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Prolonged dual hypothermic oxygenated machine preservation (DHOPE-PRO) in liver transplantation: study protocol for a stage 2, prospective, dual-arm, safety and feasibility clinical trial

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ABSTRACT

Introduction End-ischaemic preservation of a donor liver by dual hypothermic oxygenated machine perfusion (DHOPE) for 2 hours prior to transplantation is sufficient to mitigate ischaemia-reperfusion damage and fully restore cellular energy levels. Clinical studies have shown beneficial outcomes after transplantation of liver grafts preserved by DHOPE compared with static cold storage. In addition to graft reconditioning, DHOPE may also be used to prolong preservation time, which could facilitate logistics for allocation and transplantation globally.

Methods and analysis This is a prospective, pseudo-randomised, dual-arm, IDEAL-D (Idea, Development, Exploration, Assessment, Long term study-Framework for Devices) stage 2 clinical device trial designed to determine safety and feasibility of prolonged DHOPE (DHOPE-PRO). The end-time of the donor hepatectomy will determine whether the graft will be assigned to the intervention (16:00–3:59 hour) or to the control arm (4:00–15:59 hour). In total, 36 livers will be included in the study. Livers in the intervention group (n=18) will undergo DHOPE-PRO (≥4 hours) until implantation the following morning, whereas livers in the control group (n=18) will undergo regular DHOPE (2 hours) prior to implantation. The primary endpoint of this study is a composite of the occurrence of all (serious) adverse events during DHOPE and up to 30 days after liver transplantation.

Ethics and dissemination The protocol was approved by the Medical Ethical Committee of Groningen, METc2020.126 in June 2020, and the study was registered in the Netherlands National Trial Registry (<https://www.trialregister.nl/>) prior to initiation.

Trial registration number NL8740.

INTRODUCTION

Limited availability of suitable donor organs for liver transplantation remains a major concern. As a result, transplant surgeons are urged to

accept livers from suboptimal donors, such as livers from elderly donors, steatotic grafts or livers donated after circulatory death (DCD). It is well known that these organs do not tolerate long periods of static cold storage (SCS), the current standard of liver graft preservation. Therefore, preservation using ex situ machine perfusion has gained considerable interest to limit ischaemia time, resuscitate organs and facilitate enhanced utilisation of liver grafts for transplantation.^{1,2}

Over the past decade, several machine perfusion modalities have been evaluated in preclinical and clinical studies.³ Normothermic machine perfusion (NMP) is performed in a near-physiological environment with an oxygenated solution at 35°C–37°C. During NMP, the liver is metabolically active, allowing for hepatobiliary viability assessment and therapeutic interventions prior to transplantation.¹ Hypothermic oxygenated machine perfusion (HOPE) is performed at 4°C–12°C and reconditions the graft by inducing a hypometabolic state while restoring mitochondrial function through the delivery of oxygen.¹ Dual HOPE (DHOPE) is referred to HOPE performed through both the portal vein and hepatic artery, instead of single portal vein perfusion. End-ischaemic DHOPE is a relatively simple approach. Following procurement of the donor liver at the donor centre, grafts are preserved by SCS during transportation to the recipient centre. On arrival, donor livers are prepared for transplantation at the back-table and are then subjected to machine perfusion for at least 2 hours.



Studies have shown that 2 hours of end-ischaemic DHOPE is sufficient to regenerate hepatic cellular energy stores with reduced postoperative complications, compared with SCS preservation alone.⁴⁻⁶ The results of the first multicentre randomised controlled trial (RCT) comparing DHOPE versus SCS in DCD liver transplantation initiated by our group were recently published.⁷ Superior outcomes after DHOPE preservation were shown, with an almost 70% reduction in risk of clinically relevant non-anastomotic biliary strictures within 6 months after transplantation. Based on this study, DHOPE is now implemented as standard care for the transplantation of DCD donor livers in the Netherlands. In another recently completed RCT, HOPE was compared with SCS of high-risk donation after brain death (DBD) livers. Machine perfusion significantly reduced the incidence of early allograft dysfunction and complications after liver transplantation.^{8,9}

In addition to organ resuscitation and protection against ischaemia-reperfusion injury, preclinical studies have investigated the potential to prolong preservation time using machine perfusion at hypothermic temperatures.¹⁰⁻¹⁴ Organ allocation logistics, including prolonged cold ischaemia time, are important reasons to decline donor liver grafts. If machine perfusion can safely prolong preservation time, more livers could be accepted and liver transplantation may become a semi-elective procedure. Consequently, transplant surgery could be scheduled during daytime instead of during the night, since the latter has been associated with a greater risk of morbidity and mortality.¹⁵ Sleep loss has been shown to impact performance in healthcare workers employed in a wide range of medical specialties.¹⁶⁻¹⁹ Patients undergoing surgical procedures by sleep-deprived surgeons could be at risk for complications.²⁰ Moreover, the recipient will have a regular night of sleep and might be more fit to undergo surgery the next morning.

Our research group recently showed successful preservation of porcine and discarded human livers using DHOPE for up to 24 hours.¹³ Liver grafts preserved by 24 hours DHOPE had similar hepatobiliary function and injury markers after warm reperfusion compared with livers that underwent 2 or 6 hours DHOPE. To date, no clinical studies have investigated prolonged DHOPE (DHOPE-PRO) preservation. There are a few published cases in which hypothermic machine perfusion successfully extended preservation time with a maximum reported machine perfusion time of 8 hours.^{12,14,21,22}

This study is designed to assess the safety and feasibility of prolonged (≥ 4 hours) DHOPE preservation of human donor livers prior to transplantation, compared with the current standard. Livers that would have otherwise been transplanted during the night will be subjected to DHOPE-PRO to enable transplantation the next morning. We hypothesise that it is safe and feasible to prolong DHOPE with similar outcomes compared with livers preserved by 2 hours DHOPE.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Given informed consent	Simultaneous participation in another trial potentially influencing this trial
Adult patients (≥ 18 years old)	Simultaneous combined organ transplantation
Donors with a body weight ≥ 40 kg	Mental conditions rendering the subject incapable to understand the nature, scope, and consequences of the trial
DCD (n=6 per arm) or DBD (n=12 per arm) grafts	HU status
	Laboratory MELD score >30
	Recipient tested positive for HIV
	DCD Maastricht category V
	DCD donors >60 years old
	Donor with untreated HIV/HBV/HCV
	Estimated graft steatosis $>30\%$
	Split or partial liver grafts
	Domino donor livers
	Living donor liver grafts

DBD, donation after brain death; DCD, donation after circulatory death; HBV, viral hepatitis B; HCV, viral hepatitis C; HU, high urgency; LT, liver transplantation; MELD, model for end-stage liver disease.

METHODS AND ANALYSIS

Study design

This is an investigator-initiated, prospective, pseudorandomised, non-inferiority, dual-arm, clinical device trial to study the safety and feasibility of DHOPE-PRO (≥ 4 hours; intervention arm) vs regular DHOPE (2 hours; control arm) for 36 human donor livers, and the donor liver recipients. The study is conducted at a single site (University Medical Center Groningen (UMCG), Groningen, The Netherlands). IDEAL-D (Idea, Development, Exploration, Assessment, Long-Term Study) framework and recommendations for stage 2 clinical device trials (development phase) were adhered to.^{23,24} The study protocol was written in accordance to the Consolidated Standards of Reporting Trials and Standard Protocol Items: Recommendations for Interventional Trials recommendations.^{25,26} Inclusion of patients for this trial started on 1 September 2020, and is expected to take place until September 2023.

Eligibility criteria

Patients ≥ 18 years of age, eligible for liver transplantation will be screened for participation in this trial (table 1). In each arm, 6 DCD (Maastricht category III) and 12 DBD grafts are included. Exclusion criteria include patients who simultaneously participate in another trial potentially influencing this trial, patients undergoing combined organ transplantation, mental conditions rendering the patient incapable to understand the nature, scope and consequences of the trial, patients with a high-urgency status or a laboratory Model for End-stage Liver Disease (MELD) score >30 , and patients tested positive for HIV. Other exclusion criteria include DCD donors of Maastricht category V, DCD donors >60 years old, donors with untreated HIV, viral hepatitis B or C, grafts with estimated

steatosis >30%, split or partial grafts, domino donors and living donors.

Intervention

Liver grafts in the intervention group will undergo DHOPE \geq 4 hours (DHOPE-PRO). Liver grafts preserved by 2 hours DHOPE will serve as controls (DHOPE-CON). Livers are assigned to the intervention group if the donor hepatectomy is finished between 16:00 and 3:59 hour. Thus, livers that would have otherwise been transplanted during the night are now subjected to DHOPE-PRO and subsequent transplantation will be scheduled the next morning. Donor livers of which the donor hepatectomy is finished between 4:00 and 15:59 hour will undergo regular DHOPE (2 hours) prior to immediate transplantation and serve as controls. Livers will be transported to the UMCG using SCS preservation.

Study endpoints

The primary safety endpoint is: the incidence of serious adverse device events (SADEs) and serious adverse events (SAEs) during regular (control) and prolonged (intervention) DHOPE up to 30 days after liver transplantation. This endpoint is defined as the average number of SA(D)Es through the 30 days after liver transplantation per subject. The SADEs and SAEs in [table 2](#) will be evaluated. [Table 2](#) also shows the expected incidence of these SADEs and SAEs, which is based on empirical data from our centre and literature research.

The primary feasibility endpoint is: the proportion of patients who were assigned and successfully received a DHOPE-PRO-perfused liver graft.

The secondary endpoints are

- ▶ Biliary complications (including anastomotic and non-anastomotic biliary strictures) leading to a surgical or endoscopic intervention within 12 months after liver transplantation.
- ▶ Actuarial graft and patient survival at 12 months after liver transplantation.
- ▶ Incidence of acute kidney injury according to the KDIGO (Kidney Disease Improving Global Outcomes) criteria.²⁷
- ▶ Biochemical analysis of graft function and ischaemia-reperfusion injury at postoperative day 0–10, and at 1, 3, 6, and 12 months after transplantation, including serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, and international normalised ratio, and with the calculation of graft function assessment scores, including Model for Early Allograft Function²⁸ and Liver Graft Assessment Following Transplantation.²⁹
- ▶ Length of stay at the intensive-care-unit and total hospital length of stay.
- ▶ Perfusion characteristics during DHOPE, including vascular flow, pressure, resistance and oxygenation and temperature of the perfusate at every 30 min.

Table 2 Serious adverse (device) events (SADEs)

Complications	Expected incidence (%) up to 30 days after liver transplantation
SADEs	
Device error*	0
Deviation from the perfusion protocol†	0
SAEs	
Increased hepatic resistance‡	0–2
Post-reperfusion syndrome§	10–70
Primary non-function¶	4–8
Early allograft dysfunction**	15–30
Vascular complications	
Portal vein thrombosis††	1–4
Hepatic artery thrombosis‡‡	2–5
Massive biliary necrosis§§	1–5

*Any device error leading to termination of the perfusion (eg, motor pump failure).

†Any deviation from the perfusion protocol unable to be resolved within 30 min including: temperature >12°C, oxygenation <70 kPa, pressure >5 mm Hg in the portal vein or >25 mm Hg in the hepatic artery to ensure adequate portal (50–150 mL/min) and arterial (20–80 mL/min) volumetric flow rates.

‡Increased vascular resistance after initiation of machine perfusion illustrated by a hepatic artery volumetric flow rate <20 mL/min or a portal venous flow rate <50 mL/min in the absence of technical or mechanical issues.

§Haemodynamic instability after reperfusion defined as postreperfusion syndrome with a decrease in mean arterial pressure >30% below baseline, lasting for \geq 1 min, within 5 min after reperfusion (Aggarwal criteria⁴⁰), or as vasoplegia with a fall in mean arterial pressure on reperfusion to <50 mm Hg either sustained >30 min and/or requiring >0.15 μ g/kg/min norepinephrine, >2 U/hour vasopressin, or infusion of epinephrine (significant hypotension resistant to pressors).⁴¹

¶Non-life-sustaining graft function leading to graft loss or retransplantation within 7 days after liver transplantation.

**Presence of 1 or more of the following: bilirubin \geq 10 mg/dL on postoperative day 7, INR \geq 1.6 on postoperative day 7, lactate \geq 2 mmol/L on postoperative day 7 in the absence of vascular complications (modified Olthoff criteria).⁴²

††Radiologically or surgically proven thrombosis of the portal vein.

‡‡Radiologically or surgically proven thrombosis of the hepatic artery.

§§Radiological appearance of irregularities and beading dilatation of the intrahepatic bile ducts and/or the presence of cavitations and bile lakes leading to surgical or endoscopic intervention within 30 days.

SADE, serious adverse device event; SAE, serious adverse event.

- ▶ Postoperative complications according to the Clavien-Dindo classification as well as the comprehensive complications index³⁰ within 30 days after liver transplantation.

Participant timeline

All study subjects will receive standard care after liver transplantation. After discharge, patients will be evaluated up to 12 months post-transplantation.

A flow chart of the study is depicted in [figure 1](#) and the study design is graphically depicted in [figure 2](#).

Sample size

According to the IDEAL-D framework and recommendations for stage 2 clinical device trials (development

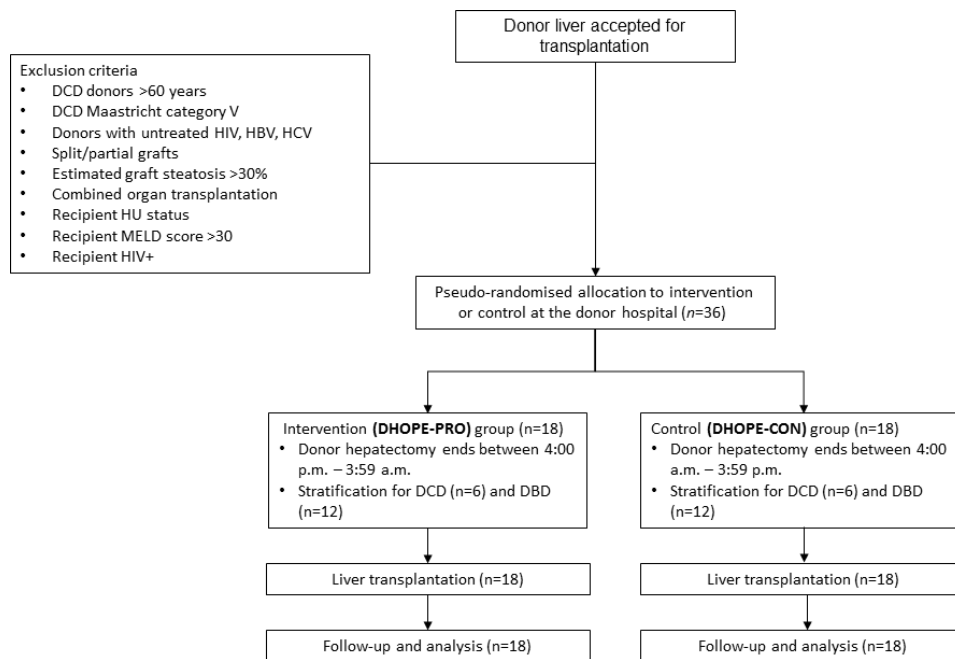


Figure 1 Flow chart of this study. DBD, donation after brain death; DCD, donation after circulatory death; DHOPE-PRO, prolonged dual hypothermic oxygenated machine perfusion; DHOPE-CON, regular dual hypothermic oxygenated machine perfusion; HBV, viral hepatitis B; HCV, viral hepatitis C; HU, high-urgency; MELD, model for end-stage liver disease.

phase), no formal sample size calculations are made because the study outcome is safety and feasibility (ie, procedural and technical success).²³ As a rule of thumb, sample size of such studies is in the 10s. Based on this, we expect to meet our primary endpoints (safety and feasibility) after including 36 liver transplants (18 in each arm) in the study.

Recruitment

Patients who are on the waiting list for liver transplantation and eligible for the study will be asked for consent. Patients will be informed about the study by the transplant surgeon, hepatologist or trial coordinator. A patient information folder has been designed for this study and an online video explaining machine perfusion of donor livers is available. Enrolment will continue until 36 livers

have been assigned to a study group and undergone machine perfusion.

Allocation

Pseudorandomisation will take place based on the end of donor hepatectomy time (independently determined by the off-site organ donation professional). Liver grafts are assigned to DHOPE-PRO if the donor hepatectomy is finished between 16:00 and 3:59 hour. Donor livers of which the donor hepatectomy is finished between 4:00 and 15:59 hour are assigned to DHOPE-CON.

Stratification

Both arms of this study will include 12 livers derived from DBD and 6 livers from DCD donors. If 12 DBD or 6 DCD

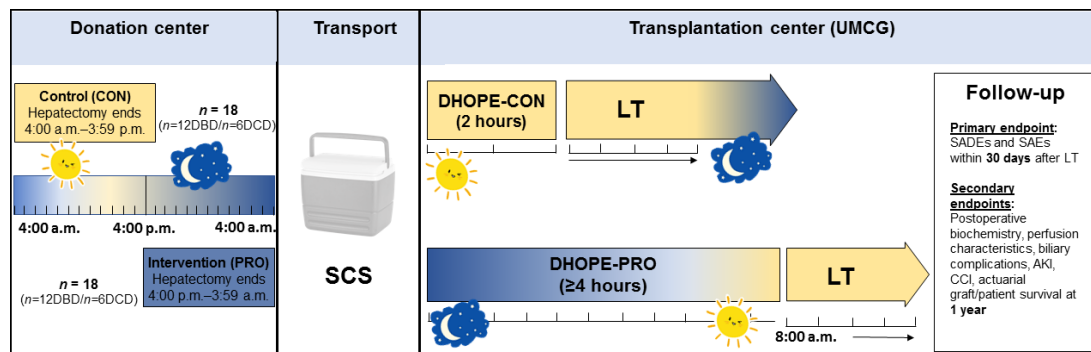


Figure 2 Study design. AKI, acute kidney injury; CCI, Comprehensive Complications Index; DBD, donation after brain death; DCD, donation after circulatory death; DHOPE-PRO, prolonged dual hypothermic oxygenated machine perfusion; DHOPE-CON, regular dual hypothermic oxygenated machine perfusion; LT, liver transplantation; SADE, serious adverse device event; SAE, serious adverse event; SCS, static cold storage; UMCG, University Medical Centre Groningen.



grafts are included in a study arm, inclusions for that group are stopped.

Blinding

Blinding of the transplant team and investigators is not possible because of the nature of the study, which includes a different timing of the transplant surgery in either group. However, the data safety monitoring board (DSMB) will provide SA(D)E adjudication for the primary safety endpoint, blinded for treatment assignment.

Organ allocation and procurement

Organs will be allocated in compliance with Eurotransplant regulations. Acceptance of a donor liver will follow current clinical practice based on position on the waiting list, size-match and clinical judgement by the transplant and hepatology staff, and will therefore not be influenced by participation in the trial.

Donor livers will be procured by one of the national multiorgan procurement teams. A standardised technique of in situ cold (4°C) flush via the aorta with at least 4 L of University of Wisconsin (UW) cold storage solution supplemented with 50 000 IU of heparin will be used. If possible, the donor liver is procured with a segment of 3–5 cm supratriuncal aorta left attached to the coeliac trunk. After procurement, the liver will be flushed with at least 1 L of UW cold storage solution through the portal vein until the effluent is clear. The cystic duct will be ligated, and the bile duct will be gently flushed with a syringe filled with UW cold storage solution.

In the receiving transplant centre, the donor liver will be procured on the backtable to dissect the caval vein, portal vein and hepatic artery, including the coeliac trunc and a supratriuncal segment of the aorta. Arterial reconstruction will be performed, if necessary. The gallbladder will be removed. Liver weights will be recorded. Cannulas (25 Fr, XVIVO, Groningen, The Netherlands) will be placed in the portal vein and the supratriuncal segment of the aorta. The liver will be flushed with 2 L of cold (4°C) UW machine perfusion solution 'PumpProtect' (Carnamedica, Warsaw, Poland), of which 1.5 L via the portal cannula and 0.5 L via the arterial cannula, just prior to connection to the perfusion device.

Investigational medical device

The Liver Assist (XVIVO, Groningen, The Netherlands) is a CE-marked machine perfusion device (European Union certification of safety, health and environmental requirements). The device enables perfusion of the liver via both the hepatic artery and the portal vein using two centrifugal pumps to provide a pulsatile and continuous flow, respectively. The system is pressure-controlled, which results in autoregulation of the flow through the liver. The temperature can be set from 8°C to 37°C. The system can be filled with any preservation solution. The organ is oxygenated by two hollow fibre membrane oxygenators providing oxygen to the perfusion fluid. The

oxygenators also ascertain removal of carbon dioxide from the perfusion fluid.

The disposable set of the Liver Assist is currently CE-marked for a usage time up to 6 hours based on normothermic perfusion using an oxygenated blood-based perfusate. In this study, machine perfusion is prolonged and may exceed 6 hours, but at hypothermic temperatures (10°C) and with an acellular solution. Use of the disposable set outside its intended use (>6 hours) was approved for this study by the national regulatory authorities (The Health and Youth Care Inspectorate) and approved by the METc.

The machine will be primed with 3 L of UW machine perfusion solution (Carnamedica, Warsaw, Poland). The perfusion pressure will be limited to a mean of 25 mm Hg for the hepatic artery and 5 mm Hg for the portal vein, based on previous preclinical and clinical studies.^{6 13} During the perfusion, pressures will be lowered to the lowest possible arterial and portal pressure to ascertain sufficient flows. A volumetric flow rate of at least 20 mL/min through the hepatic artery and 50 mL/min through the portal vein has to be maintained without exceeding the pressure limit. The temperature will be set at 10°C. The oxygen flow rate is set at 500 mL/min of 100% oxygen on both membrane oxygenators. During the perfusion, oxygen levels will be monitored every half hour to ensure adequate oxygenation throughout the entire perfusion period (partial oxygen pressure of at least 70 kPa).⁶ Arterial and portal venous volumetric flow rates are registered and the data is automatically stored by the device. No additional viability assessment is carried out during DHOPE, thus, all livers subjected to machine perfusion will be transplanted regardless of perfusion parameters. An organ perfusionist will be present during machine perfusion at all times. A bowl with sterile ice and UW preservation solution is located on a table with sterile drape near the perfusion device to allow a rapid transition to SCS preservation in case a device error causes the perfusion to stop. All organ perfusionist are trained to disconnect the liver from the machine and place the liver on ice (SCS) in a sterile fashion.

Liver grafts in the intervention group will undergo DHOPE-PRO (≥4 hours) until at least 08:00 hour the next morning (when the recipient procedure will be started), but should not be less than 4 hours. In the control group, the recipient surgery is started immediately and grafts will undergo regular (2 hours) DHOPE. In both groups, DHOPE will continue until the recipient hepatectomy is (near-) finished. This way, similar cold ischaemia times are ensured in both groups. Implantation and reperfusion of the graft will be performed as usual.

Sample collection and storage

Table 3 provides an overview of the study parameters collected per time point. Additional measurements specifically for this study are denoted in bold. Perfusion characteristics, such as volumetric flow rate, pressure and temperature, are noted at the start of machine

**Table 3** Study parameters, samples and biopsies collected per time point

	Baseline characteristics donor/recipient	Perfusion characteristics	Biopsies liver and bile duct	Preservation solution culture	Haemodynamic status recipient	Serum analysis recipient	Clinical follow-up
Before LT	X					X	
After SCS			X	X			
During machine perfusion		X					
At the end of machine perfusion		X	X	X			
During LT		X	X		X	X	
After reperfusion			X		X	X	
POD 0–10						X	X
POD 30						X	X
POM 3							X
POM 6							X
POM 9							X
POM 12						X	X

Additional measurements specifically for this study are denoted in bold.

LT, liver transplantation; POD, postoperative day; POM, postoperative month; SCS, static cold storage.

perfusion and every 15 min thereafter. Perfusate samples are taken before connection of the liver to the machine and every hour during machine perfusion thereafter. Blood gas analysis is performed every half hour during machine perfusion. A biopsy of the liver parenchyma and common bile duct will be taken before machine perfusion (after SCS) and at the end of machine perfusion. A sample from the SCS solution and from the perfusion fluid at the end of machine perfusion will be collected for microbial culture.

During liver transplantation, blood samples are routinely taken before incision, during the anhepatic phase, immediately after reperfusion, 30 min after reperfusion, and at the end of surgery. In addition, a biopsy of the liver parenchyma and common bile duct are routinely taken after reperfusion. No additional blood samples or biopsies are collected in the context of this clinical trial. After liver transplantation, patients are routinely monitored during their hospital stay, and subsequently at routine visits (1, 3, 6, 9 and 12 months post-transplantation). Blood samples will be collected to determine levels of liver transaminases, alkaline phosphates, gamma-glutamyl transferase, total bilirubin, international normalised ratio, lactate dehydrogenase, creatinine and albumin. No additional blood samples will be collected for study purposes.

Liver parenchyma and bile duct biopsies are snap-frozen in liquid nitrogen and later stored in -80°C , as well as stored in formalin and later embedded in paraffin for histological analysis. Perfusate and serum samples are centrifuged and the supernatant is stored in -80°C .

Statistics

The primary safety endpoint, a composite of the rate of SADEs and SAEs, will be presented as a percentage (proportion), mean, and actual numbers. Also, for each group, a 95% CI for the mean based on the t-distribution will be presented. A 95% CI based on the t-distribution will be presented for the difference in means between the two groups. The χ^2 test will be applied to test for significant differences. The primary feasibility endpoint, the rate of patients who have successfully received the intervention, will be presented as a percentage (proportion) and actual numbers will be provided. The secondary endpoints including postoperative (biliary) complications and acute kidney injury will be presented as a percentage, mean, and actual numbers, and compared with the χ^2 test. For each group, a 95% CI for the mean based on the t-distribution will be presented. A 95% CI based on the t-distribution will be presented for the difference in means between the two groups. The aforementioned primary and secondary endpoints will also be presented as risk ratios with 95% CI. Kaplan-Meier curves will be used to graphically depict graft and patient survival, and the groups will be compared using the log-rank test. Fixed sequence testing will be used for the secondary endpoints, eliminating the need for adjustment for multiplicity. The secondary endpoints will be tested in the order as presented above. Predetermined subgroup analyses (stratification) will be performed for different graft types (ie, DBD vs DCD). The primary and secondary endpoint analysis will be performed using IBM SPSS Statistics V.23.

Safety considerations

All AEs reported by the subject, or observed by the investigator, or staff will be recorded in the case report form (CRF). The investigator will report all SAEs to the primary investigator without undue delay after obtaining knowledge of the event. SAEs will be reported through the web portal 'ToetsingOnline' to the accredited METc within 7 days of first knowledge for SAEs resulting in death or are life-threatening followed by a period of maximum 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after first knowledge of the event. SAEs should be reported in the same timespan as SAEs.

Data monitoring and trial safety stopping rule

A case-by-case analysis was performed by a designated DSMB for the first 6 subjects in the intervention group. These patients were evaluated up to 2 weeks after liver transplantation, meaning that inclusions for the intervention group were temporarily halted during that period. The trial may be terminated prematurely due to (1) unacceptable safety concerns, such as repeat technical difficulties with machine perfusion or a significant amount of (S)AEs or (S)ADEs in the included patients, or (2) in case new external information arised that convincingly answered the study question or raised serious safety issues. An interim analysis of the first six subjects in the intervention group was performed by the DSMB and presented to the METc. On 3 November 2021, the METc concluded that the trial could be continued without the case-by-case analysis and 2-week stopping rule, and an amendment to the protocol was granted to also include patients listed for retransplantation. After completion of the trial, the DSMB will provide SA(D)E adjudication for the primary safety endpoint, blinded for treatment assignment.

In accordance to section 10, subsection 1, of the Dutch law 'Wet Medisch Wetenschappelijk Onderzoek (WMO)', the investigator will inform the subjects and the reviewing accredited METc if anything occurs on the basis of which 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 METc.

Monitor visits will be conducted by a monitor from the UMCG and take place before inclusion of the first patient, after inclusion of the first three patients and at the end of the study.

Consent

Informed consent will be obtained by the transplant surgeon, hepatologist or trial coordinator. Both the patient and one of the aforementioned contributors have to sign the informed consent form with a wet signature, named and dated.

Data access

The investigator will register all data for each patient in a CRF. The data on the CRF will be entered in the Research Electronic Data Capture system. Each participant will be assigned a unique number. Personal data will be stored separately from the study data. Subjects will be informed about data protection and that data will be pseudonymised. Encoded data will only be provided on request to authorised parties, such as the investigators, members of the health inspection, members of the METc and the study monitor. Study data and human material will be stored up to 15 years after collection. Research data will be handled with due observance of the Dutch Law for Protection of Personal data and the privacy statement of the centre.

Dissemination policy

The study results will be communicated to the physicians involved and will be released via publication. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

DISCUSSION

The introduction of machine perfusion as a way to preserve donor organs has been one of the most significant advances in the field of liver transplantation in the last decade. Our group and others have described end-ischaemic DHOPE to recharge cellular ATP levels already within 2 hours of machine perfusion,^{4 6 31} and favourable outcomes have been reported after transplantation of grafts preserved by (D)HOPE compared with SCS.^{6-8 22} Based on the results of the first RCT in the field of hypothermic machine perfusion, end-ischaemic DHOPE for 2 hours is currently being used as standard care for DCD livers in our country.⁷

The DHOPE-PRO trial aims to study the safety and feasibility of prolonged (>4 hours) DHOPE. Prolonged machine perfusion could be used globally to facilitate transplant logistics. Preclinical studies have shown feasibility of prolonged preservation by NMP for 24–86 hours.³²⁻³⁵ Recently, investigators from the transplant group in Zurich showed preserved liver function of discarded human livers up to 1 week using NMP.³⁶ We have recently shown successful preservation of porcine and discarded human livers using DHOPE for up to 24 hours.¹³ Prolonged preservation by DHOPE, compared with NMP, can be advantageous since the organ is maintained in a hypometabolic state with less production of waste products, such as urea, or coagulation proteins, reducing the need to make adjustments to the perfusate and minimising labour.^{37 38} Also, in case the perfusion system fails, the graft would still be preserved in SCS, minimising the risk of graft loss. Altogether, DHOPE-PRO is easier and may be a safer method to extend ex situ organ preservation time than prolonged NMP.



The primary safety endpoint of this study is a composite of the occurrence of (S)ADEs and (S)AEs during machine perfusion up to 30 days after liver transplantation. The primary feasibility endpoint is the proportion of patients who were assigned and successfully received a DHOPE-PRO-perfused liver graft. The reasons for selecting these endpoints are twofold. First, we sought to investigate safety and feasibility using the perfusion system outside its intended use (>6 hours). Although considered very unlikely, device errors (eg, pump failure) or deterioration of elements of the disposable set (eg, oxygenators) may occur, potentially leading to early termination of the perfusion. In case a device error arises during DHOPE, the organ will immediately be transferred to cold storage preservation. Second, DHOPE-PRO may cause damage to the graft leading to complications during machine perfusion (eg, increased hepatic resistance due to oedema) or after transplantation (eg, graft dysfunction). However, this risk is considered low. Vascular shear stress can be avoided by adjusting machine perfusion pressures to ≤ 5 mm Hg for the portal vein and ≤ 25 mm Hg for the hepatic artery.^{6 13}

The exclusion criteria are chosen to exclude patients whose condition can deteriorate within hours and, thus, for whom prolonged preservation of the donor graft is undesired. Therefore, subjects with a high-urgency status or MELD score >30 points are excluded for this study. Also, donors with untreated HIV, hepatitis B or C are excluded from participating. In the Netherlands, livers from DCD donors above 60 years of age are by protocol always resuscitated and tested by either in situ normothermic regional perfusion in the donor or ex situ end-ischaemic NMP.³⁹ Hence, grafts from DCD donors aged >60 years are excluded for this study.

Although we have designed our trial carefully, non-blinding of the transplant team for the intervention is a limitation. This may be accounted to logistical reasons as, in the case of DHOPE-PRO, transplantation is rescheduled for the next morning instead of during the night. However, the primary safety endpoint will be assessed by the adjudication committee, blinded for treatment assignment. The present trial also has some significant strengths. Livers in the intervention group, as well as in the control group, are subjected to DHOPE to resuscitate grafts prior to transplantation. In addition, the trial includes livers from both DCD and DBD donors. Even though the benefits of DHOPE are suggested to be most advantageous for livers from DCD compared with DBD donors, DHOPE-PRO may be beneficial to both DCD and DBD grafts. Finally, livers are pseudo-randomised based on the end of the donor hepatectomy time, which is independently determined by the off-site transplant coordinator at the donor hospital and not affected by the investigators.

At time of writing, eight livers have been assigned to the experimental group and ten livers to the control group, all for primary liver transplantations. We expect to finish including patients in September 2023.

In summary, we aim to study the safety and feasibility of DHOPE-PRO in order to schedule subsequent liver transplantation the following morning instead of during the night. DHOPE-PRO will be considered safe if we do not observe significantly more SADEs and/or SAEs during machine perfusion and up to 30 days after liver transplantation, compared with regular short-term DHOPE. DHOPE-PRO will be considered feasible if all patients who were assigned to the intervention group successfully received a DHOPE-PRO-perfused liver graft.

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Ethics approval This trial will be conducted in accordance with the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and according to the latest revised version of the WMO. The procedures set out in this study protocol are designed to ensure that the sponsor and investigator abide by the guidelines of Good Clinical Practice of the European Community (ICH topic E6, CPMP/ICH/135/95, Directive 2001/20/EC) and the Declaration of Helsinki in the conduct, evaluation and documentation of this study. The METc of the UMGc has approved this protocol (METc2020.126). Protocol modifications will be communicated with the METc and registered in the trial registry.

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