported to the MREC, DMC and device manufacturer once every half year together with all other events. The USADEs and device deficiencies will be reported to the device manufacturer within 7 days.

All events are recorded by the Trial Coordinator in an overview list (line-listing) that will be submitted once every half year to the MREC. This line-listing provides an overview of all

events accompanied by a brief report highlighting the main points of concern. The line-listing reporting of the events through the web portal *ToetsingOnline* to the accredited MREC is sufficient as notification to the competent authority. The Trial Coordinator will report line-listed events to the competent authorities in other Member States, according to the requirements of the Member States.

9.7 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

9.8 Data Monitoring Committee

According to the ICH-GCP Guidelines (2005/28 EG) on-site monitoring is not mandatory. However, the Sponsor chooses to install a Data Monitoring Committee (DMC) in agreement with the advice put out by the Nederlandse Federatie van Universitair Medische Centra (the Dutch Federation of University Medical Centers) (37). The advice is based on a risk assessment for patients participating in this trial, which is considered to be minimal: small chance of minor injury based on previous experiences with the Investigational Medical Device.

The DMC charter contains the details of the monitoring plan. The DMC consists of two independent members of the Trial Coordinating Center of the University Medical Center Groningen and the Trial Coordinator. The advice(s) of the DMC will be sent to the Coordinating Investigator. Should the Coordinating Investigator decide not to fully implement the advice of the DMC, the Coordinating Investigator will send the advice to the reviewing MREC including a note to substantiate why (part of) the advice of the DMC will not be followed.

10. STATISTICAL ANALYSIS

Statistical analyses will be performed using the statistical software package SPSS 19.0 (SPSS Inc, Chicago, IL). The analyses of the trial will be an intention-to-treat analyses of the livers comparing intervention (DHOPE and SCS) against control (SCS only) for all primary and secondary outcomes. The primary endpoint, incidence of NAS at 6 months after transplantation, will be analyzed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. The secondary parameters will be reported overall and by trial site. Binary outcomes will be assessed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table and logistic regression to adjust for prognostic factors. Continuous outcomes will be compared using paired T-test if normally distributed, otherwise using the Wilcoxon signed rank test or a Mann Whitney U test depending on paired or non-paired data. Group and paired data will be analyzed using the repeated measurement ANOVA. Time-to-event outcomes will be analyzed using survival

analysis methods, including Kaplan-Meier plots and Cox proportional hazards regression model with calculation of hazard ratios or alternative validated methods if the proportional hazards assumption is not met. Test will be reported with 95% confidence intervals and two-sided p-values to 3 decimal places. In case of repetitive measurements multiple testing corrections will be applied. A Statistical Analysis Plan (SAP) containing a full description of the statistical methods will be drafted as a separate document early in the trial and finalized prior to the final data lock.

In case of missing data in spite of efforts for obtaining them, we will perform a missing data analysis including the cause of the absent data, the value of the data, the distribution of the missing data and subsequently determine the method of handling the missing data.

10.1 Interim analysis

One interim analysis will be performed by the DMC after 1 year of inclusions to determine the incidence of NAS in the control group in order to determine if the assumption of 29% of NAS in the control group is adequate. There will be no statistical test performed. The DMC will only perform an interim analysis on adverse events if there are concerns about safety as described in the DMC charter. The Pocock sequential boundary will be used to determine statistical significance of adverse events between the two groups, dictating a Z-value of 2 and thus a P-value of 0.045.

The trial may be stopped early due to one of the following situations:
- Unacceptable safety concerns: The analysis shows significant (serious) adverse events in the treatment group compared to the control group.
- In case new external information arises that convincingly answers the study question or raises serious safety issues.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (59th WMA General Assembly, Seoul, October 2008) and according to the latest revised version of the Medical Research Involving Human Subjects (WMO). The procedures set out in this study protocol are designed to ensure that the Sponsor and investigator abide by the principles of the GCP guidelines of the European Community (ICH topic E6, CPMP/ICH/135/95, Directive 2001/20/EC) and the Declaration of Helsinki (in the latest revised version) in the conduct, evaluation and documentation of this study. Copies of the ICH-GCP-Guidelines and the Declaration of Helsinki are included in the Investigator's Study File.

11.2 Recruitment and consent

Since the time frame for obtaining informed consent is limited once the patient is allocated a liver and this period is stressful, it is not the appropriate time to ask for informed consent. Therefore eligible patients will be asked for informed consent while they are on the waiting list. New patients will be asked when they are put on the waiting list. Most patients waiting for a liver transplantation will receive a liver from a donation after brain death (DBD) donor (65%). However, we want patients to be able to decide on participation in this trial. Also, we believe that it is less harmful to ask patients that will not be included in the study due to transplantation with DBD livers or other reasons, for informed consent than it is to tell a patient or relative about a possible complications due to DHOPE when they were not well informed or were too stressed to take in the information. When the patient is summoned to the hospital for the transplantation, the patient will be checked for inclusion and exclusion criteria again. The patient will be informed whether they are included in the trial.

The person who obtains consent must be:

- Suitably qualified and capable of providing information about the study;
- Capable of answering questions about the study or ensuring that such questions are answered by a suitable qualified individual;
- Authorized to do so by the principal investigator or the local investigator.

The written and verbal versions of the information form and informed consent form will be presented to the patients detailing the exact nature of the study, the implications and constraints of the study, the known side effects and any risks involved in taking part. All information sheets and consent forms have been written in English and Dutch. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

When the patient chooses to participate, he/she must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Written informed consent will be documented by means of a dated signature of the participant and a dated signature of the person who gave the verbal information. The original signed form will be retained at the study site and placed in the medical notes. A copy will be given to the participant.

In case the patient doesn't consent to the study, they will receive a liver transplantation according to standard allocation and will be given routine standard of care. Data will not be recorded.

11.3 Benefits and risks assessment, group relatedness

The hypothesized benefit of DHOPE, which is performed in half of the patients included in this trial, is a reduced risk of a NAS. This complication to the biliary tree is of major impact to

the patient since it often implies a multitude of costly and debilitating interventions and hospital admission that may be unsuccessful resulting in retransplantation. Additionally experience with DHOPE in clinical liver transplantations indicate it is beneficial because of improved organ integrity and function after reperfusion but also significantly increases survival after transplantation (in experimental animal models) with a reduction in biochemical markers of preservation injury in clinical pilot studies.

The risk associated with participation in this trial are associated with the procedure DHOPE itself and with the MRCP performed to determine the secondary endpoints.

Risk associated with DHOPE
The risk assessment of DHOPE is regarded as minimal but could be associated with the timing, the perfusion fluid, the pressure used, and the device. DHOPE is technically relatively easy procedure which can be performed by any liver surgeon after training. Since perfusion is performed in the transplantation center while the recipient undergoes surgery, there is no change in transportation of the organ, which will be in the conventional SCS method. The two hours of DHOPE performed at the end of SCS will cause a minor delay in the transplantation process because the preparation of the liver for transplantation and the DHOPE perfusion will be performed by an extra surgical team. Normally, preparation of the liver is performed by the surgeons who perform the transplant operation, but this will be performed simultaneously by another team during this trial.

DHOPE is performed using a machine, which could display a technical failure during perfusion. When this happens, an alarm goes off and the liver is immediately removed from the machine by standby surgeon in a sterile manner. It will be cooled down in preservation fluid from 12ºC (in DHOPE) to 4ºC as is the case in SCS. For some minutes before the liver is cooled down, it has a metabolism of 19% instead of 11% as is the case in SCS. There is no reason to believe that this event will cause any significant injury to the liver grafts because this difference in metabolism is very small, is of short duration, and the liver is saturated with oxygen before the perfusion failure. In case of a malfunction of the Liver Assist cannot be corrected, the donor liver will be stored in cold preservation fluid as usual (SCS). Analysis of the study results, however, will be performed as an intention-to-treat analysis, and the liver / recipient will remain in the intervention group.

The pressure with which the liver is perfused may theoretically cause harm to the organ. However, the pressures used in this trial are very low (lower than physiological) and are reported to be used safely without causing any harm to the organ or the vasculature (38, 39).

The preservation fluid used is similar to the one used in the control group. The only difference is that the sodium and the potassium concentration ratio is inverted. Therefore the components that might cause an allergic reaction in the patient receiving the liver are not different from standard practice.

Risks associated with MRCP

The MRCP will be performed at six months after transplantation in patients with no history of NAS or graft loss. The risks associated with this imaging modality are insignificant. It is a non-invasive test taking about 45 minutes in which the patient lies still while the MRI scan is made. The MRCP will be planned to take place on the same day that the patient has a routine 6-months check-up in the outpatient department to minimize patient's traveling.

In conclusion, the potential risk and burden to the patients in this trial are minimal and therefore are overshadowed by the possibility of our hypothesis being correct; that DHOPE reduces the risk of patients developing NAS after liver transplantation. Additionally, the hypothesis for this trial can only be tested in this group of patients because they are undergoing liver transplantation.

11.4 Compensation for injury

Each participating center has an insurance which is in accordance with the legal requirements in the the country of the participating center. In the Netherlands this is according to Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million and five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. All participating patients will be informed of this insurance in writing through the patient information form. All participating centers outside of the Netherlands are responsible for adequate insurance policy for the patients participating in this trial in their center, according the legal requirements in their country.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

12.1.1 Data collection form

- The data collection protocol and data collection form (eCRF) will be accessed online through a secured website. This will improve protocol adherence, data accuracy, user acceptability, and timeliness of receiving data.
- Validation rules prior to submission will ensure that data is entered in the correct format, within valid ranges and minimise the chance of missing data.

- All data reports will be prepared using a unique study ID, preventing the identification of individual subjects from the report data. The study ID will consist of center abbreviation followed by DHOPE or SCS whichever is applicable and followed by a two digit number which increases per included patient (e.g. UMCG-DHOPE-01). A list of study ID and corresponding name, address and date of birth will be stored separately from the study data per site. The Principle Investigator safeguards this list with a code, known to the Trial Coordinator and the Coordinating Investigator.
- All internet-based forms will be properly secured. Each individual responsible for data entry will receive a personal password and will only be allowed to complete and see those data sections related to his/her level of responsibility and permissions within the trial. When the user logs on to the first time, he/she will have to change the password. The password allowed has to adhere to the following rules: passwords contain at least eight characters, contain both alphabets and numbers, contain a mixture of both upper case and lower case characters, contain at least one numeric character, contain a minimum of five different characters, the maximum sequential repetition of a character allowed is two, and passwords are changed at least once a year.
- The Super User will be the Trial Coordinator and is responsible for assigning user name and passwords to individuals requiring access to the data collection form.
- A tutorial will be provided with the eCRF for all users. The user must complete the tutorial before access to the eCFR is granted and will be displayed when the user first logs in to the eCFR. The tutorial will be designed by the Trial Database Programmer.
- Data will be uploaded to a central database maintained at the UMCG.

12.1.2 Data entry

- The Principal Investigator is ultimately responsible for data entry and data completeness at his/her center. He/she can also delegate this task but remains responsible for data entry and data completeness.
- Validation rules prior to submission will ensure that data is entered in the correct format, within valid ranges and minimise the chance of missing data. Data already entered will be retrievable for viewing through the data entry system. The database and forms will be password protected. Each individual responsible for data entry will receive a personal password and will only be allowed to complete and see those data sections related to his/her level of responsibility and permissions within this trial.
- All randomised recipients completing the 6 month follow-up assessment will be regarded as having completed the study. All recipients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments and that patients do not incur extra financial costs as a result of study participation.
- The Trial Coordinator will oversee data entry for discrepancies and missing data. She will be responsible for the database, and if such discrepancies are identified she will be responsible for identifying the problem and contacting the local center to ensure resolution. She will be responsible for the production of monthly reports to each

participating center containing information and details of missing data requiring completion. Every possible effort will be made to try to attain any missing data.
- However, it is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. In the event of early study termination, the date for withdrawal must be documented and the reason for withdrawal if given. Patients are asked to give consent for historical data available from patient file, which must be documented.
- However, in case a participant discontinues the trial or is lost to follow-up, last follow-up data will be used to attain secondary endpoints if permission is given and missing data will be appropriately handled.

12.1.3 Data management

Research data will be handled with due observance of the Dutch Law for Protection of Personal data (Wet Bescherming Persoonsgegevens). All data will be transmitted over a secure connection and stored in a password-protected central database, the password for which will be regularly changed. Regular backups of the main database will be made to an off-site location. All data in the central database will be stored for at least 15 years following study close-out. The database will be accessible via an “https” website only so that the connection between the server and client is encrypted.
The statistician in the DMC has access to the database to check on completeness and missing data. Before completion of the trial, the statistician of the DMC, members of the National Patient Safety Agency and members of the Medical Ethical Committee are allowed access to the data. After completion, all investigators will be given access to the data.

12.2 Monitoring and Quality Assurance

The monitoring will be performed by the DMC. The DMC will periodically review accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. The DMC will advise the Sponsor if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms. A separate DMC charter will contain full details of the committee and its roles and reporting structure.

12.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the MREC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial

All substantial amendments will be notified to the MREC and to the competent authority. Non-substantial amendments will not be notified to the accredited MREC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited MREC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/reactions, serious adverse device effects, other problems, and amendments.

12.5 End of study report

The investigator will notify the accredited MREC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the investigator will notify the accredited MREC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited MREC.

12.6 Public disclosure and publication policy

The trial will be published in a public trial registry before the first patient is recruited. Since the Sponsor is the Coordinating Investigator, there are no restrictions for disclosure of research data. The results of this study are expected to be published as a collective article from all participating centers in an international medical journal. Centers that enroll at least twenty patients will be represented by (a) co-author(s), as described in section 8 of the Clinical Trial Agreement. Depending on the eventual number of participating and co-publishing centers as well as journal regulations, medium enrolling centers (more than thirty inclusions) will be given the opportunity to list a second co-author, and high enrolling centers (more than 40 inclusions) will be given the opportunity to list a third co-author in the published article. Participating centers may only present study data derived from work packages of this study after consultation of the Sponsor-Principal Investigator. Work package leadership and authorship is agreed upon in the Clinical Trial Agreement (appendix 5).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

The Liver Assist is a CE-marked machine to be used for DHOPE, which is within its indication. Therefore section 13.1 is skipped. See section 12.3 for risk versus benefit assessment.

14. REFERENCES

1. Wertheim JA, Petrowsky H, Saab S, Kupiec-Weglinski JW, Busuttil RW. Major challenges limiting liver transplantation in the United States. Am J Transplant. 2011 Sep;11(9):1773-84.

2. Orman ES, Barritt ASt, Wheeler SB, Hayashi PH. Declining liver utilization for transplantation in the United States and the impact of donation after cardiac death. Liver Transpl. 2013 Jan;19(1):59-68.

3. Jay CL, Skaro AI, Ladner DP, Wang E, Lyuksemburg V, Chang Y, et al. Comparative effectiveness of donation after cardiac death versus donation after brain death liver transplantation: Recognizing who can benefit. Liver Transpl. 2012 Jun;18(6):630-40.

4. Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73.

5. van der Hilst CS, Ijtsma AJ, Bottema JT, van Hoek B, Dubbeld J, Metselaar HJ, et al. The price of donation after cardiac death in liver transplantation: a prospective cost-effectiveness study. Transpl Int. 2013 Apr;26(4):411-8.

6. Op den Dries S, Sutton ME, Lisman T, Porte RJ. Protection of bile ducts in liver transplantation: looking beyond ischemia. Transplantation. 2011 Aug 27;92(4):373-9.

7. Brunner SM, Junger H, Ruemmele P, Schnitzbauer AA, Doenecke A, Kirchner GI, et al. Bile duct damage after cold storage of deceased donor livers predicts biliary complications after liver transplantation. J Hepatol. 2013 Jun;58(6):1133-9.

8. Hansen T, Hollemann D, Pitton MB, Heise M, Hoppe-Lotichius M, Schuchmann M, et al. Histological examination and evaluation of donor bile ducts received during orthotopic liver transplantation--a morphological clue to ischemic-type biliary lesion? Virchows Arch. 2012 Jul;461(1):41-8.

9. Dutkowski P, de Rougemont O, Clavien PA. Machine perfusion for 'marginal' liver grafts. Am J Transplant. 2008 May;8(5):917-24.

10. Fujita S, Hamamoto I, Nakamura K, Tanaka K, Ozawa K. Isolated perfusion of rat livers: effect of temperature on O2 consumption, enzyme release, energy store, and morphology. Nihon Geka Hokan. 1993 Mar 1;62(2):58-70.

11. Koetting M, Luer B, Efferz P, Paul A, Minor T. Optimal time for hypothermic reconditioning of liver grafts by venous systemic oxygen persufflation in a large animal model. Transplantation. 2011 Jan 15;91(1):42-7.

12. Schlegel A, Rougemont O, Graf R, Clavien PA, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. J Hepatol. 2013 Feb;58(2):278-86.

13. Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved rat liver by hypothermic oxygenated machine perfusion. Am J Transplant. 2006 May;6(5 Pt 1):903-12.

14. Koetting M, Minor T. Donation after cardiac death: dynamic graft reconditioning during or after ischemic preservation? Artif Organs. 2011 Jun;35(6):565-71.

15. Kim JS, Nitta T, Mohuczy D, O'Malley KA, Moldawer LL, Dunn WA, Jr., et al. Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. Hepatology. 2008 May;47(5):1725-36.

16. Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg. 2006 Dec;244(6):968-76; discussion 76-7.

17. Guarrera JV, Henry SD, Samstein B, Reznik E, Musat C, Lukose TI, et al. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. Am J Transplant. 2015 Jan;15(1):161-9.

18. Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant. 2010 Feb;10(2):372-81.

19. Dutkowski P, Schlegel A, de Oliveira M, Mullhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. J Hepatol. 2014 Apr;60(4):765-72.

20. Groen H, Moers C, Smits JM, Treckmann J, Monbaliu D, Rahmel A, et al. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. Am J Transplant. 2012 Jul;12(7):1824-30.

21. Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.

22. D'Alessandro A M, Hoffmann RM, Knechtle SJ, Odorico JS, Becker YT, Musat A, et al. Liver transplantation from controlled non-heart-beating donors. Surgery. 2000 Oct;128(4):579-88.

23. Chan EY, Olson LC, Kisthard JA, Perkins JD, Bakthavatsalam R, Halldorson JB, et al. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. Liver Transpl. 2008 May;14(5):604-10.

24. Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10.

25. Dezza MC, Berrevoet F, Sainz-Barriga M, Rossetto A, Colenbie L, Haentjens I, et al. The choice of recipient does not have a bearing on early outcome in liver transplant patients receiving grafts from non-heart-beating donors: a reappraisal? Transplant Proc. 2007 Oct;39(8):2675-7.

26. Stahl JE, Kreke JE, Malek FA, Schaefer AJ, Vacanti J. Consequences of cold-ischemia time on primary nonfunction and patient and graft survival in liver transplantation: a meta-analysis. PLoS One. 2008;3(6):e2468.

27. Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63.

28. Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53.

29. op den Dries S, Karimian N, Sutton ME, Westerkamp AC, Nijsten MW, Gouw AS, et al. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. Am J Transplant. 2013 May;13(5):1327-35.

30. van der Plaats A, t Hart NA, Verkerke GJ, Leuvenink HG, Ploeg RJ, Rakhorst G. Hypothermic machine preservation in liver transplantation revisited: concepts and criteria in the new millennium. Ann Biomed Eng. 2004 Apr;32(4):623-31.

31. Uemura T, Randall HB, Sanchez EQ, Ikegami T, Narasimhan G, McKenna GJ, et al. Liver retransplantation for primary nonfunction: analysis of a 20-year single-center experience. Liver Transpl. 2007 Feb;13(2):227-33.

32. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

33. Slankamenac K, Nederlof N, Pessaux P, de Jonge J, Wijnhoven BP, Breitenstein S, et al. The comprehensive complication index: a novel and more sensitive endpoint for assessing outcome and reducing sample size in randomized controlled trials. Ann Surg. 2014 Nov;260(5):757-62; discussion 62-3.

34. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999 Mar 16;130(6):461-70.

35. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care. 2007;11(2):R31.

36. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003 Jan;26 Suppl 1:S5-20.

37. Centra NFvUM. Kwaliteitsborging mensgebonden onderzoek 2.0.

38. op den Dries S, Sutton ME, Karimian N, de Boer MT, Wiersema-Buist J, Gouw AS, et al. Hypothermic oxygenated machine perfusion prevents arteriolonecrosis of the peribiliary plexus in pig livers donated after circulatory death. PLoS One. 2014;9(2):e88521.

39. Schlegel A, Dutkowski P. Role of hypothermic machine perfusion in liver transplantation. Transpl Int. 2014 May 23.

15. Appendices

15.1 Appendix 1. Patient information form

15.2 Appendix 2. Patient informed consent form

15.3 Appendix 3. Data collection parameters

15.4 Appendix 4. Data Monitoring Committee Charter

15.5 Appendix 5. Clinical Trial Agreement

2. Final Version of Study Protocol

A Multicenter Randomized Controlled Trial to Compare the Efficacy of End-ischemic Dual Hypothermic Oxygenated Perfusion with Standard Static Cold Storage of Liver Grafts Donated after Circulatory Death in Preventing Non-anastomotic Biliary Strictures after Transplantation

DHOPE-DCD Trial

A Multicenter Randomized Controlled Clinical Trial
Version 5.0 – 25 February 2020

PROTOCOL TITLE:

A Multicenter Randomized Controlled Trial to Compare the Efficacy of End-ischemic Dual Hypothermic Oxygenated Perfusion with Standard Static Cold Storage of Liver Grafts Donated after Circulatory Death in Preventing Non-anastomotic Biliary Strictures after Transplantation

Title in Dutch: Een gerandomiseerd, vergelijkend onderzoek om de effectiviteit van hypotherme geoxygeneerde perfusie van de lever te vergelijken met de standaard koude bewaarmethode in het voorkomen van vernauwingen in de galwegen van de lever na transplantatie wanneer de donor lever afkomstig is van een donor overleden aan een hartstilstand

Protocol ID	DHOPE-DCD Trial / ISRCTNxxx
Short title	End-ischemic DHOPE in DCD liver transplantation
EudraCT number	N/A
Version	Version 5.0 – 25 February 2020
Coordinating investigator/ project leader	R.J. Porte, r.j.porte@umcg.nl, +31503619027 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Sponsor	R.J. Porte, r.j.porte@umcg.nl, +31 50 3619027, University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Trial Coordinator	R. van Rijn, r.van.rijn@umcg.nl, +31652724616 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands
Principal investigator(s) per site	R.J. Porte, r.j.porte@umcg.nl, +31503619027 University Medical Center Groningen, Department of Surgery, P.O. Box 30.001, 9700 RB Groningen, the Netherlands J. de Jonge, j.dejonge.1@erasmusmc.nl, +31633330449 Erasmus Medical Center, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands B. van Hoek, b.van_hoek@lumc.nl, +31715263507, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands D. Monbaliu, diethard.monbaliu@uzleuven.be, +3216348727, University Hospitals Leuven, Herestraat 49, 3000 Leuven, België R. Troisi, Roberto.troisi@ugent.be, +3293325519 Ghent University Hospital, De Pintelaan 185, 9000 Gent, Belgium N. Heaton, nigel.heaton@nhs.net, +472032994801 King's College Hospital NHS Trust, London SE5 9RS, United Kingdom

Subsidising party	Participating centers Foundation NutsOhra Bridge to Life
Independent expert (s)	Philipp Dutkowski, MD, PhD, philipp.dutkowski@usz.ch, +41442554236, UniversitätsSpital Zürich, Klinik für Viszeral- und Transplantationschirurgie, Rämistrasse 100, 8091 Zürich, Zwitserland
Company name	Organ Assist B.V., Aarhusweg 4-7, 9723 JJ, Groningen, the Netherlands, +31503131905 Bidge to Life Ltd., 128 Suber Rd., Columbia, SC 29210, USA, +18035450080
Laboratory sites	Surgical Research Laboratory, CMC V, Y2144, BA44, Hanzeplein 1, 9713 GZ Groningen, the Netherlands LETIS Erasmus MC Rotterdam, Nb1018, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands
Pharmacy	Pharmacy, Clinical Pharmacology and Pharmacy UMCG, Groningen, P.O. Box 30.001, 9700 RB Groningen, the Netherlands, Tel: +31503614071

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor / Coordinating investigator		
Prof.dr. R.J. Porte, MD HPB and Liver Transplant Surgeon University Medical Center Groningen		
Principal investigator per site:		
Prof.dr. R.J. Porte, MD HPB and Liver Transplant Surgeon University Medical Center Groningen		
Dr. J. de Jonge, MD HPB and Transplant Surgeon Erasmus Medical Center, Rotterdam		
Prof.dr. B. van Hoek, MD Gastroenterologist Leiden University Medical Center, Leiden		
Prof.dr. D. Monbaliu, MD Transplant Surgeon University Hospitals Leuven, Leuven		
Prof.dr. R.I. Troisi, MD Transplant Surgeon Ghent University Hospital		
Prof.dr. N. Heaton, MD Consultant Transplant Surgeon King's College Hospital NHS Trust, London		
Local Investigators per site:		
Dr. W.J. Polak, MD Erasmus Medical Center		
Prof. dr. J.N.M. Ijzermans, MD Erasmus Medical Center		
Prof.dr. X. Rogiers, MD Ghent University Hospital		
Ms. Miriam Consuelo Cortes, MD Consultant Transplant Surgeon King's College Hospital NHS Trust, London		
Trial Coordinator:		
R. van Rijn, MD University Medical Center Groningen		
Independent Experts:		
Prof. Dr. P. Dutkowski, MD HBP and Liver Transplant surgeon		

UniversitätsSpital Zürich		
Adjudication Committee:		
R.J. de Haas, MD Radiologist University Medical Center Groningen		
Dr. J.J.G. Slangen, MD Radiologist University Medical Center Groningen		
Nanda Krak, MD Radiologist Erasmus Medical Center, Rotterdam		
Data Safety Monitoring Board:		
Dr. Erik A.M. Verschuuren Pulmonologist University Medical Center Groningen		
Dr. Cyril Moers Surgeon University Medical Center Groningen		
Dr. Mostafa el Moumni Surgeon, Epidemiologist Universtiy Medical Center Groningen		
Company:		
M. van Voorden Organ Assist B.V., Groningen		
A. Gilchrist Bridge to Life Ltd, USA		

INVESTIGATOR SIGNATURE PAGE

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

I understand that I may terminate or suspend enrolment of the study at any time if it becomes necessary to protect the best interests of the study subjects as advised by the DSMB. This study may be terminated by the University Medical Center of Groningen, with or without cause.

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

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

I will ensure that the requirements relating to the Ethics Committee (EC) review and approval are met. I will provide the University Medical Center of Groningen with any material that is provided to the EC for ethical approval.

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

I agree to promptly report to the DSMB, EC, and sponsor any changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without EC approval, except where necessary to ensure the safety of study participants.

Name…… **Signature……**

Date…….

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AlkP	Alkaline Phosphatase
AKIN	Acute Kidney Injury Network
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-3L	European Quality of Life Instrument 5D-3L
CCI	Comprehensive Complication Index
CV	Curriculum Vitae
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
ECD	Extended Criteria Donor
ERCP	Endoscopic Retrograde Cholangiopancreaticography
EU	European Union
GCP	Good Clinical Practice
γGT	Gamma-glutamyl Transferase
HMGB	High Mobility Group Box-1
DHOPE	Dual Hypothermic Oxygenated Perfusion
ICU	Intensive Care Unit
INR	International Normalized Ratio
IPF	Initial Poor Function
MDRD	Modification of Diet in Renal Disease
MELD	Model for End-stage Liver Disease
miRNA	Micro Ribonucleic acid
MP	Machine Perfusion
MREC	Medical Research Ethics Committee (in Dutch: METc)
MRCP	Magnetic Resonance Cholangiopancreatography
NA	Not Applicable
NAS	Non-anastomotic Biliary Strictures
NODAT	New Onset Diabetes After Transplantation
OLT	Orthotopic Liver Transplantation
PNF	Primary Non-Function
PT	Prothrombin Time
RIFLE	Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
(S)ADE	(Serious) Adverse Device Effects
(S)AE	(Serious) Adverse Event
SCS	Static Cold Storage
SOP	Standard Operation Procedure
UMCG	University Medical Center Groningen
USADE	Unanticipated Serious Adverse Device Effects
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Recent publications report good results of controlled donation after circulatory death (DCD) Maastricht category III liver transplantation when strict donor-recipient matching is applied and ischemia times are kept to a minimum. However a major concern remains the high rate of biliary complications after transplantation of DCD livers. Non-anastomotic biliary strictures (NAS) occur in 29% of patients receiving a DCD graft whereas the incidence of NAS in recipients of donation after brain death (DBD) liver grafts is 11%. NAS are associated with higher morbidity and increased cost of liver transplantation. Injury to the biliary epithelium and the peribiliary vascular plexus occurring during donor warm ischemia and static cold storage (SCS) has been identified as a major risk factor for development of NAS. Machine perfusion has been proposed as an alternative strategy for organ preservation, offering the opportunity to improve the quality of the organ by providing oxygen to the graft. Experimental studies have shown that end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) helps liver grafts to recover from ischemia by restoring mitochondrial function. Moreover, DHOPE has been shown to provide better preservation of peribiliary vascular plexus of the bile ducts, which could be an important step forward in reducing the incidence of NAS after transplantation.
Objective: To study the efficacy of end-ischemic DHOPE in reducing the incidence of NAS within six months after controlled DCD (Maastricht category III) liver transplantation.
Study design: An international, multicenter, prospective, randomized, controlled, interventional, clinical trial with a two parallel arm approach (treatment/control).
Study population: Adult patients (≥18 yrs old) undergoing a liver transplantation with a liver graft procured from a controlled DCD donor (Maastricht category III) with a body weight ≥40 kg.
Intervention: In the intervention group liver grafts will be subjected to two hours of hypothermic, oxygenated perfusion at the end of SCS and before implantation. In the control group donor liver grafts will be preserved in accordance to standard practice by SCS only.
Main study parameters/endpoints: The incidence and severity of NAS as diagnosed by an Adjudication committee (who are blinded for the group assignment) by means of magnetic resonance cholangiopancreatography (MRCP) at six months after DCD liver transplantation.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients participating in this trial will experience minimal burden. There are only three difference in follow-up compared with the routine practice: livers undergo DHOPE and patients fill in a questionnaire and undergo a MRCP. The intervention, DHOPE, is associated with a non-significant risk of injury of the isolated liver due to perfusion pressure or perfusion failure. The perfusion pressures in this protocol are very low and are reported to cause no harm to the organ. In case of perfusion failure, the liver can easily and quickly (within minutes) be brought to the same conditions as in the control group. In these bridging minutes the organ has a metabolism of 19% instead of the 11%. This is non-significant especially because the organ is saturated with oxygen before the possible event. There is a minimal burden but there are no risks related to the questionnaire or the MRCP, which is planned during a routine hospital visit. When the intervention is effective in reducing the incidence of NAS, the patients participating in this trial benefit substantially when they are randomized to the intervention group. This study can only be performed in these patients because they undergo a DCD liver transplantation.

ADMINISTRATIVE INFORMATION

Investigators

Coordinating Investigator / Project Leader

The Coordinating Investigator / Project Leader is the Sponsor and will have oversight of:
- Design and conduct of the trial
- Preparation of protocols and revisions
- Preparation of standard operating procedures (SOPs)
- Preparation of electronic case report forms (eCRF)
- Organizing Investigator Meetings
- Publication of study reports
- Appoint members of the Data Safety Monitoring Board
- Review adverse events for immediate risk to patients

Principal Investigators

In each center a Principal Investigator will be identified, to be responsible for follow-up of recruitment, data collection and completeness of eCRFs in his/her center. The Principal Investigator is the liaison between the Local Investigators and the Trial Coordinator. Responsibilities will include:
- Obtaining local Ethics Committee and Research Governance approval (aided by the Trial Coordinator)
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants
- Publication of study reports of the own work package

Local Investigators

In each participating center Local Investigator(s) (transplant surgeon and/or hepatologist) will be identified. Responsibilities include:
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants

Trial Coordinator

The Trial Coordinator is responsible for tasks delegated to him/her including:
- Preparation of randomization tool
- Identification and recruitment of patients to the study
- Oversee data entry and monthly report on missing data
- Review adverse events for immediate risk to patients
- Report adverse events according to section 9.6

Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) is responsible for:
- Agreeing a charter for the conduct of the DSMB (see Appendix 4)
- Reviewing data from the study according to the schedule set out in the charter
- Reviewing serious adverse events (device related or not) and any device deficiencies

Adjudication Committee

The Adjudication Committee is responsible for timely assessment of Magnetic Resonance Cholangiopancreatography (MRCP) images obtained in all participants. The Committee consists of clinical experts in the specific clinical area: two independent radiologists from the Sponsor site and one from the Erasmus MC. In order to allow for an unbiased endpoint assessment the members are blinded to treatment assignment when assessing the images. If there is disparity between the members, they will discuss the case to reach an unequivocal decision.

Company

Organ Assist is responsible for the production and delivery of the Liver Assist machine perfusion devices. They are responsible for the training of all individuals that operate the device according to its intended use. They are responsible for any malfunction, for repairs and are obligated to deliver a replacement device within reasonable time (24 hours) after a defect device has been reported to Organ Assist.

1. INTRODUCTION AND RATIONALE

Limited organ availability for orthotopic liver transplantation (OLT) remains to be a major concern (1). Utilization of livers with marginal quality or so called “extended criteria” donors (ECD) including older donors, donors with fatty livers and donation after circulatory death (DCD) donors have reduced organ deficit in recent years. In fact, the percentage of DCD donors in the United States of America has increased from 1.1% in 1995 to 11.2% in 2010 (2). However, poor post-transplant outcomes of these grafts have concurrently limited the utilization of these livers. The percentage of unused grafts, mainly attributed to the increasing number of DCD donors, increased from 9% in 2004 to 28% in 2010 (2). Longer hospital stays and increased costs have been associated with DCD transplants (3-5). Non-anastomotic biliary strictures (NAS) are a major complication after OLT and occur in 29% of patients receiving a DCD donor graft, compared to 11% incidence among recipients of donation after brain death (DBD) liver grafts in the University Medical Center Groningen (UMCG) (unpublished data).

Among the variety of risk factors described to be associated with NAS, ischemia/reperfusion related injury is one of the most important concerns. Donor warm ischemia, as well as cold ischemia injury caused during static cold storage (SCS), has been associated with the development of NAS after DCD (4, 6). Also, injury of the peribiliary vascular plexus is thought to play an important role in development of NAS (7, 8).

The need for an increased number of usable donor livers and a better use of ECD liver grafts necessitates the development of more qualified preservation methods. Machine perfusion (MP) is an alternative strategy that offers a more dynamic preservation and provides opportunities to improve the quality of organs derived from ECD (9). One of the most important benefits of MP compared to conventional SCS is the ability to provide oxygen to the graft. Even at very low temperatures of 12°C liver metabolism still requires 0.467 µmol oxygen/min/g liver tissue (10), which can be supplied by oxygenated MP. Other benefits are that nutrients are supplied to the graft and that toxic waste products are diluted and removed. A relatively simple technique to revitalize grafts after a time period of cold ischemia is end-ischemic hypothermic oxygenated perfusion (DHOPE) at the hospital of the recipient (11, 12).

Experimental animal studies have demonstrated that oxygenated machine perfusion can help organ recovery by improving cellular energy homeostasis. The restoration of mitochondrial function by oxygenated MP, resulting in an increased adenosine triphosphate (ATP) tissue concentration, leads to less cellular death after warm reperfusion (11-13), a better hepatocyte function, and enhanced energy dependent bile production (14). It has been shown that hypothermic oxygenated reconditioning of grafts leads to better preserved autophagic pathways (15) and thus a significant decrease of hepatocellular necrosis. To summarize, experimental animal studies have shown that 1-2 hours of hypothermic oxygenated perfusion of liver grafts after SCS not only improves organ integrity and function after reperfusion but also significantly increases survival after transplantation (16).

After these experimental studies, the method was investigated in the clinical setting of human liver transplantation in centers in New York and Zurich. These first clinical experiences have shown that hypothermic machine perfusion is safe, may improve graft function, and attenuates classical biochemical markers of liver preservation injury. Early results suggest there is a reduction in preservation injury that may be responsible for better early allograft function and fewer complications post transplantation, like less biliary complications, and shorter hospital stay in comparison to patients receiving a liver preserved with SCS alone (17, 18). The Zurich group has reported extensively on the feasibility and safety of DHOPE of the portal vein and they have reported excellent early and long-term liver graft function with low serum parameters for liver injury and cholestasis, short ICU stay, and no NAS after six months after transplantation (19). Feasibility and safety of DHOPE of both the portal vein and hepatic artery was recently demonstrated in a pilot study in Groningen (NTR4493; van Rijn et al. unpublished data)

Considering these promising results along with the relative simplicity and safety of this technique (20), end-ischemic DHOPE seems to be a suitable approach to be applied in the clinical setting. However, it is not routinely applied and the efficacy of DHOPE in reducing postoperative biliary complications has not yet been evaluated. Preliminary results have indicated that DHOPE may ameliorate vascular endothelial injury (12) and provide a better preservation of peribiliary vascular plexus (16). Since these injuries are associated with development of NAS, it is hypothesized that DHOPE reduces the incidence of NAS after liver transplantation. Especially in DCD liver

transplantation, NAS is a major concern. Therefore, this graft type may have the greatest benefit from DHOPE. The present clinical trial is designed to establish the efficacy of two hours of end-ischemic DHOPE prior to implantation of a DCD (Maastricht category III) liver graft in reducing the postoperative incidence of NAS.

2. OBJECTIVES

Hypothesis:
End-ischemic DHOPE after SCS is a better method for preservation of the biliary tree resulting in a lower incidence of symptomatic NAS after DCD liver transplantation than SCS alone.

Primary Objective:

To study the efficacy of end-ischemic DHOPE in reducing the incidence of NAS after DCD Maastricht category III liver transplantation at 6 months after transplantation.

Secondary Objective(s):
To study the effect of the intervention (end-ischemic DHOPE after SCS), in comparison to the control group (SCS only), concerning:

1. The overall incidence of symptomatic and asymptomatic NAS
2. The severity of NAS after transplantation
3. The graft and recipient survival
4. The incidence of primary non-function (PNF)
5. The incidence of initial poor function (IPF)
6. The biochemical analysis of graft function and ischemia-reperfusion injury
7. The hemodynamic status of the recipient after graft reperfusion
8. Length of stay in the ICU and hospital
9. The incidence of postoperative complications, including infections and use of antibiotics
10. The renal function
11. The perfusion characteristics during DHOPE (in the intervention group only)
12. The perfusate analysis during DHOPE (in the intervention group only)
13. Prognostication of NAS, based on micro ribonucleic acid (miRNA) profiles in perfusion fluid (in the intervention group only)
14. Pathobiology of liver and bile duct parenchyma
15. Metabolic function, including new onset diabetes after transplantation (NODAT)
16. Overall cost of treatment within 6 months (in/excluding return to work)
17. Overall health related quality of life after transplantation

3. STUDY DESIGN

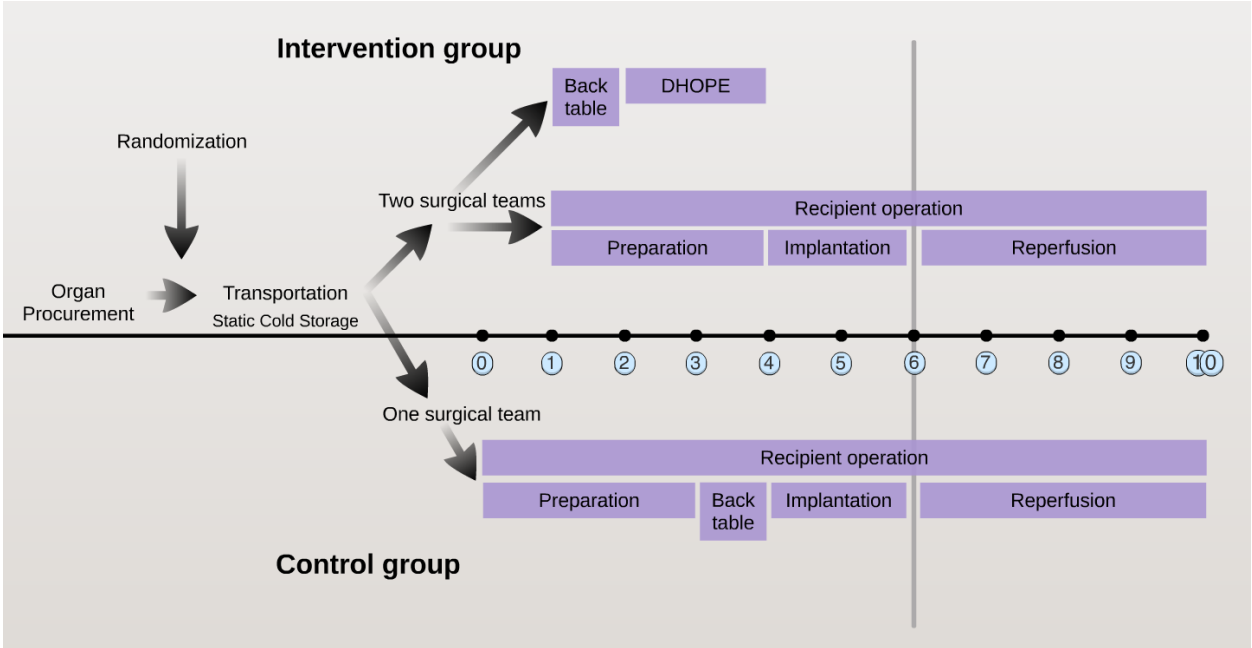
The trial is designed as a prospective, randomized, controlled, multicenter, superiority, clinical trial with two parallel groups. Primary endpoint is the incidence of NAS at six months after liver transplantation. Liver grafts in the treatment group will be preserved with SCS followed by DHOPE. Liver grafts in the control group will be preserved by SCS alone without any further intervention. **Figure-1** provides an overview of the study design.

The study setting is standard practice of OLT. The surgical procedure, post-operative care and follow-up are identical to the routine OLT-practice in each participating center. The surgical procedure will start a little earlier in the intervention group compared to the control group in an attempt to keep total preservation time equivalent.

Patients are followed during the first twelve months post-transplantation, during hospital stay and after discharge, via their routine hospital visits at 1 month, 3 months, 6 months, and 12 months after transplantation. Whenever patients included in this trial are suspected of NAS based on clinical parameters as judged by their physician, they will undergo routine examinations which include MRCP imaging of the biliary tree. The study duration is 12 months for each participant. This time interval is chosen because diagnosis of NAS is reported at a median of 3 to 4 months after transplantation (21, 22). Some studies have reported an occurrence of 100% of the cases of NAS within 4 months (22-25).

In case a patient in this trial is not diagnosed with NAS within 6 months, a MRCP is performed at six months after transplantation to assess the biliary tree for asymptomatic injury. All MRCP images will be evaluated by an Adjudication Committee in order to harmonise and standardise endpoint assessment. The Adjudication Committee consists of three independent radiologists. In order to allow for an unbiased endpoint assessment the members are blinded to study group assignment.

Figure 1.



4. STUDY POPULATION

4.1 Population (base)

Adult patients (≥ 18 years old) with end-stage chronic liver disease awaiting liver transplantation are screened for participation in this trial.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adult patients (≥ 18 years old)
- Signed informed consent
- Willing and able to attend follow-up examinations
- Donor liver graft from a controlled donation after circulatory death (Maastricht category III)
- Donors with a body weight ≥40 kg

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Simultaneous participation in another clinical trial that might possibly influence this trial
- Mental conditions rendering the subject incapable to understand the nature, scope and consequences of the trial
- Listed for liver transplantation due to fulminant liver failure or retransplantation because of PNF
- Recipient positive test for HIV antigen or HIV antibody
- Donor positive for HIV antigen, HIV antibody, Hepatitis B core antibody or hepatitis B surface antigen, or hepatitis C antibody
- Patients with contra-indications for MRCP (i.e. pacemaker)
- Simultaneous transplantation of another organ

4.4 Sample size calculation

The study is powered to detect a clinically relevant difference in incidence of NAS between the two study groups. The incidence of NAS is 29% after DCD liver transplantation and is 11% after DBD liver transplantation in patients transplanted in the UMCG from 2008 to 2013 (unpublished data). This is similar to incidence reported by Abt *et. al.* (27% in DCD versus 2% in DBD transplantation), Dubbeld *et. al* (24% versus 8%), Croome *et. al.* (22% versus 4%, and Meurisse *et. al.* (33% versus 12%) (4, 24, 27, 28). With the intervention (DHOPE) we aim to reduce the incidence of NAS after DCD liver transplantation to the level observed after DBD liver transplantation (absolute difference of 29-11=18%). We base this presumed reduction on our results in the pilot study in which 1 of 10 (10%) of the patients with a DHOPE treated liver developed NAS. The other previously reported pilot studies observed no NAS in any of the patients receiving a liver treated with end-ischemic hypothermic machine perfusion. For a power of 80% (β=0.80) and a 5% significance level (2-sided test) in two independent cohorts, using a Chi-squared test, 77 livers are needed to be included in each arm, calculated with nQuery + nInterim 3.0. Although there is a (very) small likelihood of lost to follow-up, we still want to include

an extra patient per study arm. In conclusion,, the total number of patients to be included in this study will be 156 (77*2+2).

4.5 Study duration

Based on expected numbers to be enrolled in the participating centers per country per year we estimate an inclusion period of 24 months, depending on participating centers. With a follow-up period of 12 months, the study duration total to 30 months.

An estimate for the number of inclusions per country per year is based on 2013:
- The Netherlands: 48 donors per year, 96 in two years

Potential partners outside the Netherlands (to be confirmed):
- Birmingham and Edinburgh, UK: 40 in two years
- Montreal and Halifax, Canada: 20 in two years
- Cordoba, Spain:
- Paris, France:

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The patients randomized to the control group will receive a liver graft preserved by conventional SCS without any further intervention. In the treatment group donor livers will be subjected to two hours of DHOPE applied by a medical device named the Liver Assist® (see section 6). During transportation from the donor hospital to the recipient hospital, the liver is preserved via SCS. The intervention is restricted to the liver graft after arrival in the transplant center and before implantation. A detailed description of DHOPE is given in section 6.5.

5.2 Use of co-intervention and concomitant care

Donors will be managed by local standard of care and protocols formulated by the local organ procurement organizations. No changes in donor management will be made for the sake of this trial. *In situ* flushing of the liver during organ retrieval will be done according to local standard of care and protocols. Concomitant care of the recipient, including the implantation procedure, postoperative care, immunosuppression and other medications will reflect the current standard of care at the recipient hospital. Local immunosuppression protocols will not be altered for the sake of this trial. There is no restriction in behaviour, in use of co-interventions or medication for patients in this trial.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

The Liver Assist is a dedicated machine for *ex-vivo* liver perfusion during storage. It is a CE marked (European Union Certification of Safety, Health and Environmental Requirements) device that is designed, produced, and delivered by Organ Assist (Groningen, The Netherlands) and has been

used in a pilot study (NTR 4493, van Rijn et al. unpublished data). Organ Assit will also be responsible for training of the relevant individuals on how to operate the device for its intended use as well as for maintenance and repairs to the machine as required. Organ Assist will deliver a replacement device within 24 hours in the event of a defect being reported to them. Training on the use of the Liver Assist machine will be provided in advance of recruitment of the first patient. A record of all device training will be maintained.

The Liver Assist enables dual perfusion via the portal vein and the hepatic artery using two centrifugal pumps to provide a continuous venous flow and a pulsatile arterial flow at 60 bpm. The system is pressure controlled which allows autoregulation of the flow through the liver, with constant pressure at variable flow rates. The perfusion fluid can be oxygenated by two hollow fiber membrane oxygenators and carbon dioxide van be removed. The temperature of the preservation fluid can be adjusted between 10 and 38°C. The system can be filled with any preferred perfusion fluid.

6.2 Description and justification of method of use in this trial

The instruction manual of the Liver Assist will be submitted as a separate document, but a summary of how the machine will be used in the trial is described below.

Preparation of the liver in both study groups
After circulatory death of the donor, the stand-by surgical team performs a median laparotomy and aortic cannulation to perfuse the abdominal organs with at least 4000 ml of cold (0-4°C) preservation fluid, preferably Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL) with 50.000 IU of heparin. The liver is procured with a segment of 5 cm circular supratruncal aorta left attached to the coelic trunk if possible. The portal vein and common bile duct is kept as long as possible. After procurement the liver is flushed via the portal vein with at least 1 liter of preservation fluid, preferably Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL), without applying pressure. The cystic duct is ligated and the bile ducts are flushed with preservation fluid, preferably Cold Storage Solution – Belzer UW (Bridge-to-Life, Ltd., Northbrook, IL) preferably at the donor hospital. The gallbladder is preferably left in situ. The liver is transported to the recipient hospital, where the conventional back table procedure is performed by the surgeon.

Preparation in intervention group only
Subsequently, the portal vein and the supratruncal aorta are cannulated. The supratruncal aorta is cannulated so that the hepatic artery is not damaged due to cannulation. The side branches of the hepatic artery are ligated or clipped during the back table preparation. Short before connection to the Liver Assist, the liver is flushed via the portal vein cannula with 1000 mL cold (0-4°C) Belzer machine perfusion solution (Bridge-to-Life, Ltd., Northbrook, IL) until the caval effluent is clear. Normally the back table preparation takes one hour on average.

Preparation of the Liver Assist
Simultaneously with the back table procedure, the Liver Assist is prepared for use. The disposable is connected to the machine and is filled with 4000 mL ice-cold machine perfusion solution Belzer

UW ® (Bridge-to-Life, Ltd., Northbrook, Ireland), with additional 3 mmol/L glutathione (Biomedica, Foscama Group, Roma, Italy). The gluthatione is a component of the machine perfusion solution – Belzer UW, which is added at recommendation of Bridge-to-Life because it may have become inactive during shelf-time. The system will be pressure controlled with the pressure limited to a mean of 25 mmHg for the hepatic artery and 5 mm Hg for the portal vein. These pressure settings are based on previous studies and are lower than physiological pressures to avoid shear stress of the cold endothelium of the hepatic vasculature (12, 16, 29). The temperature of the perfusion fluid will be 12°C, when the temperature is set to 10°C. The thermoregulator of the Liver Assist is filled with crushed ice in the reservoir of the cooling unit to achieve the desired temperature. The oxygen flow is set at 500 mL/min of 100% oxygen on each of the two membrane oxygenators. This flow is adequate to obtain a pO2 which has been reported to be effective in increasing ATP and not harmful to the graft (10, 30). The Liver Assist is ready for perfusion once the temperature is reached and the solution is oxygenated for at least 15 minutes with oxygen. The preparation of the Liver Assist takes 30 minutes on average.

Perfusion of liver with Liver Assist
The surgeon connects the cannulas to the disposable tubings of the Liver Assist after which the pumps of the device are started. During the connection of the liver to the machine, the perfusion pressure will be adjusted manually in the first five minutes after connection so that a minimum of 100 ml/min flow via the portal vein is maintained, but without exceeding a portal vein pressure of 7 mm Hg. Perfusion fluid and liver will be cooled to 12°C by the thermoregulator. The reservoir of the cooling unit of the Liver Assist must be filled with crushed ice that is regularly replaced. The Liver Assist continuously registers flow rates and temperature and gives alarms in case of high flow or temperature. A surgeon supervises this procedure and is in the vicinity.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary endpoint of this trial is the incidence of symptomatic NAS at six months after DCD liver transplantation. The diagnosis of symptomatic NAS is defined as all of the following criteria (21):

- any irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
- which are diagnosed by cholangiogram (preferably by MRCP)
- in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
- and as assessed by the Adjudication Committee

- when imaging is indicated by clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up

This endpoint is selected as it is considered to reflect a clinically relevant sign of biliary injury caused by ischemia-reperfusion (21). Also, it is reproducibly attainable at all study sites and therefore can be objectified by blinded assessment by the Adjudication Committee including three independent radiologists. Moreover, the imaging modality is minimally invasive and is part of the routine diagnostic work-up in case of clinical suspicion of NAS.

8.1.2 Secondary study parameters/endpoints

1. The overall incidence of NAS is based on symptomatic NAS (see primary study parametersendpoint) and asymptomatic NAS. Asymptomatic NAS is defined as all of the following:
    a. irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
    b. which are diagnosed by cholangiogram (preferably by MRCP)
    c. in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
    d. in the absence of clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up
2. The severity and location of NAS will be determined by the Adjudication Committee, based on:
    a. Assessment of the images of the MRCP obtained in all patients at six months after transplantation (time window of 15 days) which will be performed based on a scoring system described by Buis et. al. (21)
    b. Required treatment for NAS (i.e. ursodeoxycholic acid, ERCP, retransplantation)
3. Graft (censored and uncensored for patient death) and patient survival at 7 days, 1, 3, 6, and 12 months after transplantation
4. PNF is defined as liver failure requiring retransplantation or leading to death within seven days after transplantation without any identifiable cause such as surgical problems, hepatic artery thrombosis, portal vein thrombosis and acute rejection (31).
5. IPF is defined based on a modification of the Olthoff criteria: Prothrombin time/INR >1.6 and or serum total bilirubin >10 mg/dL on postoperative day 7 (32). If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.
6. Biochemical analysis of graft function and ischemia-reperfusion injury is determined with serum levels of alanine aminotransferase (ALT), AST, alkaline phosphatase (AlkP), gamma-glutamyl transferase (γGT), and total bilirubin at postoperative day 0 – 7 and 1, 3, 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.
7. Hemodynamic status (blood pressure, heart rate and vasopressor dosage) will be recorded 5 min before reperfusion, as well as 10 and 20 minutes after reperfusion
8. Length of initial ICU and initial hospital stay is determined in days of admission following liver transplantation. Duration of follow-up hospital stay is determined in days of hospital admission after discharge and up to six months after liver transplantation.

9. Postoperative complications are graded according to the comprehensive complication index (CCI) (33). Special interest will be given to predefined infectious complications and the total length of use and cumulative doses of antibiotics.
10. Renal function is defined as estimated glomerular filtration rate (eGFR) according to the 4-variable Modification of Diet in Renal Disease (MDRD) equation (34) at day 7, and 1, 3, 6 months after transplantation. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered. Kidney injury is scored according to acute kidney injury network (AKIN) and risk, injury, failure, loss of kidney function and end-stage kidney disease (RIFLE) criteria (35). In selected centers, urinary kidney injury markers (kidney injury molecule (15), tissue inhibitor of matrix metalloproteinases-2 [TIMP2], Insulin-like Growth Factor Binding Protein-7 [IGFBP7], and neutrophil gelatinase-associated lipocalin [NGAL]) are determined preoperatively, at arrival in the ICU and at day 1, 3, and 5 after transplantation.
11. Perfusion characteristics during DHOPE include flow, pressure and resistance at every fifteen minutes.
12. In selected centers, perfusate analyses will be performed to study the dynamics of experimental markers of tissue and mitochondrial injury. The perfusate at the start and end of DHOPE procedure, and every half hour in between will be analysed for pH, sodium, potassium, bicarbonate, lactate, ALT, AST, AlkP, γGT, urea, total bilirubin, thrombomodulin, high mobility group box-1 (HMBG) protein, cytochrome C.
13. In selected centers, prognostication of NAS is based on miRNA's: CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a determined in perfusate.
14. In selected centers, biopsies of liver parenchyma and bile duct, which are routinely taken during transplantation, are also taken in this trial at the time points: before DHOPE, after DHOPE, and after reperfusion at the time of bile duct anastomosis during anesthesia. The purpose is to underpin the histopathological status of the liver and bile ducts in both study groups. In addition, mechanistic research into molecular mechanisms of injury and repair during DHOPE will be done to identify pathophysiological pathways that might have potential to predict function and outcomes after transplantation.

15. Metabolic function, including new onset diabetes after transplantation (NODAT) in the first 90 days after transplantation. NODAT is defined according to the WHO criteria (36).

    - Symptoms of diabetes and random plasma glucose ≥11.1 mmol/L. Symptoms include polyuria, polydipsia, and unexplained weight loss. OR

    - Fasting plasma glucose ≥7.0 mmol/L. Fasting is defined as no caloric intake for at least eight hours. OR

    - Two-hour plasma glucose ≥11.1 mmol/L during an oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

16. In selected centers, overall cost of treatment within 6 months (in/excluding return to work) is calculated according to the Cost and Outcome analysis of Liver Transplantation (COLT) study (5).

17. Health related quality of life will be determined using an EQ-5D-3L questionnaire obtained when the patient before transplantation and at 6 months after transplantation.

8.1.3 Other study parameters: Baseline values and parameters at inclusion

- Patient general demographics (age, gender, weight, height)
- Patient medical history including
    - Model for end-stage liver disease (MELD) score
    - Indication for OLT
    - Viral status
    - Current residence (home, hospital ward or ICU)
    - Kidney function
    - Medication
- Donor and liver graft demographics (age, gender, weight before and after DHOPE, height, cause of death, viral status, liver function, donor risk index, etc.)
- DCD characteristics such as time interval between withdrawal of life support and circulatory arrest, time interval between circulatory arrest and start cold perfusion *in situ,* etc.
- Surgical methods and technical difficulties or abnormalities
- Cold ischemia time, total preservation time (SCS and DHOPE) and warm ischemia time during implantation

8.2 Randomisation, blinding and treatment allocation

When a DCD donor liver becomes available, transplant centers involved in the trial will be informed by their local organ procurement organization. It is important to note that absolutely no changes will be made to national and international liver allocation rules. The standard local liver allocation rules will be followed. The study does not interfere or change the process of accepting or declining a liver offered to a certain patient in any way. Once a suitable recipient for the liver is identified, the recipient will be invited to the relevant transplant center for the surgical procedure as per routine procedure. Standard care at individual transplant centers is provided. After acceptance of the liver for a certain patient, the inclusion, exclusion criteria and informed consent are checked (see section 8.3).

Only when the liver is definitively deemed suitable for transplantation by the liver transplant surgeon, and the rest of the OR team including the anesthetist is informed about the exact starting time of the transplantation, and all inclusion and exclusion criteria are met, the patient will be randomized. The procurement surgeon must be blinded for the study group during the donor operation.

The patient will be randomized by the local investigator or the trial coordinator using an online randomization tool. A computer-generated list of random assignments (block randomization per site) is prepared in advance by Clinical Trial Center Maastricht. All personnel involved in randomization will be trained in the use of the online randomization by the Trial Coordinator or the Principal Investigator of each site. When a patient is registered to the website, an inclusion number is generated consisting of study-center-inclusion; for example the first patient in UMCG will have inclusion number UMCG-DHOPE-01. The local investigator will register which graft the patient has received. This information will be stored, so in all cases, it will be possible to determine the study

group the patient belongs to. The randomization website will send inclusion information to the mailbox of the Trial Coordinator, containing the inclusion number and the date and time.

Blinding

After randomization, the transplanting surgeon will be informed whether the patient is randomized to receive a graft after DHOPE or SCS only. The procurement surgeon is blinded to the study group. Patients are blinded to study group. The study is blinded for assessment of the primary endpoint by the Adjudication Committee. When there is a breach of blinding, this is described in the eCRF and the Sponsor is notified.

8.3 Study procedures

Before transplantation

- Patient general demographics (age, gender, weight, height)
- Patient medical history including
    - MELD score
    - Indication for OLT
    - Viral status
    - Current residence (home, hospital ward or ICU)
    - Medication
    - Laboratory serum analyses:
        - Serum creatinine for the eGFR based on the MDRD-equation
- Donor and liver graft demographics (age, gender, weight, height, cause of death, viral status, donor risk index, latest serum values AST, ALT, total bilirubin, INR, APTT, etc.)
- DCD characteristics such as time interval between withdrawal of life support and circulatory arrest, time interval between circulatory arrest and start cold perfusion *in situ,* etc.

During transplantation

- Cold ischemia time, total preservation time (SCS + DHOPE) and warm ischemia time during implantation
- Surgical methods and technical difficulties or abnormalities
- Hemodynamic status is recorded routinely and continuously. Items recorded for this study are systolic and diastolic blood pressure, heart rate and vasopressor dosage. These will be recorded 5 min before portal reperfusion, as well as 10 and 20 minutes after reperfusion.
- Biopsies of the caudal part of the common bile duct (a ring of 1 mm) will be taken and stored in formaline. Biopsies are routinely taken during transplantation.
- Wedge biopsies of the liver parenchyma will be taken from the edge of segment III of the liver (1x1x1xcm) and divided into three pieces storing one of each piece in formaline for pathohistology and immunohistology and two pieces snap frozen in liquid nitrogen for ATP analysis or in isopenthane on dry ice. Biopsies are routinely taken during transplantation.

Intervention group only

- Perfusion characteristics are obtained during DHOPE by the personnel monitoring the perfusion. At the start of perfusion and every fifteen minutes thereafter the flow is noted. These data are noted directly in the eCRF.
- Perfusate will be sampled for a point of care analysis to determine the pH, sodium, potassium, bicarbonate, and lactate using standard biochemical methods. The time points will be before the start of DHOPE, at the end of DHOPE, and every half hour in between, equaling to 5 time points. Per time point 0.4 ml will be taken adding up to a total of 20 ml.
- Perfusate will also be sampled to determine ALT, AlkP, γGT, urea, total bilirubin, thrombomodulin, HMBG, and cytochrome c using standard biochemical methods. Also the amount of miRNA's CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a will be determined. The time points will include before the start of DHOPE, at the end of DHOPE, and every half hour in between, adding up to 5 time points. Per time point 20 ml will be taken adding up to a total of 100 ml.

After transplantation

- All patients are routinely followed during their admission postoperatively, and in the outpatient department at 1, 3, 6, and 12 months after transplantation.
- Routine serum levels of ALT, AST, AlkP, γGT, total bilirubin, INR, PT, APTT, glucose, creatinine, leukocytes and CRP are assessed at day 0-7 and at 1, 3, and 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day.
- Renal function expressed as eGFR and the AKIN/RIFLE criteria will be determined by observation of oliguria and routine serum analyses including creatinine following transplantation.
- The urinary kidney injury markers will be determined in urine samples taken from the urinary catheter placed under anaesthesia routinely during OLT. For this end point, samples of 10 ml urine will be drawn from this catheter at time points: after induction of anesthesia, at arrival in the ICU and at day 1, 3, and 5 after transplantation. A total of 50 ml of urine will be taken and stored at -80ºC.

At six months

- The incidence of NAS will be calculated per group. The diagnosis is defined in section 8.1.1. To obtain this diagnosis, no additional procedures are necessary. Patients in this study will undergo a MRCP when there is a suspicion of NAS, which is part of the routine diagnostic work-up. The images of the MRCP will be reviewed by the Adjudication Committee in a later stage.
  - Severity of NAS will be determined by the type and frequency of interventions required.
- MRCP: In all patients who haven't been diagnosed with NAS within six months and haven't suffered from graft loss, a MRCP will be performed at six months after transplantation (time window of 15 days). The MRCP is an extra procedure for this study which is performed regardless of symptoms. A MRCP is a MRI scan of the bile ducts. It is a non-invasive imaging modality that does not use radiation. It is a painless and safe procedure which takes 30 to 60 minutes. It will be performed during a routine follow-up visit to the out-patient department.

- The images of the MRCP: the Adjudication Committee blinded for group treatment will assess all MRCP images of all patients (including those with diagnosed NAS) to objectively score the bile duct for strictures as defined by Buis et. al. (22).
  - the amount of lesions
  - the localization
  - the severity
- Graft and patient survival will be determined
- Incidence of PNF and IPF will be determined
- Incidence of postoperative complications graded according to CCI will be determined, including NODAT (see section 8.1.2)
- Length of hospital stay and stay in the ICU department directly following transplantation and in the follow-up period will be determined
- Cost-effectiveness will be determined based on data which are already obtained
- Health related quality of life will be determined based on EQ-5D-3L questionnaire obtained before transplantation and at six months after transplantation

Table 1. An overview of study endpoints per time point.

Endpoint / Time point	Baseline parameters	Perfusion characteristics	Hemodynamic status	Serum and urine analysis	Clinical follow-up	MRCP
Before OLT	X			x		
During DHOPE		x				
During OLT		x	x			
After reperfusion			x	x		
Day 0-7				x	x	
Month 1				x	x	
Month 3				x	x	
Month 6				x	x	x
Month 12					x*	

A non-exhaustive list of data retrieved per eCRF can be found in Appendix 3: Data collection parameters.

*) patient and graft survival data will be noted

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. It can be possible that a patient has given informed consent, but does not fulfill the inclusion/exclusion criteria at the time of transplantation. In this case the patient is not be included in the trial and will not be randomized.

In case a participant wishes to withdraw consent before the procedure, they must contact the local investigator by e-mail, telephone or in person. In such an event, his/her liver will not be perfused and data will not be collected. In case the consent is withdrawn during follow-up and after the intervention, the participant will be asked if historical data and data from the patient file may be obtained. This case will be entered in the intention-to-treat analysis and an additional patient must be included.

8.5 Premature termination of the study

The study will be terminated prematurely if we find a higher rate of adverse events than expected (see section 9.4) that is possibly related to the study product.

9. SAFETY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited MREC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 Definition of AEs, SAEs, ADEs, SADEs, and USADEs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to DHOPE. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. This definition includes physical signs, symptoms and laboratory test values. At study enrolment, laboratory values that fall outside the relevant reference range will not be reported as AEs.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:
- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Is any other important medical event that may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above;
- Is a device deficiency that might have led to a serious adverse event if: a) suitable action had not been taken or b) intervention had not been made or c) circumstances had been less fortunate.

- Is not an anticipated (serious) adverse event defined in section 9.4

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

9.2.3 Adverse Device Effects (ADEs)

An adverse event related to the use of an investigational medical device. This definition includes any events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device. This definition also includes any event resulting from user error or from intentional misuse of the investigational device.

9.2.4 Serious Adverse Device Effects (SADEs)

Any untoward medical occurrence that can be attributed wholly or partly to the device, which resulted in any of the characteristics of a serious adverse event as described above.

9.2.5 Unanticipated Serious Adverse Device Effects (USADEs)

Any serious adverse device effect which, by its nature, incidence, severity or outcome, has not been identified in section 9.4.

9.2.6 Device Deficiency

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling. Device deficiencies resulting in SADEs will be managed as detailed in section 9.3.
Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate will also be managed as detailed in section 9.5.

9.2.7 Use error

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

9.3 Causality of an AE related to the investigational medicinal device

- *Highly probable*: Apparent relationship in time between AE and intervention. Relationship between AE and intervention is already known or expected and there is an appropriate temporal relationship between therapy and AE.
- *Probable*: Known effect of the intervention with no possible other cause and appropriate temporal association.
- *Possible*: AE likely to be associated with the intervention and no other explanation for the AE, or known effect of intervention that could also be associated with another concomitant therapy, illness or external cause.
- *Unlikely*: Unlikely to be causally related; e.g. reaction occurred after intervention or is more likely to be due to another concomitant therapy, illness or external cause.

- *Definitely not*: AE known to be caused by another concomitant therapy, illness or external cause.
- *Not assessable*: Likelihood of AE not known, or relationship of AE to intervention, another concomitant therapy, illness or external cause is not clear. This category should be used very scarcely.

9.3.1 Grade of severity

- *Mild (grade 1):* patient is aware of symptoms but tolerates them easily. Symptoms do not interfere with daily activity.
- *Moderate (grade 2*): patient experiences discomfort that interferes with normal activity. No treatment is required except acetaminophen/paracetamol.
- *Severe (grade 3):* patient is unable to carry out normal activity. Treatment is required.
- *Life-threatening (grade 4):* emergency room visit, disabling or hospitalization.

9.4 Anticipated adverse events

All participants in this trial undergo liver transplantation, which is a surgical procedure with significant morbidity and mortality. This implies an intrinsic risk of AEs and SAEs anticipated after liver transplantation defined as:

Complications			**Incidence (%)**
Acute Rejection	Requiring biopsy or medication		7%
Initial poor function			16%
Kidney dysfunction	Requiring alteration of medication up to dialysis		10%
Infectious complications			
	Wound Infection requiring opening		20%
	Infected ascites or intraabdominal abcess requiring drainage		30%
	Pneumonia requiring antibiotics		8%
	Urinary tract infection requiring antibiotics		20%
	Viral infection requiring virostatica		35%
	Blood stream infection requiring antibiotics		21%
Bleeding Complications	Surgical site bleeding requiring reoperation or transfusion		10%
Biliary Complications	Biliary leakage requiring drainage or endoscopy		22%
	NAS requiring intervention		29%
	Anastomotic biliary strictures requiring intervention		10%
Thrombotic and Ischemic Complications	Systemic thromboemboliic events (deep venous thrombosis, pulmonary embolism)*		3%
	Cardiovascular events	Requiring medication or electroversion	6%
		Infarction	2%
		Arrhythmia	3%
		New congestive heart failure	2%
	Cerebrovascular events		3%
		Stroke	2%
		Hemorrhage	0,3-0,6%

Central nervous system	Including delirium requiring medication	10%
NODAT	Requiring insulin	7%

*This does not include portal venous thrombosis or hepatic artery thrombosis (these thromboembolic events should always be reported according to section 9.5).

These events are to be reported as AE (section 9.5). However, if in the opinion of the investigator, the frequency or severity of the event is greater than would be expected based on the described incidence, the event must be reported to the Sponsor. The investigator will exercise his/her medical judgment in deciding whether an adverse event, a postoperative laboratory finding falling outside the relevant reference range or other abnormal assessment is clinically significant.

9.5 Recording of adverse events

It is the responsibility of the Principal Investigator to ensure that all adverse events (including ADEs) and device deficiencies occurring during the course of the study are collected. This will include but not be limited to:

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

Adverse events will not be recorded or reported in case the event is both graded as a grade 1 complication according to the classification of Clavien Dindo as well as graded as mild or moderately severe (section 9.3.1). The following events are not recorded or reported:

- Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions. This includes but is not limited to:
  - Therapeutic regimens such as antiemetics, antipyretics, analgetics, diuretics, electrolytes, and physiotherapy
  - Wound infections opened at the bedside that do not require intervention such as medication (modification), intervention or surgery
  - Transient hyper/hypocalcemia, hyper/hyponatremia, hyper/hypokalaemia, hyper/hypophosphataemia, hypomagnesemia
  - Peripheral edema and hypoalbuminemia in the peri-operative period related to filling status, peri-operative management and recovering liver function (until first 3 months after liver transplantation)
  - Anaemia, leukopenia or thrombocytopenia related to immunosuppression
  - Hypertension as a pre-existing disease or induced by immunosuppression
  - Gastrointestinal problems (nausea, constipation and/or diarrhoea) related to the use of immunosuppression (such as mycophenolate acid derivatives)

- Post- liver transplantation delirium related to pre-existing encephalopathy and post-operative and ICU delirium

Adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects. In addition each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms within the eCFR.

9.6 Reporting of adverse events

Reporting of all Serious Adverse Events will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010). It is the responsibility of the Principle Investigator to ensure that all adverse events which fall into the category of SAEs, SADEs, USADEs and device deficiencies are reported to the Coordinating Investigator as soon as possible after becoming aware of the event but no later than 24 hours. Details to be included in the report are described in Section 9.5.

All (serious) adverse events and (unanticipated) (serious) device effects or device deficiencies will be reported via the appropriate form in the eCRF, in which all but AEs and ADEs will be automatically forwarded to the clinical reviewers by e-mail. The clinical reviewers are the Coordinating Investigator and the Trial Coordinator. Reporting by Fax will provide a backup system (+31503619050) in the event that the online data collection tool is unavailable. The Fax machine is located at the Sponsor site and is manned during normal office hours only. Within the following 5 working days, the Principal Investigator should provide any additional information on the initial event in the appropriate electronic form using the same form submitted initially – do not create a new form for follow up information. This should include a copy of the completed form, and any other diagnostic or relevant information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the coordinating center and clinical reviewers using the same electronic form.

The clinical reviewers will review the events and, if they feel they pose an immediate risk to patient health or safety, they will report them to the DSMB immediately and within 2 calendar days of becoming aware of the event to the device manufacturer and MREC via the webportal *ToetstingOnline*. The Coordinating Investigator will also inform all Principal Investigators concerned of relevant information about events that could adversely affect the safety of participants. All other events will be reported to the DSMB within 7 calendar days of notification, if appropriate, except for events defined in section 9.4. These will be reported to the MREC, DSMB and device manufacturer once every half year together with all other events. The USADEs and device deficiencies will be reported to the device manufacturer within 7 days.

All events are recorded by the Trial Coordinator in an overview list (line-listing) that will be submitted once every half year to the MREC. This line-listing provides an overview of all events accompanied by a brief report highlighting the main points of concern. The line-listing reporting of the events through the web portal *ToetsingOnline* to the accredited MREC is sufficient as notification to the

competent authority. The Trial Coordinator will report line-listed events to the competent authorities in other Member States, according to the requirements of the Member States.

9.7 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

9.8 Monitoring

According to the ICH-GCP Guidelines (2005/28 EG) on-site monitoring is not mandatory. However, the Sponsor chooses to install monitoring in agreement with the advice put out by the Nederlandse Federatie van Universitair Medische Centra (the Dutch Federation of University Medical Centers) (37). The advice is based on a risk assessment for patients participating in this trial, which is considered to be minimal: small chance of minor injury based on previous experiences with the Investigational Medical Device.

The monitoring will be performed by certified monitors collaborating with the Trial Coordinating Center of the University Medical Center Groningen and with the Trial Sites. The advice(s) of the monitor will be sent to the Coordinating Investigator. Should the Coordinating Investigator decide not to fully implement the advice of the monitor, the Coordinating Investigator will send the advice to the reviewing MREC including a note to substantiate why (part of) the advice of the monitor will not be followed.

10. STATISTICAL ANALYSIS

Statistical analyses will be performed using the statistical software package SPSS 19.0 (SPSS Inc, Chicago, IL). The analyses of the trial will be an intention-to-treat analyses of the livers comparing intervention (DHOPE and SCS) against control (SCS only) for all primary and secondary outcomes. The primary endpoint, incidence of NAS at 6 months after transplantation, will be analyzed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. The secondary parameters will be reported overall and by trial site. Binary outcomes will be assessed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table and logistic regression to adjust for prognostic factors. Continuous outcomes will be compared using paired T-test if normally distributed, otherwise using the Wilcoxon signed rank test or a Mann Whitney U test depending on paired or non-paired data. Group and paired data will be analyzed using the repeated measurement ANOVA. Time-to-event outcomes will be analyzed using survival analysis methods, including Kaplan-Meier plots and Cox proportional hazards regression model with calculation of hazard ratios or alternative validated methods if the proportional hazards assumption is not met. Test will be reported with 95% confidence intervals and two-sided p-values to 3 decimal places. In case of repetitive measurements multiple testing corrections will be applied. A Statistical

Analysis Plan (SAP) containing a full description of the statistical methods will be drafted as a separate document early in the trial and finalized prior to the final data lock.

In case of missing data in spite of efforts for obtaining them, we will perform a missing data analysis including the cause of the absent data, the value of the data, the distribution of the missing data and subsequently determine the method of handling the missing data.

10.1 Interim analysis

One interim analysis will be performed by the DSMB after half of patients (n=78) are included to determine the incidence of NAS in the control group in order to determine if the assumption of 29% of NAS in the control group is adequate. The analysis will take place after 78 patients have completed 6 months follow-up. There will be no statistical test performed. The DSMB will only perform an interim analysis on adverse events if there are concerns about safety as described in the DSMB charter. The Pocock sequential boundary will be used to determine statistical significance of adverse events between the two groups, dictating a Z-value of 2 and thus a P-value of 0.045.

The trial may be stopped early due to one of the following situations:
- Unacceptable safety concerns: The analysis shows significant (serious) adverse events in the treatment group compared to the control group.
- In case new external information arises that convincingly answers the study question or raises serious safety issues.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (59th WMA General Assembly, Seoul, October 2008) and according to the latest revised version of the Medical Research Involving Human Subjects (WMO). The procedures set out in this study protocol are designed to ensure that the Sponsor and investigator abide by the principles of the GCP guidelines of the European Community (ICH topic E6, CPMP/ICH/135/95, Directive 2001/20/EC) and the Declaration of Helsinki (in the latest revised version) in the conduct, evaluation and documentation of this study. Copies of the ICH-GCP-Guidelines and the Declaration of Helsinki are included in the Investigator's Study File.

11.2 Recruitment and consent

Since the time frame for obtaining informed consent is limited once the patient is allocated a liver and this period is stressful, it is not the appropriate time to ask for informed consent. Therefore eligible patients will be asked for informed consent while they are on the waiting list. New patients will be asked when they are put on the waiting list. Most patients waiting for a liver transplantation will receive a liver from a donation after brain death (DBD) donor (65%). However, we want patients to be able to decide on participation in this trial. Also, we believe that it is less harmful to ask patients that will not be included in the study due to transplantation

with DBD livers or other reasons, for informed consent than it is to tell a patient or relative about a possible complications due to DHOPE when they were not well informed or were too stressed to take in the information. When the patient is summoned to the hospital for the transplantation, the patient will be checked for inclusion and exclusion criteria again. The patient will be informed whether they are included in the trial.

The person who obtains consent must be:
- Suitably qualified and capable of providing information about the study;
- Capable of answering questions about the study or ensuring that such questions are answered by a suitable qualified individual;
- Authorized to do so by the principal investigator or the local investigator.

The written and verbal versions of the information form and informed consent form will be presented to the patients detailing the exact nature of the study, the implications and constraints of the study, the known side effects and any risks involved in taking part. All information sheets and consent forms have been written in English and Dutch. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

When the patient chooses to participate, he/she must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Written informed consent will be documented by means of a dated signature of the participant and a dated signature of the person who gave the verbal information. The original signed form will be retained at the study site and placed in the medical notes. A copy will be given to the participant.

In case the patient doesn't consent to the study, they will receive a liver transplantation according to standard allocation and will be given routine standard of care. Data will not be recorded.

11.3 Benefits and risks assessment, group relatedness

The hypothesized benefit of DHOPE, which is performed in half of the patients included in this trial, is a reduced risk of a NAS. This complication to the biliary tree is of major impact to the patient since it often implies a multitude of costly and debilitating interventions and hospital admission that may be unsuccessful resulting in retransplantation. Additionally experience with DHOPE in clinical liver transplantations indicate it is beneficial because of improved organ integrity and function after reperfusion but also significantly increases survival after transplantation (in experimental animal models) with a reduction in biochemical markers of preservation injury in clinical pilot studies.

The risk associated with participation in this trial are associated with the procedure DHOPE itself and with the MRCP performed to determine the secondary endpoints.

Risk associated with DHOPE
The risk assessment of DHOPE is regarded as minimal but could be associated with the timing, the perfusion fluid, the pressure used, and the device. DHOPE is technically relatively easy procedure

which can be performed by any liver surgeon after training. Since perfusion is performed in the transplantation center while the recipient undergoes surgery, there is no change in transportation of the organ, which will be in the conventional SCS method. The two hours of DHOPE performed at the end of SCS will cause a minor delay in the transplantation process because the preparation of the liver for transplantation and the DHOPE perfusion will be performed by an extra surgical team. Normally, preparation of the liver is performed by the surgeons who perform the transplant operation, but this will be performed simultaneously by another team during this trial.

DHOPE is performed using a machine, which could display a technical failure during perfusion. When this happens, an alarm goes off and the liver is immediately removed from the machine by standby surgeon in a sterile manner. It will be cooled down in preservation fluid from 12ºC (in DHOPE) to 4ºC as is the case in SCS. For some minutes before the liver is cooled down, it has a metabolism of 19% instead of 11% as is the case in SCS. There is no reason to believe that this event will cause any significant injury to the liver grafts because this difference in metabolism is very small, is of short duration, and the liver is saturated with oxygen before the perfusion failure. In case of a malfunction of the Liver Assist cannot be corrected, the donor liver will be stored in cold preservation fluid as usual (SCS). Analysis of the study results, however, will be performed as an intention-to-treat analysis, and the liver / recipient will remain in the intervention group.

The pressure with which the liver is perfused may theoretically cause harm to the organ. However, the pressures used in this trial are very low (lower than physiological) and are reported to be used safely without causing any harm to the organ or the vasculature (38, 39).

The preservation fluid used is similar to the one used in the control group. The only difference is that the sodium and the potassium concentration ratio is inverted. Therefore the components that might cause an allergic reaction in the patient receiving the liver are not different from standard practice.

Risks associated with MRCP
The MRCP will be performed at six months after transplantation in patients with no history of NAS or graft loss. The risks associated with this imaging modality are insignificant. It is a non-invasive test taking about 45 minutes in which the patient lies still while the MRI scan is made. The MRCP will be planned to take place on the same day that the patient has a routine 6-months check-up in the outpatient department to minimize patient's traveling.

In conclusion, the potential risk and burden to the patients in this trial are minimal and therefore are overshadowed by the possibility of our hypothesis being correct; that DHOPE reduces the risk of patients developing NAS after liver transplantation. Additionally, the hypothesis for this trial can only be tested in this group of patients because they are undergoing liver transplantation.

11.4 Compensation for injury

Each participating center has an insurance which is in accordance with the legal requirements in the the country of the participating center. In the Netherlands this is according to Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003~~)~~. This

insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million and five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. All participating patients will be informed of this insurance in writing through the patient information form. All participating centers outside of the Netherlands are responsible for adequate insurance policy for the patients participating in this trial in their center, according the legal requirements in their country.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

12.1.1 Data collection form

- The data collection protocol and data collection form (eCRF) will be accessed online through a secured website. This will improve protocol adherence, data accuracy, user acceptability, and timeliness of receiving data.
- Validation rules prior to submission will ensure that data is entered in the correct format, within valid ranges and minimise the chance of missing data.
- All data reports will be prepared using a unique study ID, preventing the identification of individual subjects from the report data. The study ID will consist of center abbreviation followed by DHOPE or SCS whichever is applicable and followed by a two digit number which increases per included patient (e.g. UMCG-DHOPE-01). A list of study ID and corresponding name, address and date of birth will be stored separately from the study data per site. The Principle Investigator safeguards this list with a code, known to the Trial Coordinator and the Coordinating Investigator.
- All internet-based forms will be properly secured. Each individual responsible for data entry will receive a personal password and will only be allowed to complete and see those data sections related to his/her level of responsibility and permissions within the trial. When the user logs on to the first time, he/she will have to change the password. The password allowed has to adhere to the following rules: passwords contain at least eight characters, contain both alphabets and numbers, contain a mixture of both upper case and lower case characters, contain at least one numeric character, contain a minimum of five different characters, the maximum sequential repetition of a character allowed is two, and passwords are changed at least once a year.
- The Super User will be the Trial Coordinator and is responsible for assigning user name and passwords to individuals requiring access to the data collection form.

- A tutorial will be provided with the eCRF for all users. The user must complete the tutorial before access to the eCFR is granted and will be displayed when the user first logs in to the eCFR. The tutorial will be designed by the Trial Database Programmer.
- Data will be uploaded to a central database maintained at the UMCG.

12.1.2 Data entry

- The Principal Investigator is ultimately responsible for data entry and data completeness at his/her center. He/she can also delegate this task but remains responsible for data entry and data completeness.
- Validation rules prior to submission will ensure that data is entered in the correct format, within valid ranges and minimise the chance of missing data. Data already entered will be retrievable for viewing through the data entry system. The database and forms will be password protected. Each individual responsible for data entry will receive a personal password and will only be allowed to complete and see those data sections related to his/her level of responsibility and permissions within this trial.
- All randomised recipients completing the 6 month follow-up assessment will be regarded as having completed the study. All recipients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments and that patients do not incur extra financial costs as a result of study participation.
- The Trial Coordinator will oversee data entry for discrepancies and missing data. She will be responsible for the database, and if such discrepancies are identified she will be responsible for identifying the problem and contacting the local center to ensure resolution. She will be responsible for the production of monthly reports to each participating center containing information and details of missing data requiring completion. Every possible effort will be made to try to attain any missing data.
- However, it is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. In the event of early study termination, the date for withdrawal must be documented and the reason for withdrawal if given. Patients are asked to give consent for historical data available from patient file, which must be documented.
- However, in case a participant discontinues the trial or is lost to follow-up, last follow-up data will be used to attain secondary endpoints if permission is given and missing data will be appropriately handled.

12.1.3 Data management

Research data will be handled with due observance of the Dutch Law for Protection of Personal data (Wet Bescherming Persoonsgegevens). All data will be transmitted over a secure connection and stored in a password-protected central database, the password for which will be regularly changed. Regular backups of the main database will be made to an off-site location. All data in the central database will be stored for at least 15 years following study close-out. The database will be accessible via an “https” website only so that the connection between the server and client is encrypted.

The statistician in the DSMB has access to the database to check on completeness and missing data. Before completion of the trial, the statistician of the DSMB, members of the National Patient Safety Agency and members of the Medical Ethical Committee are allowed access to the data. After completion, all investigators will be given access to the data.

12.2 Monitoring and Quality Assurance

The monitoring will be performed by qualified monitors associated with the Trial Coordinating Center of the UMCG or of the participating Trial Site. Theywill periodically review accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. They will advise the Sponsor if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms.

12.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the MREC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial

All substantial amendments will be notified to the MREC and to the competent authority. Non-substantial amendments will not be notified to the accredited MREC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited MREC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/reactions, serious adverse device effects, other problems, and amendments.

12.5 End of study report

The investigator will notify the accredited MREC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit. In case the study is ended prematurely, the investigator will notify the accredited MREC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited MREC.

12.6 Public disclosure and publication policy

The trial will be published in a public trial registry before the first patient is recruited. Since the Sponsor is the Coordinating Investigator, there are no restrictions for disclosure of research data. The results of this study are expected to be published as a collective article from all participating centers in an international medical journal. Centers that enroll at least ten patients will be represented by two co-author(s), as described in section 8 of the Clinical Trial Agreement. Depending on the eventual number of participating and co-publishing centers as well as journal regulations, medium enrolling centers (more than twenty inclusions) will be given the opportunity to list a third co-author, and high enrolling centers (more than thirty inclusions) will be given the opportunity to list a fourth co-author in the published article. Participating centers may only present study data derived from work packages of this study after consultation of the Sponsor-Principal Investigator. Work package leadership and authorship is agreed upon in the Clinical Trial Agreement (appendix 5).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

The Liver Assist is a CE-marked machine to be used for DHOPE, which is within its indication. Therefore section 13.1 is skipped. See section 12.3 for risk versus benefit assessment.

14. REFERENCES

1. Wertheim JA, Petrowsky H, Saab S, Kupiec-Weglinski JW, Busuttil RW. Major challenges limiting liver transplantation in the United States. Am J Transplant. 2011 Sep;11(9):1773-84.

2. Orman ES, Barritt ASt, Wheeler SB, Hayashi PH. Declining liver utilization for transplantation in the United States and the impact of donation after cardiac death. Liver Transpl. 2013 Jan;19(1):59-68.

3. Jay CL, Skaro AI, Ladner DP, Wang E, Lyuksemburg V, Chang Y, et al. Comparative effectiveness of donation after cardiac death versus donation after brain death liver transplantation: Recognizing who can benefit. Liver Transpl. 2012 Jun;18(6):630-40.

4. Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73.

5. van der Hilst CS, Ijtsma AJ, Bottema JT, van Hoek B, Dubbeld J, Metselaar HJ, et al. The price of donation after cardiac death in liver transplantation: a prospective cost-effectiveness study. Transpl Int. 2013 Apr;26(4):411-8.

6. Op den Dries S, Sutton ME, Lisman T, Porte RJ. Protection of bile ducts in liver transplantation: looking beyond ischemia. Transplantation. 2011 Aug 27;92(4):373-9.

7. Brunner SM, Junger H, Ruemmele P, Schnitzbauer AA, Doenecke A, Kirchner GI, et al. Bile duct damage after cold storage of deceased donor livers predicts biliary complications after liver transplantation. J Hepatol. 2013 Jun;58(6):1133-9.

8. Hansen T, Hollemann D, Pitton MB, Heise M, Hoppe-Lotichius M, Schuchmann M, et al. Histological examination and evaluation of donor bile ducts received during orthotopic liver transplantation--a morphological clue to ischemic-type biliary lesion? Virchows Arch. 2012 Jul;461(1):41-8.

9. Dutkowski P, de Rougemont O, Clavien PA. Machine perfusion for 'marginal' liver grafts. Am J Transplant. 2008 May;8(5):917-24.

10. Fujita S, Hamamoto I, Nakamura K, Tanaka K, Ozawa K. Isolated perfusion of rat livers: effect of temperature on O2 consumption, enzyme release, energy store, and morphology. Nihon Geka Hokan. 1993 Mar 1;62(2):58-70.

11. Koetting M, Luer B, Efferz P, Paul A, Minor T. Optimal time for hypothermic reconditioning of liver grafts by venous systemic oxygen persufflation in a large animal model. Transplantation. 2011 Jan 15;91(1):42-7.

12. Schlegel A, Rougemont O, Graf R, Clavien PA, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. J Hepatol. 2013 Feb;58(2):278-86.

13. Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved rat liver by hypothermic oxygenated machine perfusion. Am J Transplant. 2006 May;6(5 Pt 1):903-12.

14. Koetting M, Minor T. Donation after cardiac death: dynamic graft reconditioning during or after ischemic preservation? Artif Organs. 2011 Jun;35(6):565-71.

15. Kim JS, Nitta T, Mohuczy D, O'Malley KA, Moldawer LL, Dunn WA, Jr., et al. Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. Hepatology. 2008 May;47(5):1725-36.

16. Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg. 2006 Dec;244(6):968-76; discussion 76-7.

17. Guarrera JV, Henry SD, Samstein B, Reznik E, Musat C, Lukose TI, et al. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. Am J Transplant. 2015 Jan;15(1):161-9.

18. Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant. 2010 Feb;10(2):372-81.

19. Dutkowski P, Schlegel A, de Oliveira M, Mullhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. J Hepatol. 2014 Apr;60(4):765-72.

20. Groen H, Moers C, Smits JM, Treckmann J, Monbaliu D, Rahmel A, et al. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. Am J Transplant. 2012 Jul;12(7):1824-30.

21. Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.

22. D'Alessandro A M, Hoffmann RM, Knechtle SJ, Odorico JS, Becker YT, Musat A, et al. Liver transplantation from controlled non-heart-beating donors. Surgery. 2000 Oct;128(4):579-88.

23. Chan EY, Olson LC, Kisthard JA, Perkins JD, Bakthavatsalam R, Halldorson JB, et al. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. Liver Transpl. 2008 May;14(5):604-10.

24. Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10.

25. Dezza MC, Berrevoet F, Sainz-Barriga M, Rossetto A, Colenbie L, Haentjens I, et al. The choice of recipient does not have a bearing on early outcome in liver transplant patients receiving grafts from non-heart-beating donors: a reappraisal? Transplant Proc. 2007 Oct;39(8):2675-7.

26. Stahl JE, Kreke JE, Malek FA, Schaefer AJ, Vacanti J. Consequences of cold-ischemia time on primary nonfunction and patient and graft survival in liver transplantation: a meta-analysis. PLoS One. 2008;3(6):e2468.

27. Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63.

28. Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53.

29. op den Dries S, Karimian N, Sutton ME, Westerkamp AC, Nijsten MW, Gouw AS, et al. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. Am J Transplant. 2013 May;13(5):1327-35.

30. van der Plaats A, t Hart NA, Verkerke GJ, Leuvenink HG, Ploeg RJ, Rakhorst G. Hypothermic machine preservation in liver transplantation revisited: concepts and criteria in the new millennium. Ann Biomed Eng. 2004 Apr;32(4):623-31.

31. Uemura T, Randall HB, Sanchez EQ, Ikegami T, Narasimhan G, McKenna GJ, et al. Liver retransplantation for primary nonfunction: analysis of a 20-year single-center experience. Liver Transpl. 2007 Feb;13(2):227-33.

32. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

33. Slankamenac K, Nederlof N, Pessaux P, de Jonge J, Wijnhoven BP, Breitenstein S, et al. The comprehensive complication index: a novel and more sensitive endpoint for assessing outcome and reducing sample size in randomized controlled trials. Ann Surg. 2014 Nov;260(5):757-62; discussion 62-3.

34. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999 Mar 16;130(6):461-70.

35. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care. 2007;11(2):R31.

36. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003 Jan;26 Suppl 1:S5-20.

37. Centra NFvUM. Kwaliteitsborging mensgebonden onderzoek 2.0.

38. op den Dries S, Sutton ME, Karimian N, de Boer MT, Wiersema-Buist J, Gouw AS, et al. Hypothermic oxygenated machine perfusion prevents arteriolonecrosis of the peribiliary plexus in pig livers donated after circulatory death. PLoS One. 2014;9(2):e88521.

39. Schlegel A, Dutkowski P. Role of hypothermic machine perfusion in liver transplantation. Transpl Int. 2014 May 23.

15. Appendices

15.1 Appendix 1. Patient information form

15.2 Appendix 2. Patient informed consent form

15.3 Appendix 3. Data collection parameters

15.4 Appendix 4. Data Safety Monitoring Charter

15.5 Appendix 5. Clinical Trial Agreement

3. Summary of Amendments to Study Protocol

During study progress, the protocol was amended a few times. The first and final version of the protocol are included in this document and changes are summarized in the table below.

The main changes were:

- The original sample size calculation in 2015 was based on a one-sided test for the primary end point. Based on advice from one of the national regulatory authorities, this was changed in two-sided testing in 2018.
- The original version of the study protocol specified graft and patient survival analyses at 6 months, which was later adjusted to 12 months.

Protocol version	Date	Comment
1.0	21-09-2015	– Original version
2.0	12-01-2016	– Exclusion criteria 'simultaneous transplantation of another non-renal organ' was added. – The tasks for trial monitoring were defined separately while this was previously defined as task of the data safety monitoring committee.
3.0	07-07-2016	– Exclusion criteria 'expected cold ischemia time of far more than 8 hours' was removed. – Patients were able to give consent for participation after 1 day instead of 2 days after receiving information about the trial. – Donor surgeon procuring the donor liver is blinded for randomization group.
4.0	06-09-2018	– Sample size calculation was changed to a 2-sided Chi square test instead of a 1-sided test. This did not lead to a greater sample size as graft loss was erroneously accounted for in the previous sample size calculation. – Timing of the interim analysis was changed to 'after inclusion of half of the patients' instead of 'after 1 year', as only 8% of patients were included after 1 year.
5.0	25-02-2020	– Duration of follow-up for determination of patient and graft survival was changed from 6 months to 12 months

4. Original Version of Statistical Analysis Plan

Statistical Analysis Plan

1 Administrative information

TRIAL FULL TITLE	A multicenter randomized controlled trial to compare the efficacy of end-ischemic dual hypothermic oxygenated machine perfusion with static cold storage in preventing non-anastomotic biliary strictures after transplantation of liver grafts donated after circulatory death: DHOPE-DCD Trial
CLINICALTRIALS.GOV REGISTRATION NUMBER	NCT02584283
CLINICALTRIALS.GOV REGISTRATION DATE	22-10-2015
SAP VERSION 1 - DATE	19-05-2019
TRIAL PRINCIPLE INVESTIGATOR	R.J. Porte (r.j.porte@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
TRIAL COORDINATOR	R. van Rijn (r.van.rijn@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
SAP AUTHOR / EPIDEMIOLOGIST	V.E. de Meijer (v.e.de.meijer@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
DSMB EPIDEMIOLOGIST	M. El Moumni (m.el.moumni@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands

This SAP adheres to the Guidelines for the content of statistical analysis plans in clinical trials (SAPIT)[1]

[1] Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-43.

1.1 SAP Signatures

I give my approval for the attached SAP entitled DHOPE-DCD Trial dated 19-05-2019.

Principle Investigator

Name: Robert J. Porte

Signature: _____

Date: 20-05-2019

SAP Author/Epidemiologist

Name: Vincent E. de Meijer

Signature: _____

Date: 19-05-2019

DSMB Member/Epidemiologist

Name: Mostafa el Moumni

Signature: _____

Date: _____

1.2 Table of Contents

1.3 Abbreviations and Definitions

AE	Adverse Event
AlkP	Alkaline Phosphatase
AKIN	Acute Kidney Injury Network
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
CCMO	Central Committee on Research Involving Human Subjects
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-3L	European Quality of Life Instrument 5D-3L
CCI	Comprehensive Complication Index
CV	Curriculum Vitae
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
ECD	Extended Criteria Donor
ERCP	Endoscopic Retrograde Cholangiopancreaticography
EU	European Union
GCP	Good Clinical Practice
γGT	Gamma-glutamyl Transferase
HMGB	High Mobility Group Box-1
DHOPE	Dual Hypothermic Oxygenated Perfusion
ICU	Intensive Care Unit

INR	International Normalized Ratio
IPF	Initial Poor Function
MDRD	Modification of Diet in Renal Disease
MELD	Model for End-stage Liver Disease
miRNA	Micro Ribonucleic acid
MP	Machine Perfusion
MREC	Medical Research Ethics Committee
MRCP	Magnetic Resonance Cholangiopancreatography
NA	Not Applicable
NAS	Non-anastomotic Biliary Strictures
NODAT	New Onset Diabetes After Transplantation
OLT	Orthotopic Liver Transplantation
PNF	Primary Non-Function
PT	Prothrombin Time
RIFLE	Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
(S)ADE	(Serious) Adverse Device Effects
(S)AE	(Serious) Adverse Event
SAP	Statistical Analysis Plan
SCS	Static Cold Storage
SOP	Standard Operation Procedure
UMCG	University Medical Center Groningen
USADE	Unanticipated Serious Adverse Device Effects
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act

2 Introduction

2.1 Background and rationale

Recent publications report good results of controlled donation after circulatory death (DCD) liver transplantation when strict donor-recipient matching is applied and ischemia times are kept to a minimum. However a major concern remains the high rate of biliary complications after transplantation of DCD livers. Non-anastomotic biliary strictures (NAS) occur in 29% of patients receiving a DCD graft whereas the incidence of NAS in recipients of donation after brain death (DBD) liver grafts is 11%. NAS are associated with higher morbidity and increased cost of liver transplantation. Injury to the biliary epithelium and the peribiliary vascular plexus occurring during donor warm ischemia and static cold storage (SCS) has been identified as a major risk factor for development of NAS. Machine perfusion has been proposed as an alternative strategy for organ preservation, offering the opportunity to improve the quality of the organ by providing oxygen to the graft. Experimental studies have shown that end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) helps liver grafts to recover from ischemia by restoring mitochondrial function and by preventing of reperfusion injury due to production of radical oxygen species and danger associated molecular patterns. Moreover, DHOPE has been shown to provide better preservation of peribiliary vascular plexus of the bile ducts, which could be an important step forward in reducing the incidence of NAS after transplantation.

2.2 Objectives

Primary Objective:

To study the efficacy of end-ischemic DHOPE in reducing the incidence of symptomatic NAS at 6 months after controlled DCD liver transplantation.

Secondary Objective(s):

To study the effect of the intervention (end-ischemic DHOPE after SCS), in comparison to the control group (SCS only), concerning:

1. The overall incidence of symptomatic and asymptomatic NAS
2. The severity of NAS after transplantation
3. The graft and recipient survival
4. The incidence of primary non-function (PNF)
5. The incidence of early allograft dysfunction (EAD)
6. The biochemical analysis of graft function and ischemia-reperfusion injury
7. The hemodynamic status of the recipient after graft reperfusion to assess post-reperfusion syndrome
8. Length of stay in the ICU and hospital
9. The incidence of postoperative complications, including infections and use of antibiotics
10. The renal function, including need for renal replacement therapy
11. The perfusion characteristics during DHOPE (in the intervention group only)
12. The perfusate analysis during DHOPE (in the intervention group only)
13. Prognostication of NAS, based on micro ribonucleic acid (miRNA) profiles in perfusion fluid (in the intervention group only)
14. Pathobiology of liver and bile duct parenchyma
15. Metabolic function, including new onset diabetes after transplantation (NODAT)
16. Overall cost of treatment within 6 months (in/excluding return to work)
17. Overall health related quality of life after transplantation

3 Study Methods

3.1 General Study Design and Plan

The trial is designed as a prospective, randomized, controlled, multicenter, superiority, clinical trial with two parallel groups. Primary endpoint is the incidence of symptomatic NAS at six months after liver transplantation. Liver grafts in the treatment group will be preserved with SCS followed by DHOPE. Liver grafts in the control group will be preserved by SCS alone without any further intervention. **Figure-1** provides an overview of the study design.

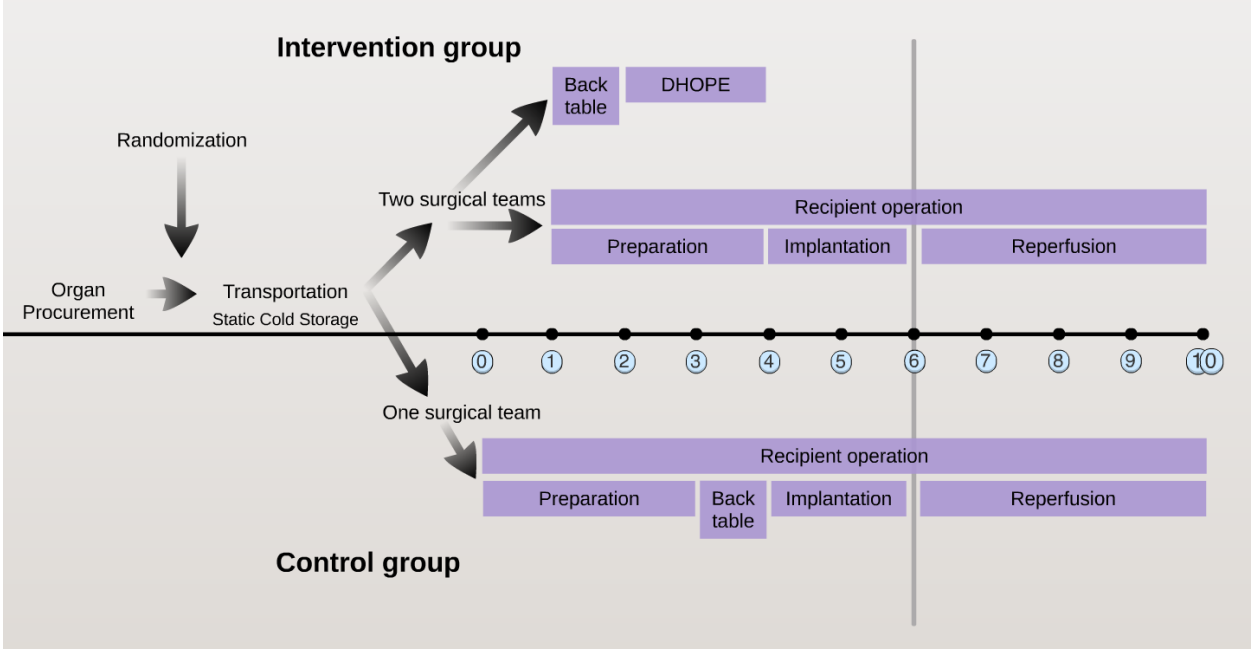


Figure 1. Study flow chart.

The study setting is standard practice of OLT. The surgical procedure, post-operative care and follow-up are identical to the routine OLT-practice in each participating center.

Patients are followed during the six months post-transplantation, during hospital stay and after discharge, via their routine hospital visits at 1 month, 3 months, 6 months after transplantation.

Whenever patients included in this trial are suspected of NAS based on clinical parameters as judged by their treating physician, they will undergo routine examinations which includes MRCP imaging of the biliary tree. In case of radiologically confirmed symptomatic NAS, this is primary outcome event is registered in the electronical case report form (eCRF).

In case a patient in this trial is not diagnosed with NAS within 6 months, a MRCP is performed at six months after transplantation to assess the biliary tree for asymptomatic radiological abnormalities. This time interval is chosen because diagnosis of NAS is reported at a median of 3 to 4 months after transplantation. Some studies have reported an occurrence of 100% of the cases of NAS within 4 months. All biliary imaging, including the trial MRCP at 6 months, will be evaluated by an Adjudication Committee in order to harmonize and standardize endpoint assessment. The Adjudication Committee consists of three independent radiologists. In order to allow for an unbiased endpoint assessment the members are blinded to study group assignment.

3.2 Randomization and Blinding

Randomization

When a DCD donor liver becomes available, transplant centers involved in the trial will be informed by their local organ procurement organization. It is important to note that absolutely no changes will be made to national and international liver allocation rules. The standard local liver allocation rules will be followed. The study does not interfere or change the process of accepting or declining a liver offered to a certain patient in any way. Once a suitable recipient for the liver is identified, the recipient will be invited to the relevant transplant center for the surgical procedure as per routine procedure. Standard care at individual transplant centers is provided. After acceptance of the liver for a certain patient, the inclusion, exclusion criteria and informed consent are once more checked.

Only when the liver is definitively deemed suitable for transplantation by the liver transplant surgeon, and the rest of the OR team including the anesthetist is informed about the exact starting time of the transplantation, and all inclusion and

exclusion criteria are met, the patient will be randomized. The procurement surgical team remains blinded for the study group during the donor operation.

The patient will be randomized by the local investigator or the trial coordinator using an online randomization tool. A computer-generated list of random assignments (block randomization) is prepared in advance by Clinical Trial Center. Block randomization is stratified for trial site and for patients with primary sclerosing cholangitis (PSC). All personnel involved in randomization will be trained in the use of the online randomization by the Trial Coordinator or the Principal Investigator of each site. When a patient is registered to the website, an inclusion number is generated consisting of study-center-inclusion; for example the first patient in UMCG will have inclusion number UMCG-DHOPE-01. The local investigator will register which graft the patient has received. This information will be stored, so in all cases, it will be possible to determine the study group the patient belongs to. The randomization website will send inclusion information to the mailbox of the Trial Coordinator, containing the inclusion number and the date and time.

Blinding

After randomization, the transplanting surgeon will be informed whether the patient is randomized to receive a graft after DHOPE or SCS only. The procurement surgeon is blinded to the study group. Patients are blinded to study group. The study is blinded for assessment of the primary endpoint by the Adjudication Committee. When there is a breach of blinding, this is described in the eCRF and the Sponsor is notified.

3.3 Sample Size

The study is powered to detect a clinically relevant difference in incidence of symptomatic NAS between the two study groups. The incidence of NAS is 29% after DCD liver transplantation and is 11% after DBD liver transplantation in patients transplanted in the UMCG from 2008 to 2013 (unpublished data). This is similar to incidence reported by Abt *et. al.* (27% in DCD versus 2% in DBD

transplantation)[2], Dubbeld *et. al* (24% versus 8%)[3], Croome *et. al.* (22% versus 4%)[4], and Meurisse *et. al.* (33% versus 12%)[5]. With the intervention (DHOPE) we aim to reduce the incidence of NAS after DCD liver transplantation to the level observed after DBD liver transplantation (i.e., risk reduction of 62%; absolute difference of 29-11=18%). We base this presumed reduction on our results in the pilot study in which 1 of 10 (10%) of the patients with a DHOPE treated liver developed NAS. The other previously reported pilot studies observed no NAS in any of the patients receiving a liver treated with end-ischemic hypothermic machine perfusion. For a power of 80% (β=0.80) and a 5% significance level (2-sided test) in two independent cohorts, using a Chi-squared test, 77 livers are needed to be included in each arm, calculated with nQuery + nInterim 3.0. Although there is a (very) small likelihood of lost to follow-up, we still want to include an extra patient per study arm. Therefore, the total number of patients to be included in this study will be 156 (77*2+2).

3.4 Interim analysis

One interim analysis will be performed by the DSMB after half of patients (n=78) are included to determine the incidence of NAS in the control group in order to determine if the assumption of 29% of NAS in the control group is adequate. The analysis will take place after 78 patients have completed 6 months follow-up. There will be no statistical test performed. The DSMB will only perform an interim analysis on adverse events if there are concerns about safety as described in the DSMB charter. The Pocock sequential boundary will be used to determine statistical significance of adverse events between the two groups, dictating a Z-value of 2 and thus a P-value of 0.045.

---

[2] Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63

[3] Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53

[4] Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10

[5] Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73

The trial may be stopped early due to one of the following situations:

- Unacceptable safety concerns: The analysis shows significant (serious) adverse events in the treatment group compared to the control group.
- In case new external information arises that convincingly answers the study question or raises serious safety issues.

3.5 Stopping guidance

The study will be terminated prematurely if we find a higher rate of adverse events (AEs) than expected (see study protocol; section 9.4) that is possibly related to the study. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to DHOPE. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. This definition includes physical signs, symptoms and laboratory test values. At study enrolment, laboratory values that fall outside the relevant reference range will not be reported as AEs.

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

3.6 Timing of final analysis

All data will be gathered when all subjects have completed at least the 6-months follow-up assessment, or have dropped out prior to this. All data from all study sites will be checked on completeness and missing data. Queries will be sent to the participating centres in order to complete any (initially) missing data. After completion and cleaning, the database will be locked and the investigators will be given access to the data. All outcomes will be analyzed collectively.

3.7 Timing of outcome assessments

Table 1. Overview of study variables per time point.

Endpoint / Time point	Baseline parameters	Perfusion characteristics	Hemodynamic status	Serum and urine analysis	Clinical follow-up	MRCP
Before OLT	X			X		
During DHOPE		X				
During OLT		X	X			
After reperfusion			X	X		
Day 0-7				X	X	
Month 1				X	X	
Month 3				X	X	
Month 6				X	X	X

* Patient still alive (yes/no) and/or retransplanted (yes/no)

A non-exhaustive list of data retrieved per eCRF can be found in Appendix 3 of the Study Protocol: Data collection parameters.

4 Statistical principles

4.1 Confidence intervals and *P* values

Data will be presented as numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with interquartile range) for continuous variables. The Kolmogorov Smirnov test will used to assess whether continuous data are normally distributed. Tests will be reported with 95% confidence intervals and two-sided p-values. A P-value of less than 0.05 will be considered to indicate statistical significance. No correction for multiple testing will be performed.

4.2 Analysis populations

Full Analysis Intention to Treat Population

- All subjects who were randomized and who participated in at least one post-baseline assessment

Per Protocol Population

- All subjects who were randomized and who have completed at least the 6-months follow-up assessment (including trial MRCP in patients not previously diagnosed with symptomatic NAS).

Safety Population

- All subjects who were randomized (including control) and are confirmed as providing complete follow-up regarding adverse event information.

5 Trial population

5.1 Inclusion-Exclusion Criteria and General Study Population

Study Population

Adult patients (≥ 18 years old) with end-stage chronic liver disease awaiting liver transplantation are screened for participation in this trial.

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adult patients (≥ 18 years old)
- Signed informed consent
- Willing and able to attend follow-up examinations
- Donor liver graft from a controlled donation after circulatory death
- Donors with a body weight ≥40 kg

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Simultaneous participation in another clinical trial that might possibly influence this trial
- Mental conditions rendering the subject incapable to understand the nature, scope and consequences of the trial
- Listed for liver transplantation due to fulminant liver failure or retransplantation because of PNF
- Recipient positive test for HIV antigen or HIV antibody
- Donor positive for HIV antigen, HIV antibody, Hepatitis B core antibody or hepatitis B surface antigen, or hepatitis C antibody
- Patients with contra-indications for MRCP (i.e. pacemaker)
- Simultaneous transplantation of another organ

5.2 Recruitment / Proposed Consort Flow Diagram [6]

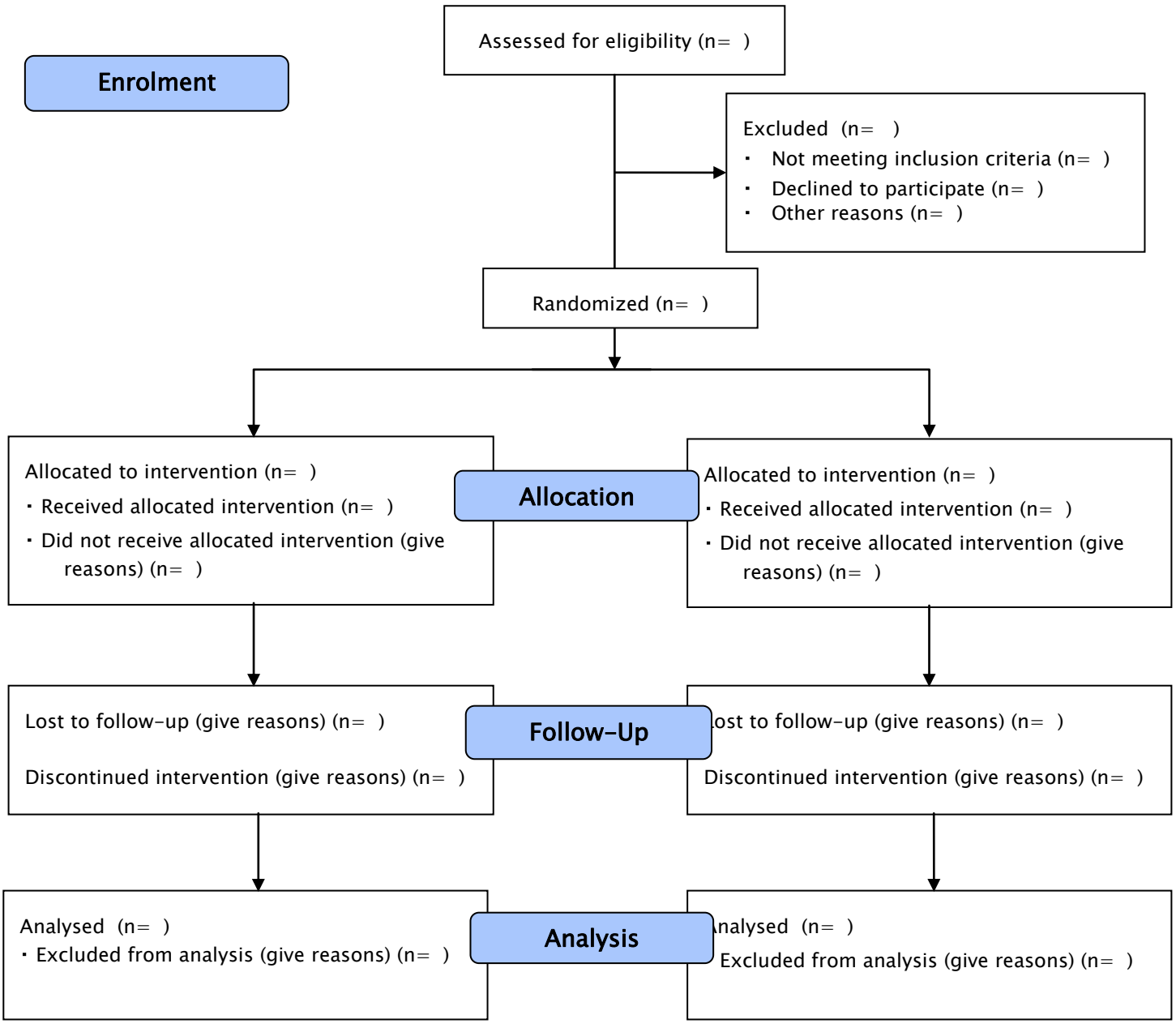


Figure 2. Proposed Consort flow diagram

[6] http://www.consort-statement.org/

5.3 Baseline patient characteristics

Table 2. **Characteristics of the Donors and Recipients at Baseline.**			
Variable	**Machine Perfusion (N=)**	**Static Cold Storage (N=)**	**P Value**
Donor characteristics			
Age (yr)			
Male sex , n (%)			
Cause of death			
Cerebrovascular accident, n (%)			
Postanoxic brain injury, n (%)			
Trauma, n (%)			
Miscellaneous, n (%)			
Donor risk index			
Body mass index (kg/m^2)			
Preservation characteristics			
Time from donor death to aortic flush-out (min)			
Time from withdrawal of life support to aortic flush-out (min)			
Static cold ischaemia time, excluding DHOPE (hr)			
DHOPE perfusion time (hr:min)			
Total preservation time from aortic perfusion in donor to reperfusion in recipient (hr:min)			

Recipient characteristics			
Age (yr)			
Gender (male) , n (%)			
Indication for transplantation			
Cryptogenic liver cirrhosis, n (%)			
Hepatitis B/C, n (%)			
Hepatocellular carcinoma , n (%)			
Metabolic liver disease, n (%)			
Non-alcoholic steato-hepatitis, n (%)			
Post-alcoholic liver cirrhosis, n (%)			
Primary biliary cholangitis, n (%)			
Primary sclerosing cholangitis, n (%)			
Polycystic liver disease, n (%)			
Retransplantation, n (%)			
Miscellaneous[b], n (%)			
Laboratory MELD score			
Renal replacement therapy, n (%)			

The Donor Risk Index (DRI) will be calculated based on donor cause of death, donor race, donor height, graft type, and share type, using the following formula:

$$DRI = \exp[((0.154 \text{ if } 40 \leq \text{age} < 50) + (0.274 \text{ if } 50 \leq \text{age} < 60) + (0.424 \text{ if } 60 \leq \text{age} < 70) + (0.501 \text{ if } 70 \leq \text{age}) + (0.079 \text{ if COD} = \text{anoxia}) + (0.145 \text{ if COD} = \text{CVA}) + (0.184 \text{ if COD} = \text{other}) + (0.176 \text{ if race} = \text{African American}) + (0.126 \text{ if race} = \text{other}) + (0.411 \text{ if DCD}) + (0.422 \text{ if partial/split}) + (0.066*((170-\text{height [cm]})/10)) + (0.105 \text{ if regional share}) + (0.44 \text{ if national or international share}) + (0.010 \times \text{cold time [h]} - 8 \text{ h})$$

Recipient laboratory MELD score will be calculated from serum creatinine, bilirubin and INR according to the following formula:

$$MELD\ Score = (0.957 \times \ln[serum\ creatinine(mg/dL)] + 0.378 \times \ln[serum\ bilirubin(mg/dL)] + 1.120 \times \ln[INR] + 0.643) \times 10$$

The following modifications to the score were applied:

- If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0
- Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any positive value below 1 would yield a negative result).

Data will be presented as numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with interquartile range) for continuous variables. The Kolmogorov Smirnov test will used to assess whether continuous data are normally distributed. Tests will be reported with 95% confidence intervals and two-sided p-values.

6 Analysis

6.1 Outcome definitions

Primary Endpoint:

The primary endpoint of this trial is the incidence of symptomatic NAS at six months after DCD liver transplantation. The diagnosis of symptomatic NAS is defined as all of the following criteria:

- any irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
- which are diagnosed by cholangiogram (preferably by MRCP)
- in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
- and as confirmed by the Adjudication Committee
- when imaging is indicated by clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up

This endpoint is selected as it is considered to reflect a clinically relevant sign of biliary injury caused by ischemia-reperfusion.[7] Also, it is reproducibly attainable at all study sites and therefore can be objectified by blinded assessment by the Adjudication Committee including three independent radiologists. Moreover, the imaging modality is minimally invasive and is part of the routine diagnostic work-up in case of clinical suspicion of NAS.

[7]Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.

Secondary Endpoints:

1. The overall incidence of NAS is based on symptomatic NAS (see primary study endpoint) and asymptomatic NAS. Asymptomatic NAS is defined as all of the following:

    a. irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
    b. which are diagnosed by trial MRCP at 6-months post-transplant
    c. in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
    d. in the absence of clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up

2. The severity and location of NAS will be determined by the Adjudication Committe, based on:

    a. Assessment of the images of the MRCP obtained in all patients at six months after transplantation (time window of 15 days) which will be performed based on a scoring system described by Den Dulk et al.[8]
    b. Required treatment for NAS (i.e. ursodeoxycholic acid, ERCP, retransplantation)

3. Graft (censored and uncensored for patient death) and patient survival at 7 days, 1, 3, and 6 months after transplantation

4. PNF is defined as liver failure requiring retransplantation or leading to death within seven days after transplantation without any identifiable cause such as surgical problems, hepatic artery thrombosis, portal vein thrombosis and acute rejection (31).

5. EAD is defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function

[8] Den Dulk AC, Wasser MN, Willemssen FE, Monraats MA, De Vries M, Van den Boom R, et al. Value of Magnetic Resonance Cholangiopancreatography in Assessment of Nonanastomotic Biliary Strictures After Liver Transplantation. Transplant Direct. 2015 Nov;1(10):e42.

(the Olthoff criteria)[9]: Prothrombin time/INR ≥1.6 and or serum total bilirubin ≥10 mg/dL on postoperative day 7 and serum alanine (ALT) and aspartate aminotransferases (AST) >2000 IU/L within the first 7 days. In addition, a modification of the Olthoff criteria will be used, based on serum bilirubin and INR only. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.

6. Biochemical analysis of graft function and ischemia-reperfusion injury is determined with serum levels of ALT, AST, alkaline phosphatase (AlkP), gamma-glutamyl transferase (γGT), and total bilirubin at postoperative day 0 – 7 and 1, 3, 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.

7. Hemodynamic status (blood pressure, heart rate and vasopressor dosage) will be recorded 5 min before reperfusion, as well as 10 and 20 minutes after reperfusion to determine occurrence of post-reperfusion syndrome, as defined by Aggarwal et al.[10] and Hilmi et al.[11]

8. Length of initial ICU and initial hospital stay is determined in days of admission following liver transplantation. Duration of follow-up hospital stay is determined in days of hospital admission after discharge and up to six months after liver transplantation.

9. Postoperative complications are graded according to the Clavien-Dindo classification and comprehensive complication index (CCI). Special interest will be

[9] Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

[10] Aggarwal S, Kang Y, Freeman JA, et al. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54-5.

[11] Hilmi I, Horton CN, Planinsic RM, et al. The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. Liver Transpl 2008;14:504-8.

given to predefined infectious complications and the total length of use and cumulative doses of antibiotics.

10. Renal function is defined as estimated glomerular filtration rate (eGFR) according to the 4-variable Modification of Diet in Renal Disease (MDRD) equation (34) at day 7, and 1, 3, 6 months after transplantation. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered. Kidney injury is scored according to acute kidney injury network (AKIN) and risk, injury, failure, loss of kidney function and end-stage kidney disease (RIFLE) criteria. In selected centers, urinary kidney injury markers (kidney injury molecule, tissue inhibitor of matrix metalloproteinases-2 [TIMP2], Insulin-like Growth Factor Binding Protein-7 [IGFBP7], and neutrophil gelatinase-associated lipocalin [NGAL]) are determined preoperatively, at arrival in the ICU and at day 1, 3, and 5 after transplantation. Need for renal replacement therapy will be recorded.

11. Perfusion characteristics during DHOPE include flow, pressure and resistance at every fifteen minutes.

12. In selected centers, perfusate analyses will be performed to study the dynamics of experimental markers of tissue and mitochondrial injury. The perfusate at the start and end of DHOPE procedure, and every half hour in between will be analysed for pH, sodium, potassium, bicarbonate, lactate, ALT, AST, AlkP, γGT, urea, total bilirubin, thrombomodulin, high mobility group box-1 (HMBG) protein, cytochrome C.

13. In selected centers, prognostication of NAS is based on miRNA's: CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a determined in perfusate.

14. In selected centers, biopsies of liver parenchyma and bile duct, which are routinely taken during transplantation, are also taken in this trial at the time points: before DHOPE, after DHOPE, and after reperfusion at the time of bile duct anastomosis during anesthesia. The purpose is to underpin the histopathological status of the liver and bile ducts in both study groups. In addition, mechanistic research into molecular mechanisms of injury and repair during DHOPE will be done to identify pathophysiological pathways that might have potential to predict function and outcomes after transplantation.

15. Metabolic function, including new onset diabetes after transplantation (NODAT) in the first 90 days after transplantation. NODAT is defined according to the WHO criteria.

    a. Symptoms of diabetes and random plasma glucose ≥11.1 mmol/L. Symptoms include polyuria, polydipsia, and unexplained weight loss. OR
    b. Fasting plasma glucose ≥7.0 mmol/L. Fasting is defined as no caloric intake for at least eight hours. OR
    c. Two-hour plasma glucose ≥11.1 mmol/L during an oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

16. In selected centers, overall cost of treatment within 6 months (in/excluding return to work) is calculated according to the Cost and Outcome analysis of Liver Transplantation (COLT) study.

17. Health related quality of life will be determined using an EQ-5D-3L questionnaire obtained when the patient before transplantation and at 6 months after transplantation.

6.2 Analysis methods

6.2.1 Primary efficacy analysis

The primary endpoint, incidence of clinically symptomatic NAS within 6 months after transplantation, will be analyzed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. The difference between the study arms will be reported as the result of the chi-squared test (X^2 [*degrees of freedom*, *N* = *sample size*] = *chi-square statistic value*, *p* = *p value*) as well as a rate ratio. A P-value less than 0.05 will be considered to indicate statistical significance.

6.2.2 Secondary efficacy analyses

For the primary endpoint, log-binomial regression will be performed with or without adjustment for stratification factors (transplant center and PSC) and well-known graft-related risk factors for NAS (donor warm ischemia time and donor risk index). The difference between the study arms will be reported as an adjusted risk

ratio with corresponding 95% confidence interval (CI). Advantage of log-binominal regression, compared to logistic regression, is that it generates estimates of the risk ratio (and 95% CI) instead of the odds ratio. With an expected incidence of NAS in the control group >10%, odd ratios will overestimate any treatment effect size

Additionally, time-to-event will be analyzed using the Kaplan-Meier method and significance of survival differences will be determined with the log rank test. Kaplan-Meier plot will be censored for patient death with functioning graft and for retransplantation for other causes than the primary end-point. Median or mean time to diagnosis will be reported. Furthermore, a Cox proportional hazards regression model will be performed to calculate (adjusted) hazard ratios (with 95% confidence intervals), adjusting for stratification factors (transplant center and PSC) and well-known graft-related risk factors (donor warm ischemia time and donor risk index). The proportional hazards assumption will be tested and if non-proportionality is present appropriate methodology will be used such as stratified Cox model and extended Cox model containing time-dependent variables.

6.2.3 Efficacy analyses of secondary endpoints

Binary data will be assessed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. Continuous data will be compared using unpaired T-test if normally distributed, otherwise using the Wilcoxon signed rank test or a Mann Whitney U test depending on parametric or non-parametric data.

If appropriate, binary outcome variables will also be analyzed using log-binomial regression analysis with adjustment for relevant risk factors, and (adjusted) risk ratios with 95% CI will be reported. Similar to the primary endpoint, log-binomial regression analysis of binary secondary endpoints is preferred over logistic-regression analysis as it generated estimates of the risk ratio (95% CI) instead of odds ratio (95% CI). The latter of which may overestimate the treatment effect size when the incidence of the binary outcome variable is >10%.

Time-to-event outcomes (i.e. patient and graft survival) will be analyzed using the Kaplan-Meier method (and logrank test) and/or Cox proportional hazards regression model with calculation of (unadjusted/adjusted) hazard ratios with 95% confidence intervals.

Biochemical liver function tests will be analyzed using ANOVA (with adjustment for stratification factors and/or other relevant risk factors, if appropriate). If these data are not normally distributed (non-parametric), the first approach will be log transformation of the data. If the data cannot be transformed to normal distribution, the difference in data between study arms will be analyzed using Mann-Whitney U test. If a non-parametric analysis needs to be performed there will be no adjustment for stratification factors or other covariates. In that case, multiple testing corrections will be applied for repetitive measurements.

6.3 Missing data

As patients are followed intensively after transplantation, it is expected that there will be no/few missing data. Strategies to handle missing data are, therefore, expected to be used only for secondary outcomes analyses and only if the missing rate is 10% or more. In case of missing data in spite of efforts for obtaining them, we will perform a missing data analysis including the cause of the absent data, the value of the data, the distribution of the missing data and subsequently determine the method of handling the missing data (such as multiple imputation).

6.4 Safety Analyses

6.4.1 Analysis of anticipated adverse events

All participants in this trial undergo liver transplantation, which is a surgical procedure with significant morbidity and mortality. This implies an intrinsic risk of AEs and SAEs anticipated after liver transplantation defined as:

Table 3. **List of anticipated adverse events associated with liver transplantation**

Complications		**Incidence (%)**
Acute Rejection	Requiring biopsy or medication	7%
EAD		16%
Kidney dysfunction	Requiring alteration of medication up to dialysis	10%
Infectious complications	Wound Infection requiring opening	20%
	Infected ascites or intraabdominal abcess requiring drainage	30%
	Pneumonia requiring antibiotics	8%

	Urinary tract infection requiring antibiotics		20%
	Viral infection requiring virostatica		35%
	Blood stream infection requiring antibiotics		21%
Bleeding Complications	Surgical site bleeding requiring reoperation or transfusion		10%
Biliary Complications	Biliary leakage requiring drainage or endoscopy		22%
	NAS requiring intervention		29%
	Anastomotic biliary strictures requiring intervention		10%
Thrombotic and Ischemic Complications	Systemic thromboemboliic events (deep venous thrombosis, pulmonary embolism)*		3%
	Cardiovascular events	Requiring medication or electroversion	6%
		Infarction	2%
		Arrhythmia	3%
		New congestive heart failure	2%
	Cerebrovascular events		3%
		Stroke	2%
		Hemorrhage	0,3-0,6%
Central nervous system	Including delirium requiring medication		10%
NODAT	Requiring insulin		7%

*This does not include portal venous thrombosis or hepatic artery thrombosis (these thromboembolic events should always be reported immediately).

The abovementioned events will be analysed as adverse events (AE). The investigator will exercise his/her medical judgment in deciding whether an adverse event, a postoperative laboratory finding falling outside the relevant reference range or other abnormal assessment is clinically significant.

6.4.2 Recording of adverse events

It is the responsibility of the Principal Investigator to ensure that all adverse events (including ADEs) and device deficiencies occurring during the course of the study are collected. This will include but not be limited to:

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

Adverse events will not be recorded or reported in case the event is both graded as a grade 1 complication according to the classification of Clavien Dindo as well as graded as mild or moderately severe.

Adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects. In addition each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms within the eCRF.

6.4.3 Reporting of adverse events

All events are recorded by the Trial Coordinator in an overview list (line-listing) that will be submitted once every half year to the MREC. This line-listing provides an overview of all events accompanied by a brief report highlighting the main points of concern. The line-listing reporting of the events to the accredited MREC is sufficient as notification to the competent authority. The Trial Coordinator will report line-listed events to the competent authorities in other Member States, according to the requirements of the Member States.

Reporting of all Serious Adverse Events (SAEs) will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010). It is the responsibility of the Principle Investigator to ensure that all adverse events which fall into the category of SAEs, SADEs, USADEs and device deficiencies are reported to the Coordinating Investigator as soon as possible after becoming aware of the event but no later than 24 hours.

6.4.4 Follow up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

6.5 Statistical software

Statistical analyses will be performed using the statistical software package SPSS® software version 23.0 for Windows® (IBM, Armonk, New York, USA).

7 References

1. Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-43.
2. Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63
3. Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53
4. Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10
5. Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73
6. URL: http://www.consort-statement.org/
7. Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.
8. Den Dulk AC, Wasser MN, Willemssen FE, Monraats MA, De Vries M, Van den Boom R, et al. Value of Magnetic Resonance Cholangiopancreatography in Assessment of Nonanastomotic Biliary Strictures After Liver Transplantation. Transplant Direct. 2015 Nov;1(10):e42.
9. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

10. Aggarwal S, Kang Y, Freeman JA, et al. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54-5.
11. Hilmi I, Horton CN, Planinsic RM, et al. The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. Liver Transpl 2008;14:504-8.

5. Final Version of Statistical Analysis Plan

Statistical Analysis Plan

1 Administrative information

TRIAL FULL TITLE	A multicenter randomized controlled trial to compare the efficacy of end-ischemic dual hypothermic oxygenated machine perfusion with static cold storage in preventing non-anastomotic biliary strictures after transplantation of liver grafts donated after circulatory death: DHOPE-DCD Trial
CLINICALTRIALS.GOV REGISTRATION NUMBER	NCT02584283
CLINICALTRIALS.GOV REGISTRATION DATE	22-10-2015
SAP VERSION 1 - DATE	19-05-2019
SAP VERSION 2 - DATE	28-02-2020
REVISIONS in V2	Extending follow-up for secondary end points patient and graft survival from 6 to 12 months
TRIAL PRINCIPLE INVESTIGATOR	R.J. Porte (r.j.porte@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
TRIAL COORDINATOR	R. van Rijn (r.van.rijn@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
SAP AUTHOR / EPIDEMIOLOGIST	V.E. de Meijer (v.e.de.meijer@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands
DSMB EPIDEMIOLOGIST	M. El Moumni (m.el.moumni@umcg.nl) University Medical Center Groningen, Dept. of Surgery P.O. Box 30.001, 9700 RB Groningen, the Netherlands

This SAP adheres to the Guidelines for the content of statistical analysis plans in clinical trials (SAPIT)[1]

[1] Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-43.

1.1 SAP Signatures

I give my approval for the attached SAP entitled DHOPE-DCD Trial dated 28-02-2020.

Principle Investigator

Name: Robert J. Porte

Signature: _____

Date: 2/3/2020

SAP Author/Epidemiologist

Name: Vincent E. de Meijer

Signature: _____

Date: 28/02/2020

DSMB Member/Epidemiologist

Name: Mostafa el Moumni

Signature: _____

Date: 02-03-2020

1.2 Table of Contents

1.3 Abbreviations and Definitions

AE	Adverse Event
AlkP	Alkaline Phosphatase
AKIN	Acute Kidney Injury Network
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
CCMO	Central Committee on Research Involving Human Subjects
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-3L	European Quality of Life Instrument 5D-3L
CCI	Comprehensive Complication Index
CV	Curriculum Vitae
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
ECD	Extended Criteria Donor
ERCP	Endoscopic Retrograde Cholangiopancreaticography
EU	European Union
GCP	Good Clinical Practice
γGT	Gamma-glutamyl Transferase
HMGB	High Mobility Group Box-1
DHOPE	Dual Hypothermic Oxygenated Perfusion
ICU	Intensive Care Unit

INR	International Normalized Ratio
IPF	Initial Poor Function
MDRD	Modification of Diet in Renal Disease
MELD	Model for End-stage Liver Disease
miRNA	Micro Ribonucleic acid
MP	Machine Perfusion
MREC	Medical Research Ethics Committee
MRCP	Magnetic Resonance Cholangiopancreatography
NA	Not Applicable
NAS	Non-anastomotic Biliary Strictures
NODAT	New Onset Diabetes After Transplantation
OLT	Orthotopic Liver Transplantation
PNF	Primary Non-Function
PT	Prothrombin Time
RIFLE	Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
(S)ADE	(Serious) Adverse Device Effects
(S)AE	(Serious) Adverse Event
SAP	Statistical Analysis Plan
SCS	Static Cold Storage
SOP	Standard Operation Procedure
UMCG	University Medical Center Groningen
USADE	Unanticipated Serious Adverse Device Effects
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act

2 Introduction

2.1 Background and rationale

Recent publications report good results of controlled donation after circulatory death (DCD) liver transplantation when strict donor-recipient matching is applied and ischemia times are kept to a minimum. However a major concern remains the high rate of biliary complications after transplantation of DCD livers. Non-anastomotic biliary strictures (NAS) occur in 29% of patients receiving a DCD graft whereas the incidence of NAS in recipients of donation after brain death (DBD) liver grafts is 11%. NAS are associated with higher morbidity and increased cost of liver transplantation. Injury to the biliary epithelium and the peribiliary vascular plexus occurring during donor warm ischemia and static cold storage (SCS) has been identified as a major risk factor for development of NAS. Machine perfusion has been proposed as an alternative strategy for organ preservation, offering the opportunity to improve the quality of the organ by providing oxygen to the graft. Experimental studies have shown that end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) helps liver grafts to recover from ischemia by restoring mitochondrial function and by preventing of reperfusion injury due to production of radical oxygen species and danger associated molecular patterns. Moreover, DHOPE has been shown to provide better preservation of peribiliary vascular plexus of the bile ducts, which could be an important step forward in reducing the incidence of NAS after transplantation.

2.2 Objectives

Primary Objective:

To study the efficacy of end-ischemic DHOPE in reducing the incidence of symptomatic NAS at 6 months after controlled DCD liver transplantation.

Secondary Objective(s):

To study the effect of the intervention (end-ischemic DHOPE after SCS), in comparison to the control group (SCS only), concerning:

1. The overall incidence of symptomatic and asymptomatic NAS
2. The severity of NAS after transplantation
3. The graft and recipient survival
4. The incidence of primary non-function (PNF)
5. The incidence of early allograft dysfunction (EAD)
6. The biochemical analysis of graft function and ischemia-reperfusion injury
7. The hemodynamic status of the recipient after graft reperfusion to assess post-reperfusion syndrome
8. Length of stay in the ICU and hospital
9. The incidence of postoperative complications, including infections and use of antibiotics
10. The renal function, including need for renal replacement therapy
11. The perfusion characteristics during DHOPE (in the intervention group only)
12. The perfusate analysis during DHOPE (in the intervention group only)
13. Prognostication of NAS, based on micro ribonucleic acid (miRNA) profiles in perfusion fluid (in the intervention group only)
14. Pathobiology of liver and bile duct parenchyma
15. Metabolic function, including new onset diabetes after transplantation (NODAT)
16. Overall cost of treatment within 6 months (in/excluding return to work)
17. Overall health related quality of life after transplantation

3 Study Methods

3.1 General Study Design and Plan

The trial is designed as a prospective, randomized, controlled, multicenter, superiority, clinical trial with two parallel groups. Primary endpoint is the incidence of symptomatic NAS at six months after liver transplantation. Liver grafts in the treatment group will be preserved with SCS followed by DHOPE. Liver grafts in the control group will be preserved by SCS alone without any further intervention. Figure-1 provides an overview of the study design.

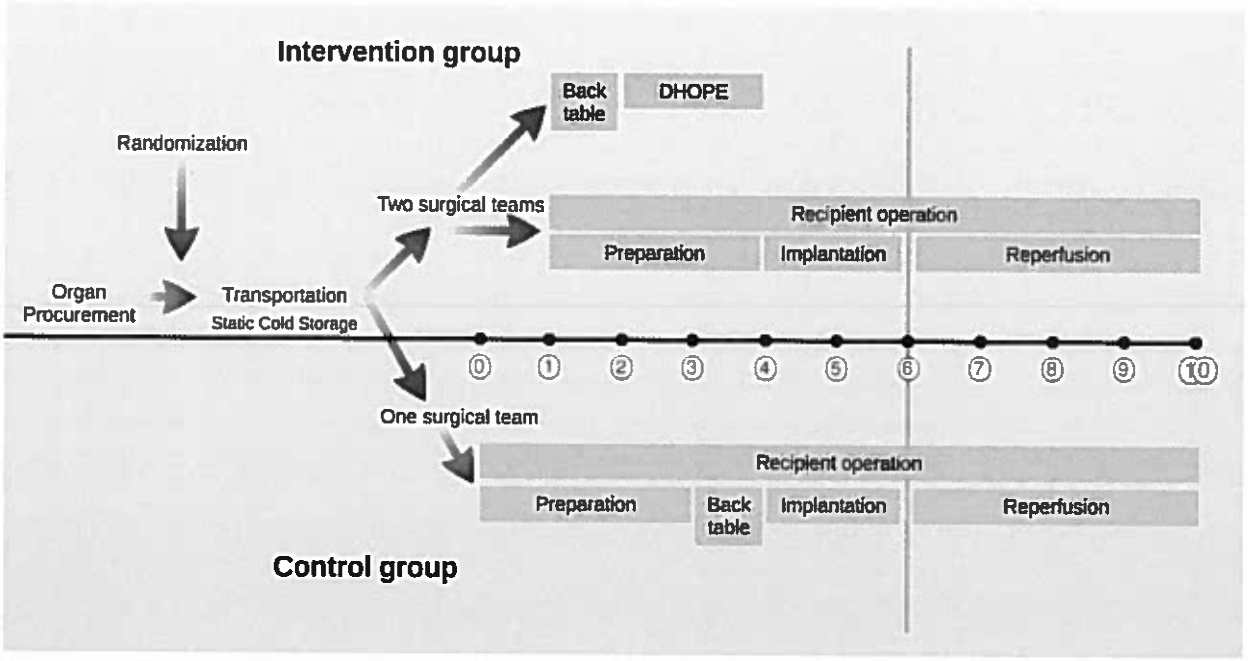


Figure 1. Study flow chart.

The study setting is standard practice of OLT. The surgical procedure, post-operative care and follow-up are identical to the routine OLT-practice in each participating center.

Patients are followed during the six months post-transplantation, during hospital stay and after discharge, via their routine hospital visits at 1 month, 3 months, 6 months after transplantation. In the final (5th) version of the trial protocol it was decided to include data on 1-year patient and graft survival as well (the SAP was

changed accordingly; version 2). Whenever patients included in this trial are suspected of NAS based on clinical parameters as judged by their treating physician, they will undergo routine examinations which includes MRCP imaging of the biliary tree. In case of radiologically confirmed symptomatic NAS, this is primary outcome event is registered in the electronical case report form (eCRF).

In case a patient in this trial is not diagnosed with NAS within 6 months, a MRCP is performed at six months after transplantation to assess the biliary tree for asymptomatic radiological abnormalities. This time interval is chosen because diagnosis of NAS is reported at a median of 3 to 4 months after transplantation. Some studies have reported an occurrence of 100% of the cases of NAS within 4 months. All biliary imaging, including the trial MRCP at 6 months, will be evaluated by an Adjudication Committee in order to harmonize and standardize endpoint assessment. The Adjudication Committee consists of three independent radiologists. In order to allow for an unbiased endpoint assessment the members are blinded to study group assignment.

3.2 Randomization and Blinding

Randomization

When a DCD donor liver becomes available, transplant centers involved in the trial will be informed by their local organ procurement organization. It is important to note that absolutely no changes will be made to national and international liver allocation rules. The standard local liver allocation rules will be followed. The study does not interfere or change the process of accepting or declining a liver offered to a certain patient in any way. Once a suitable recipient for the liver is identified, the recipient will be invited to the relevant transplant center for the surgical procedure as per routine procedure. Standard care at individual transplant centers is provided. After acceptance of the liver for a certain patient, the inclusion, exclusion criteria and informed consent are once more checked.

Only when the liver is definitively deemed suitable for transplantation by the liver transplant surgeon, and the rest of the OR team including the anesthetist is informed about the exact starting time of the transplantation, and all inclusion and

exclusion criteria are met, the patient will be randomized. The procurement surgical team remains blinded for the study group during the donor operation.

The patient will be randomized by the local investigator or the trial coordinator using an online randomization tool. A computer-generated list of random assignments (block randomization) is prepared in advance by Clinical Trial Center. Block randomization is stratified for trial site and for patients with primary sclerosing cholangitis (PSC). All personnel involved in randomization will be trained in the use of the online randomization by the Trial Coordinator or the Principal Investigator of each site. When a patient is registered to the website, an inclusion number is generated consisting of study-center-inclusion; for example the first patient in UMCG will have inclusion number UMCG-DHOPE-01. The local investigator will register which graft the patient has received. This information will be stored, so in all cases, it will be possible to determine the study group the patient belongs to. The randomization website will send inclusion information to the mailbox of the Trial Coordinator, containing the inclusion number and the date and time.

Blinding

After randomization, the transplanting surgeon will be informed whether the patient is randomized to receive a graft after DHOPE or SCS only. The procurement surgeon is blinded to the study group. Patients are blinded to study group. The study is blinded for assessment of the primary endpoint by the Adjudication Committee. When there is a breach of blinding, this is described in the eCRF and the Sponsor is notified.

3.3 Sample Size

The study is powered to detect a clinically relevant difference in incidence of symptomatic NAS between the two study groups. The incidence of NAS is 29% after DCD liver transplantation and is 11% after DBD liver transplantation in patients transplanted in the UMCG from 2008 to 2013 (unpublished data). This is similar to incidence reported by Abt *et. al.* (27% in DCD versus 2% in DBD

transplantation)[2], Dubbeld *et. al* (24% versus 8%)[3], Croome *et. al.* (22% versus 4%)[4], and Meurisse *et. al.* (33% versus 12%)[5]. With the intervention (DHOPE) we aim to reduce the incidence of NAS after DCD liver transplantation to the level observed after DBD liver transplantation (i.e., risk reduction of 62%; absolute difference of 29-11=18%). We base this presumed reduction on our results in the pilot study in which 1 of 10 (10%) of the patients with a DHOPE treated liver developed NAS. The other previously reported pilot studies observed no NAS in any of the patients receiving a liver treated with end-ischemic hypothermic machine perfusion. For a power of 80% (β=0.80) and a 5% significance level (2-sided test) in two independent cohorts, using a Chi-squared test, 77 livers are needed to be included in each arm, calculated with nQuery + nInterim 3.0. Although there is a (very) small likelihood of lost to follow-up, we still want to include an extra patient per study arm. Therefore, the total number of patients to be included in this study will be 156 (77*2+2).

3.4 Interim analysis

One interim analysis will be performed by the DSMB after half of patients (n=78) are included to determine the incidence of NAS in the control group in order to determine if the assumption of 29% of NAS in the control group is adequate. The analysis will take place after 78 patients have completed 6 months follow-up. There will be no statistical test performed. The DSMB will only perform an interim analysis on adverse events if there are concerns about safety as described in the DSMB charter. The Pocock sequential boundary will be used to determine statistical significance of adverse events between the two groups, dictating a Z-value of 2 and thus a P-value of 0.045.

---

[2] Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63

[3] Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53

[4] Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10

[5] Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73

The trial may be stopped early due to one of the following situations:

- Unacceptable safety concerns: The analysis shows significant (serious) adverse events in the treatment group compared to the control group.
- In case new external information arises that convincingly answers the study question or raises serious safety issues.

3.5 Stopping guidance

The study will be terminated prematurely if we find a higher rate of adverse events (AEs) than expected (see study protocol; section 9.4) that is possibly related to the study. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to DHOPE. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. This definition includes physical signs, symptoms and laboratory test values. At study enrolment, laboratory values that fall outside the relevant reference range will not be reported as AEs.

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

3.6 Timing of final analysis

All data will be gathered when all subjects have completed at least the 6-months follow-up assessment, or have dropped out prior to this. All data from all study sites will be checked on completeness and missing data. Queries will be sent to the participating centres in order to complete any (initially) missing data. After completion and cleaning, the database will be locked and the investigators will be given access to the data. All outcomes will be analyzed collectively.

3.7 Timing of outcome assessments

Table 1. Overview of study variables per time point.

Endpoint / Time point	Baseline parameters	Perfusion characteristics	Hemodynamic status	Serum and urine analysis	Clinical follow-up	MRCP
Before OLT	X			X		
During DHOPE		X				
During OLT		X	X			
After reperfusion			X	X		
Day 0-7				X	X	
Month 1				X	X	
Month 3				X	X	
Month 6				X	X	X
Month 12					X*	

* Patient still alive (yes/no) and/or retransplanted (yes/no)

A non-exhaustive list of data retrieved per eCRF can be found in Appendix 3 of the Study Protocol: Data collection parameters.

4 Statistical principles

4.1 Confidence intervals and *P* values

Data will be presented as numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with interquartile range) for continuous variables. The Kolmogorov Smirnov test will used to assess whether continuous data are normally distributed. Tests will be reported with 95% confidence intervals and two-sided p-values. A P-value of less than 0.05 will be considered to indicate statistical significance. No correction for multiple testing will be performed.

4.2 Analysis populations

Full Analysis Intention to Treat Population

- All subjects who were randomized and who participated in at least one post-baseline assessment

Per Protocol Population

- All subjects who were randomized and who have completed at least the 6-months follow-up assessment (including trial MRCP in patients not previously diagnosed with symptomatic NAS).

Safety Population

- All subjects who were randomized (including control) and are confirmed as providing complete follow-up regarding adverse event information.

5 Trial population

5.1 Inclusion-Exclusion Criteria and General Study Population

Study Population

Adult patients (≥ 18 years old) with end-stage chronic liver disease awaiting liver transplantation are screened for participation in this trial.

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adult patients (≥ 18 years old)
- Signed informed consent
- Willing and able to attend follow-up examinations
- Donor liver graft from a controlled donation after circulatory death
- Donors with a body weight ≥40 kg

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Simultaneous participation in another clinical trial that might possibly influence this trial
- Mental conditions rendering the subject incapable to understand the nature, scope and consequences of the trial
- Listed for liver transplantation due to fulminant liver failure or retransplantation because of PNF
- Recipient positive test for HIV antigen or HIV antibody
- Donor positive for HIV antigen, HIV antibody, Hepatitis B core antibody or hepatitis B surface antigen, or hepatitis C antibody
- Patients with contra-indications for MRCP (i.e. pacemaker)
- Simultaneous transplantation of another organ

5.2 Recruitment / Proposed Consort Flow Diagram [6]

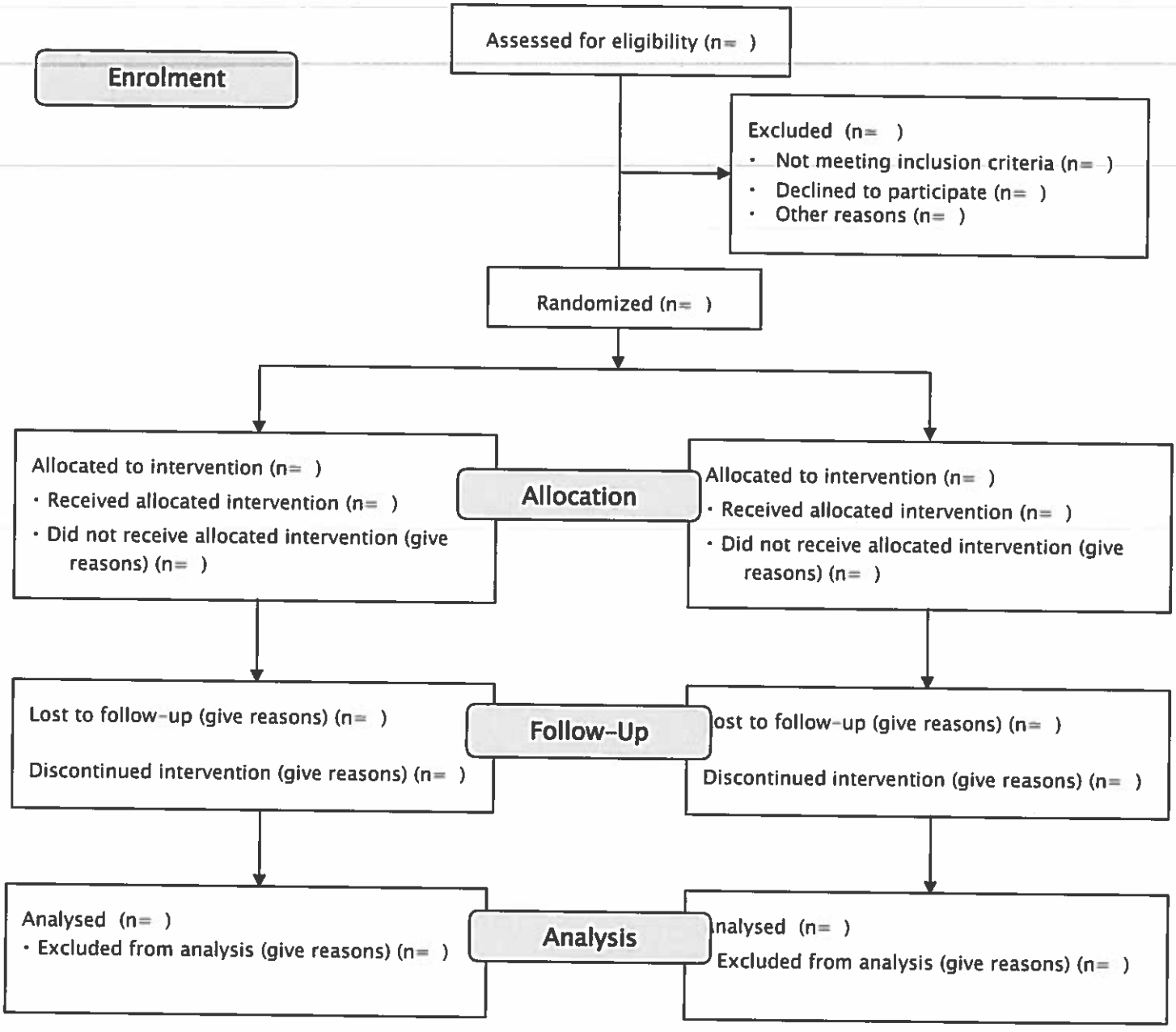


Figure 2. Proposed Consort flow diagram

[6] http://www.consort-statement.org/

5.3 Baseline patient characteristics

Table 2. Characteristics of the Donors and Recipients at Baseline.			
Variable	**Machine Perfusion (N=)**	**Static Cold Storage (N=)**	**P Value**
Donor characteristics			
Age (yr)			
Male sex , n (%)			
Cause of death			
Cerebrovascular accident, n (%)			
Postanoxic brain injury, n (%)			
Trauma, n (%)			
Miscellaneous, n (%)			
Donor risk index			
Body mass index (kg/m^2)			
Preservation characteristics			
Time from donor death to aortic flush-out (min)			
Time from withdrawal of life support to aortic flush-out (min)			
Static cold ischaemia time, excluding DHOPE (hr)			
DHOPE perfusion time (hr:min)			
Total preservation time from aortic perfusion in donor to reperfusion in recipient (hr:min)			

Recipient characteristics			
Age (yr)			
Gender (male) , n (%)			
Indication for transplantation			
Cryptogenic liver cirrhosis, n (%)			
Hepatitis B/C, n (%)			
Hepatocellular carcinoma , n (%)			
Metabolic liver disease, n (%)			
Non-alcoholic steato-hepatitis, n (%)			
Post-alcoholic liver cirrhosis, n (%)			
Primary biliary cholangitis, n (%)			
Primary sclerosing cholangitis, n (%)			
Polycystic liver disease, n (%)			
Retransplantation, n (%)			
Miscellaneous[b], n (%)			
Laboratory MELD score			
Renal replacement therapy, n (%)			

The Donor Risk Index (DRI) will be calculated based on donor cause of death, donor race, donor height, graft type, and share type, using the following formula:

DRI = exp[((0.154 if 40 ≤ age <50) + (0.274 if 50 ≤ age <60) + (0.424 if 60 ≤ age <70) + (0.501 if 70 ≤ age) + (0.079 if COD = anoxia) + (0.145 if COD = CVA) + (0.184 if COD = other) + (0.176 if race = African American) + (0.126 if race = other) + (0.411 if DCD) + (0.422 if partial/split) + (0.066*((170-height [cm])/10)) + (0.105 if regional share) + (0.44 if national or international share) + (0.010×cold time [h]-8 h)

Recipient laboratory MELD score will be calculated from serum creatinine, bilirubin and INR according to the following formula:

$$MELD\ Score = (0.957 \times \ln[serum\ creatinine(mg/dL)] + 0.378 \times \ln[serum\ bilirubin(mg/dL)] + 1.120 \times \ln[INR] + 0.643) \times 10$$

The following modifications to the score were applied:

- If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0
- Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any positive value below 1 would yield a negative result).

Data will be presented as numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with interquartile range) for continuous variables. The Kolmogorov Smirnov test will used to assess whether continuous data are normally distributed. Tests will be reported with 95% confidence intervals and two-sided p-values.

6 Analysis

6.1 Outcome definitions

Primary Endpoint:

The primary endpoint of this trial is the incidence of symptomatic NAS at six months after DCD liver transplantation. The diagnosis of symptomatic NAS is defined as all of the following criteria:

- any irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
- which are diagnosed by cholangiogram (preferably by MRCP)
- in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
- and as confirmed by the Adjudication Committee
- when imaging is indicated by clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up

This endpoint is selected as it is considered to reflect a clinically relevant sign of biliary injury caused by ischemia-reperfusion.[7] Also, it is reproducibly attainable at all study sites and therefore can be objectified by blinded assessment by the Adjudication Committee including three independent radiologists. Moreover, the imaging modality is minimally invasive and is part of the routine diagnostic work-up in case of clinical suspicion of NAS.

[7]Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.

Secondary Endpoints:

1. The overall incidence of NAS is based on symptomatic NAS (see primary study endpoint) and asymptomatic NAS. Asymptomatic NAS is defined as all of the following:
    a. irregularities or narrowing of the lumen of the intra- or extrahepatic donor bile ducts, but not at the anastomosis
    b. which are diagnosed by trial MRCP at 6-months post-transplant
    c. in the presence of a patent hepatic artery demonstrated by Doppler ultrasonography and if necessary, by computed tomography angiography
    d. in the absence of clinical signs (i.e., jaundice, cholangitis) or elevation of cholestatic laboratory parameters in blood samples taken during follow-up
2. The severity and location of NAS will be determined by the Adjudication Committe, based on:
    a. Assessment of the images of the MRCP obtained in all patients at six months after transplantation (time window of 15 days) which will be performed based on a scoring system described by Den Dulk et al.[8]
    b. Required treatment for NAS (i.e. ursodeoxycholic acid, ERCP, retransplantation)
3. Graft (censored and uncensored for patient death) and patient survival at 7 days, 1, 3, 6, and 12 months after transplantation
4. PNF is defined as liver failure requiring retransplantation or leading to death within seven days after transplantation without any identifiable cause such as surgical problems, hepatic artery thrombosis, portal vein thrombosis and acute rejection (31).
5. EAD is defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function

[8] Den Dulk AC, Wasser MN, Willemssen FE, Monraats MA, De Vries M, Van den Boom R, et al. Value of Magnetic Resonance Cholangiopancreatography in Assessment of Nonanastomotic Biliary Strictures After Liver Transplantation. Transplant Direct. 2015 Nov;1(10):e42.

(the Olthoff criteria)[9]: Prothrombin time/INR ≥1.6 and or serum total bilirubin ≥10 mg/dL on postoperative day 7 and serum alanine (ALT) and aspartate aminotransferases (AST) >2000 IU/L within the first 7 days. In addition, a modification of the Olthoff criteria will be used, based on serum bilirubin and INR only. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.

6. Biochemical analysis of graft function and ischemia-reperfusion injury is determined with serum levels of ALT, AST, alkaline phosphatase (AlkP), gamma-glutamyl transferase (γGT), and total bilirubin at postoperative day 0 - 7 and 1, 3, 6 months. Day 0 is defined as the interval between graft portal reperfusion and the midnight of that day. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered.

7. Hemodynamic status (blood pressure, heart rate and vasopressor dosage) will be recorded 5 min before reperfusion, as well as 10 and 20 minutes after reperfusion to determine occurrence of post-reperfusion syndrome, as defined by Aggarwal et al.[10] and Hilmi et al.[11]

8. Length of initial ICU and initial hospital stay is determined in days of admission following liver transplantation. Duration of follow-up hospital stay is determined in days of hospital admission after discharge and up to six months after liver transplantation.

9. Postoperative complications are graded according to the Clavien-Dindo classification and comprehensive complication index (CCI). Special interest will be

---

[9] Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

[10] Aggarwal S, Kang Y, Freeman JA, et al. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54-5.

[11] Hilmi I, Horton CN, Planinsic RM, et al. The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. Liver Transpl 2008;14:504-8.

given to predefined infectious complications and the total length of use and cumulative doses of antibiotics.

10. Renal function is defined as estimated glomerular filtration rate (eGFR) according to the 4-variable Modification of Diet in Renal Disease (MDRD) equation (34) at day 7, and 1, 3, 6 months after transplantation. If there are multiple analyses in one day, the morning sample at around 5.00 A.M. is registered. Kidney injury is scored according to acute kidney injury network (AKIN) and risk, injury, failure, loss of kidney function and end-stage kidney disease (RIFLE) criteria. In selected centers, urinary kidney injury markers (kidney injury molecule, tissue inhibitor of matrix metalloproteinases-2 [TIMP2], Insulin-like Growth Factor Binding Protein-7 [IGFBP7], and neutrophil gelatinase-associated lipocalin [NGAL]) are determined preoperatively, at arrival in the ICU and at day 1, 3, and 5 after transplantation. Need for renal replacement therapy will be recorded.

11. Perfusion characteristics during DHOPE include flow, pressure and resistance at every fifteen minutes.

12. In selected centers, perfusate analyses will be performed to study the dynamics of experimental markers of tissue and mitochondrial injury. The perfusate at the start and end of DHOPE procedure, and every half hour in between will be analysed for pH, sodium, potassium, bicarbonate, lactate, ALT, AST, AlkP, γGT, urea, total bilirubin, thrombomodulin, high mobility group box-1 (HMBG) protein, cytochrome C.

13. In selected centers, prognostication of NAS is based on miRNA's: CDmiR-30e, CDmiR-222, CDmiR-296, HDmiR-122 and HDmiR-148a determined in perfusate.

14. In selected centers, biopsies of liver parenchyma and bile duct, which are routinely taken during transplantation, are also taken in this trial at the time points: before DHOPE, after DHOPE, and after reperfusion at the time of bile duct anastomosis during anesthesia. The purpose is to underpin the histopathological status of the liver and bile ducts in both study groups. In addition, mechanistic research into molecular mechanisms of injury and repair during DHOPE will be done to identify pathophysiological pathways that might have potential to predict function and outcomes after transplantation.

15. Metabolic function, including new onset diabetes after transplantation (NODAT) in the first 90 days after transplantation. NODAT is defined according to the WHO criteria.

    a. Symptoms of diabetes and random plasma glucose ≥11.1 mmol/L. Symptoms include polyuria, polydipsia, and unexplained weight loss. OR
    b. Fasting plasma glucose ≥7.0 mmol/L. Fasting is defined as no caloric intake for at least eight hours. OR
    c. Two-hour plasma glucose ≥11.1 mmol/L during an oral glucose tolerance test. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

16. In selected centers, overall cost of treatment within 6 months (in/excluding return to work) is calculated according to the Cost and Outcome analysis of Liver Transplantation (COLT) study.

17. Health related quality of life will be determined using an EQ-5D-3L questionnaire obtained when the patient before transplantation and at 6 months after transplantation.

6.2 Analysis methods

6.2.1 Primary efficacy analysis

The primary endpoint, incidence of clinically symptomatic NAS within 6 months after transplantation, will be analyzed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. The difference between the study arms will be reported as the result of the chi-squared test (X^2 [*degrees of freedom*, N = *sample size*] = *chi-square statistic value*, p = *p value*) as well as a rate ratio. A P-value less than 0.05 will be considered to indicate statistical significance.

6.2.2 Secondary efficacy analyses

For the primary endpoint, log-binomial regression will be performed with or without adjustment for stratification factors (transplant center and PSC) and well-known graft-related risk factors for NAS (donor warm ischemia time and donor risk index). The difference between the study arms will be reported as an adjusted risk

ratio with corresponding 95% confidence interval (CI). Advantage of log-binominal regression, compared to logistic regression, is that it generates estimates of the risk ratio (and 95% CI) instead of the odds ratio. With an expected incidence of NAS in the control group >10%, odd ratios will overestimate any treatment effect size

Additionally, time-to-event will be analyzed using the Kaplan-Meier method and significance of survival differences will be determined with the log rank test. Kaplan-Meier plot will be censored for patient death with functioning graft and for retransplantation for other causes than the primary end-point. Median or mean time to diagnosis will be reported. Furthermore, a Cox proportional hazards regression model will be performed to calculate (adjusted) hazard ratios (with 95% confidence intervals), adjusting for stratification factors (transplant center and PSC) and well-known graft-related risk factors (donor warm ischemia time and donor risk index). The proportional hazards assumption will be tested and if non-proportionality is present appropriate methodology will be used such as stratified Cox model and extended Cox model containing time-dependent variables.

6.2.3 Efficacy analyses of secondary endpoints

Binary data will be assessed using a chi-squared test or a Fisher's exact test depending on the frequencies in the cross table. Continuous data will be compared using unpaired T-test if normally distributed, otherwise using the Wilcoxon signed rank test or a Mann Whitney U test depending on parametric or non-parametric data.

If appropriate, binary outcome variables will also be analyzed using log-binomial regression analysis with adjustment for relevant risk factors, and (adjusted) risk ratios with 95% CI will be reported. Similar to the primary endpoint, log-binomial regression analysis of binary secondary endpoints is preferred over logistic-regression analysis as it generated estimates of the risk ratio (95% CI) instead of odds ratio (95% CI). The latter of which may overestimate the treatment effect size when the incidence of the binary outcome variable is >10%.

Time-to-event outcomes (i.e. patient and graft survival) will be analyzed using the Kaplan-Meier method (and logrank test) and/or Cox proportional hazards regression model with calculation of (unadjusted/adjusted) hazard ratios with 95% confidence intervals.

Biochemical liver function tests will be analyzed using ANOVA (with adjustment for stratification factors and/or other relevant risk factors, if appropriate). If these data are not normally distributed (non-parametric), the first approach will be log transformation of the data. If the data cannot be transformed to normal distribution, the difference in data between study arms will be analyzed using Mann-Whitney U test. If a non-parametric analysis needs to be performed there will be no adjustment for stratification factors or other covariates. In that case, multiple testing corrections will be applied for repetitive measurements.

6.3 Missing data

As patients are followed intensively after transplantation, it is expected that there will be no/few missing data. Strategies to handle missing data are, therefore, expected to be used only for secondary outcomes analyses and only if the missing rate is 10% or more. In case of missing data in spite of efforts for obtaining them, we will perform a missing data analysis including the cause of the absent data, the value of the data, the distribution of the missing data and subsequently determine the method of handling the missing data (such as multiple imputation).

6.4 Safety Analyses

6.4.1 Analysis of anticipated adverse events

All participants in this trial undergo liver transplantation, which is a surgical procedure with significant morbidity and mortality. This implies an intrinsic risk of AEs and SAEs anticipated after liver transplantation defined as:

Table 3. **List of anticipated adverse events associated with liver transplantation**

Complications		**Incidence (%)**
Acute Rejection	Requiring biopsy or medication	7%
EAD		16%
Kidney dysfunction	Requiring alteration of medication up to dialysis	10%
Infectious complications	Wound Infection requiring opening	20%
	Infected ascites or intraabdominal abcess requiring drainage	30%
	Pneumonia requiring antibiotics	8%

	Urinary tract infection requiring antibiotics		20%
	Viral infection requiring virostatica		35%
	Blood stream infection requiring antibiotics		21%
Bleeding Complications	Surgical site bleeding requiring reoperation or transfusion		10%
Biliary Complications	Biliary leakage requiring drainage or endoscopy		22%
	NAS requiring intervention		29%
	Anastomotic biliary strictures requiring intervention		10%
Thrombotic and Ischemic Complications	Systemic thromboembolic events (deep venous thrombosis, pulmonary embolism)*		3%
	Cardiovascular events	Requiring medication or electroversion	6%
		Infarction	2%
		Arrhythmia	3%
		New congestive heart failure	2%
	Cerebrovascular events		3%
		Stroke	2%
		Hemorrhage	0,3-0,6%
Central nervous system	Including delirium requiring medication		10%
NODAT	Requiring insulin		7%

*This does not include portal venous thrombosis or hepatic artery thrombosis (these thromboembolic events should always be reported immediately).

The abovementioned events will be analysed as adverse events (AE). The investigator will exercise his/her medical judgment in deciding whether an adverse event, a postoperative laboratory finding falling outside the relevant reference range or other abnormal assessment is clinically significant.

6.4.2 Recording of adverse events

It is the responsibility of the Principal Investigator to ensure that all adverse events (including ADEs) and device deficiencies occurring during the course of the study are collected. This will include but not be limited to:

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

Adverse events will not be recorded or reported in case the event is both graded as a grade 1 complication according to the classification of Clavien Dindo as well as graded as mild or moderately severe.

Adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects. In addition each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms within the eCRF.

6.4.3 Reporting of adverse events

All events are recorded by the Trial Coordinator in an overview list (line-listing) that will be submitted once every half year to the MREC. This line-listing provides an overview of all events accompanied by a brief report highlighting the main points of concern. The line-listing reporting of the events to the accredited MREC is sufficient as notification to the competent authority. The Trial Coordinator will report line-listed events to the competent authorities in other Member States, according to the requirements of the Member States.

Reporting of all Serious Adverse Events (SAEs) will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010). It is the responsibility of the Principle Investigator to ensure that all adverse events which fall into the category of SAEs, SADEs, USADEs and device deficiencies are reported to the Coordinating Investigator as soon as possible after becoming aware of the event but no later than 24 hours.

6.4.4 Follow up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

6.5 Statistical software

Statistical analyses will be performed using the statistical software package SPSS® software version 23.0 for Windows® (IBM, Armonk, New York, USA).

7 References

1. Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017;318(23):2337-43.
2. Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation. 2003 May 27;75(10):1659-63
3. Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg. 2010 May;97(5):744-53
4. Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol. 2012 Sep;26(9):607-10
5. Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc. 2012 Nov;44(9):2868-73
6. URL: http://www.consort-statement.org/
7. Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. Liver Transpl. 2007 May;13(5):708-18.
8. Den Dulk AC, Wasser MN, Willemssen FE, Monraats MA, De Vries M, Van den Boom R, et al. Value of Magnetic Resonance Cholangiopancreatography in Assessment of Nonanastomotic Biliary Strictures After Liver Transplantation. Transplant Direct. 2015 Nov;1(10):e42.
9. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010 Aug;16(8):943-9.

10. Aggarwal S, Kang Y, Freeman JA, et al. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54-5.
11. Hilmi I, Horton CN, Planinsic RM, et al. The impact of postreperfusion syndrome on short-term patient and liver allograft outcome in patients undergoing orthotopic liver transplantation. Liver Transpl 2008;14:504-8.

6. Summary of Amendments to Statistical Analysis Plan

During study progress, the statistical analysis plan was amended once. The first and final version of the statistical analysis plan are included in the present file and changes concern the extension of the trial follow-up period to 12 months for patient and graft survival.