BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017558
Article Type:	Protocol
Date Submitted by the Author:	01-May-2017
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Primary Subject Heading:	Surgery
Secondary Subject Heading:	Intensive care
Keywords:	Hypothermic oxygenated machine perfusion, Donation After Brain Death,

	Extended Criteria Donor, HOPE, ECD, DBD

Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective randomized controlled trial (HOPE ECD-DBD)

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Running title: Hypothermic Oxygenated Perfusion (HOPE) of Human Liver Allografts from Extended Criteria Donors (ECD)

Keywords: Hypothermic oxygenated machine perfusion (HOPE), Donation After Brain Death (DBD), Extended Criteria Donor (ECD)

Trial Registration: clinicaltrials.gov (NCT03124641)

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ABREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BR	Bilirubin
CCI	Comprehensive complication index
CCS	Conventional cold storage
CE	Conformité européene (European conformity)
CK18	Circulating cytokeratine 18
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRF	Case report file
DBD	Donation after brain death
DCD	Donation after cardiac death
DGF	Delayed graft function
DSO	Deutsche Stiftung Organtransplantation (German Foundation for Organ Transplantation)
ECD	Extended criteria donor
ELISA	Enzyme-linked immunosorbent assay
ET	Eurotransplant
HOPE	Hypothermic oxygenated machine perfusion
HTK	Histidine-tryptophan-ketoglutarate solution
ICH-GCP	International conference on harmonisation-Good clinical practice
ICU	Intensive care unit
INR	International normalized ratio
I/R	Ischemia-reperfusion
MP	Machine perfusion
MPS	Machine perfusion solution
MWU	Mann-Whitney-U test
OLT	Orthotopic liver transplantation
PNF	Primary graft non-function
RCT	Randomized controlled trial
RT-PCR	Reverse transcription polymerase chain reaction
RWTH	Rheinisch-Westfälische Technische Hochschule
SD	Standard deviation
USA	United States of America
UW	University of Wisconsin

ABSTRACT

Introduction: Orthotopic liver transplantation (OLT) has emerged as the mainstay of treatment for end-stage liver disease. In an attempt to improve the availability of donor allografts and reduce waiting list mortality, graft acceptance criteria were extended increasingly over the decades. The use of extended criteria donor (ECD) allografts is associated with a higher incidence of primary graft non-function (PNF) and/or delayed graft function (DGF). As such, several strategies have been developed aiming at reconditioning poor quality ECD liver allografts. Hypothermic oxygenated machine perfusion (HOPE) has been successfully tested in pre-clinical experiments and in few clinical series of donation after cardiac death (DCD) OLT.

Methods and analysis: HOPE ECD-DBD is an investigator initiated, open-label, phase-II, prospective randomized controlled trial on the effects of HOPE on ECD-allografts in donation after brain death (DBD) OLT (HOPE ECD-DBD). Human whole organ liver grafts will be submitted to 1 hour of HOPE (n=23) via the portal vein before implantation and are going to be compared to a control-group (n=23) of patients transplanted after conventional cold storage (CCS). Primary (peak alanine aminotransferase-ALT) and secondary (aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, post-operative complications, duration of hospital and intensive care unit stay, 1-year patient- and graft survival) endpoints will be analyzed within a 12-month follow up. Extent of ischemia-reperfusion (I/R) injury will be assessed using liver tissue and serum samples taken during the perioperative phase of OLT.

Ethics and dissemination: The study was approved by the institutional review board of the RWTH Aachen University, Aachen, Germany (EK 049/17). First results are expected in 2018.

Trial registration number: NCT03124641 (clinicaltrials.gov)

ARTICLE SUMMARY

Strengths and limitations of this study:

- HOPE ECD-DBD is the first randomized controlled trial investigating the specific effects of HOPE on ECD organs in DBD transplantation.
- Tissue and blood samples, retrieved for translational research, will enable an in-depth analysis of the effects of HOPE on ischemia-reperfusion injury and inflammation.
- The stratified randomization model will allow us to achieve a homogenous distribution of patients between treatment groups based on prognostic variables.
- Open-label design is considered as a limitation of the study. Owing to the nature of the surgical procedure, it is not possible to blind the surgical team for the group allocation.

INTRODUCTION

Since Thomas Starzl`s pioneering efforts in 1963, orthotopic liver transplantation (OLT) has evolved as the standard therapy for patients with end-stage liver disease [1]. In 2015, approximately 1500 patients were listed for liver transplantation in Germany with only 894 transplants performed due to organ shortage [2]. Several strategies for donor pool expansion are being pursued concurrently. These include the use of living donors, splitting of cadaveric livers for two recipients, and the use of extended criteria donor (ECD) allografts for OLT. These ECD-allografts, however, exhibit poor tolerance to ischemia-reperfusion (I/R) injury, an important cause of liver damage. As such, I/R-injury is the underlying cause of graft dysfunction in ECD-allografts and negatively affects the process of liver regeneration in surgical conditions including hepatic resections and OLT [2].

Accordingly, several strategies have been developed aiming at reconditioning poor quality ECD-allografts [3]. Hypothermic oxygenated machine perfusion (HOPE) has been tested intensively in pre-clinical animal experiments [3]. The positive effects of HOPE in this setting have been demonstrated among others to reduce the incidence of biliary complications, mitochondrial damage and level of cellular energy-status [3]. In the clinical setting, hypothermic oxygenated organ perfusion is performed in the transplant center shortly before the actual implantation using an extra-corporal organ perfusion system with full oxygen saturation over the portal vein [3]. The first clinical study with HOPE was recently published by Dutkowski et al. in a cohort of patients undergoing OLT with donation after cardiac death (DCD) allografts [4]. In donation after brain death (DBD), the only legally accepted approach for organ donation in Germany and many other European countries, HOPE and its effect on early graft function and post-operative complications has not been reported yet [2].

The purpose of this study is to test the effects of HOPE in a prospective randomized clinical trial (RCT) on ECD liver allografts in DBD liver-transplantation compared to conventional cold storage (HOPE ECD-DBD). Primary (peak alanine aminotransferase-ALT) and secondary (peak aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, post-operative complications, hospital stay, graft- and patient survival) endpoints are going to be analyzed. I/R-injury and biomarkers of HOPE induced subcellular responses will be assessed using liver tissue and serum samples during the perioperative phase of OLT. Follow-up is determined at 12 months.

METHODS AND ANALYSIS

Study type

HOPE ECD-DBD is an investigator initiated, open-label, phase-II, prospective randomized controlled trial on ECD-allografts in DBD OLT. Figure 1 summarizes the trial design. The study protocol was written in accordance to the CONSORT- and SPIRIT-recommendations.

Eligibility

Patients above 18 years of age, suffering from end stage-liver disease and/or malignant liver tumors, listed for OLT and receiving ECD organs at the Department of Surgery and Transplantation, University Hospital RWTH Aachen, Aachen, Germany are eligible for the study. Informed consent is obtained from all subjects participating in the trial by a qualified member of the study team in the outpatient clinic. Potential study participants on the OLT-waiting list will be screened for defined in- and exclusion criteria and will be enrolled accordingly (Figure 1). Allocation of liver allografts follows national (Deutsche Stiftung Organtransplantation; DSO) and European (Eurotransplant; ET) policies. The study will not influence the allocation procedure or cause any delay in the actual implantation procedure.

Inclusion Criteria

Inclusion criteria are:

- Signed informed consent
- Patients suffering from end stage-liver disease and/or malignant liver tumors
- Listed for OLT
- Receiving ECD-allografts

Exclusion Criteria

Exclusion criteria are:

- Recipients of split or living donor OLT
- Previous OLT
- Participation in other liver related trials

- The subject received an investigational drug within 30 days prior to inclusion
- The subject is unwilling or unable to follow the procedures outlined in the protocol
- The subject is mentally or legally incapacitated
- Non-German or non-English speakers
- Family members of the investigators or employees of the participating department

Randomization

Randomization is performed by the principal investigator with an online randomizing tool for clinical trials (www.randomizer.at) at the time of admission for OLT [5, 6]. A stratified randomization model will be used to ensure balance of prognostic variables between the treatment groups. Stratums (1, Donor age: 50-65 years & expected cold ischemic time: < 9 hours; 2, Donor age: over 65 years & expected cold ischemic time: < 9 hours; 3, Donor age: 50-65 years & expected cold ischemic time: > 9 hours; 4, Donor age: over 65 years & expected cold ischemic time: > 9 hours) were created based on donor age and predicted cold ischemic time as prognostic variables.

Organ procurement, ECD criteria

All ECD liver-allografts will be retrieved by ET and DSO procurement teams within the ET network. Following cross-clamping (*in situ* flushing of the abdominal organs and begin of cold ischemia time), allografts will be removed and transported in a standardized fashion of conventional cold storage (CCS) on packed ice (4-6° C). Required data of the donor and the organ will be registered on a standard ET data sheet and will be transferred to a designated case report file (CRF).

Definition of ECD for the present study was adopted from the recommendations of the German Medical Chamber (Bundesaerztekammer) [7].

- Donors 60 years of age and older; or 50 years and older with cause of death other than trauma

- Intensive care therapy of the donor was required before donation for at least 5 days
- Obesity of the donor with a Body Mass Index > 30 kg/m²
- Fatty liver (with histology) > 40 %
- Serum-Sodium > 165 mmol/l
- Serum AST or ALT > 3 x upper limit of normal
- Serum-Bilirubin > 2 mg/dl
- Other risk factors: alcohol abuse in medical history; use of potentially hepatotoxic drugs; longer than 6 hours of expected cold ischemia if any of the above is also present

HOPE versus CCS

The present RCT comprises two groups, a perfusion (group 1; HOPE) and a control conventional cold storage (group 2; CCS) group. Patients on the waiting list for OLT with proven written consent will be recruited.

In case of randomization to group 1, HOPE will be applied to the allograft in the operation room after regular organ procurement, transport and back table preparation (Figure 2) according to a protocol previously described by Dutkowski et al. for DCD liver transplantation [4]. The application of HOPE (Liver Assist®; Organ Assist b.v., Groningen, The Netherlands) to the liver allograft will not delay the implantation. We will use machine-perfusion approved Belzer solution (Belzer MPS® UW Machine Perfusion Solution, Bridge to Life, London, United Kingdom) as perfusate. HOPE will be applied through the portal vein for 1 hour, with a perfusion rate of 0.1 ml/g liver /min in a pressure-controlled system with 2-3 mm Hg. The 4-6 °C perfusate (3-4 L) will be re-circulated. Perfusate will be oxygenized with a pO2 of 60-80 kPa by an oxygenator included as disposable in the setup. Storage, management and use of the above mentioned medical products will be performed according to the manufacturers guidelines. Patients in the CCS control group will undergo the clinically routine procedure with CCS using HTK solution (Custodiol®, Dr. Franz Köhler Chemie, Bernsheim, Germany) according to our institutional protocols.

Orthotopic liver transplantation (OLT)

OLT will be performed using the total-cava replacement technique with veno-venous bypass including the porto-mesenteric vascular bed using a roller pump system with an adjustable flow as described previously [8, 9]. Biliary reconstruction will be performed as a side-to-side common bile duct anastomosis with T-tube insertion or primary Roux-en-Y hepaticojejunostomy in cases of primary sclerosing cholangitis as previously described [10].

Sample collection and storage

I/R-injury will be assessed using liver tissue samples taken upon arrival of the organ (before HOPE or corresponding cold-storage) and at the end of implantation before closure of the abdomen, to evaluate the amount of I/R-injury (0-3 hours, Figure 3). In total, two excision biopsies (2 cm^3) will be harvested from the ECD liver allograft (segment III). Blood samples are collected as part of the daily routine during the peri- and postoperative course of OLT (Figure 3). Blood parameters of liver tissue damage and/or function (serum AST, ALT, BR, albumin, INR) as well as kidney damage and/or function (creatinine, urea, glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation) will be monitored. An additional 20 ml blood will be drawn on hospital-admission and on post-operative days 1, 2, 3, and 7 (POD 1, POD 2, POD 3, and POD 7) and will be used for translational research (Figure 3). All liver tissue and serum samples will be directly snap-frozen in liquid nitrogen (-80°C) and stored for 12 months after completion of the trial.

Post-operative care and immunosuppression

All patients are treated in accordance with the clinical routine of our institution for OLT recipients. Apart for the *ex vivo* allograft perfusion (group 1, HOPE), patients will be treated according to standard operating procedures for perioperative medical, interventional and surgical OLT management. Patients after OLT will be monitored at the intensive care unit (ICU) during the early postoperative phase and later, depending on their individual recovery, transferred to regular ward.

The used immunosuppressive regimen is based on induction therapy with intravenous basiliximab and methylprednisolone followed by corresponding oral doses of prednisolone, tacrolimus, and mycophenolate mofetil [8, 9].

Study endpoints

Primary endpoints

Early graft function as assessed by peak alanine aminotransferase-ALT in DBD-OLT using ECD allografts undergoing HOPE or CCS during the first 7-days post-OLT.

Secondary endpoints

1. Incidence of post-operative complications as assessed by the Clavien-Dindo complication score and the comprehensive complication index (CCI) [11]
2. Further laboratory parameters such as serum AST, BR, INR, platelet count, albumin, creatinine, urea, glomerular filtration rate using the CKD-EPI equation
3. Duration of intensive care stay
4. Duration of hospital stay
5. One-year recipient- and graft survival
6. Biomarkers of I/R-injury and inflammation as translational aspects of the project

Power of the study

A sample size of 23 patients per group (Σn=46) was calculated with the G*Power software (Heinrich-Heine-University, Düsseldorf, Germany) using the following settings: α=0.05; 1-β(power)=0.8; 2-sided t-test, including 15% drop-out and invalid data; peak AST and ALT levels 48 hours post-transplantation. Power calculation was performed based on the previous data from Guarrera et al. [12]. A reduction of 65% (AST) – 59% (ALT) is expected in the mean peak transaminase levels following machine perfusion treatment (peak AST: MP, 1154 ± 355.5 SD IU/mL, CCS, 3339 ± 3376.9.1 SD IU/mL; peak ALT: MP, 560.0 ± 355.5 SD IU/mL, CCS, 1358 ± 1208.4 SD IU/mL) [12].

Data collection and statistics

All collected data are documented on CRFs and considered as source data. Members of the study team document the required information into the CRF system following previous training. A study database will be created based on the CRFs and data correction, record keeping, archiving and subsequently the destruction of study documents will be performed according to the ICH-GCP guidelines. Subjects will be informed about data protection and that data will be pseudonymized. Furthermore, data will be handed out to third party only anonymized. Encoded data will only be provided to authorized persons (clinical monitor, authorized study staff, authorities, institutional review board). The study will be prematurely terminated for an individual subject in case of study related complications or if the subject withdraws informed consent. Values of p-value less than 0.05 will be considered significant. Unpaired t-test and Mann-Whitney-U test are going to be applied in case of normally and non-normally distributed data, respectively. For time course analysis of laboratory parameters two-way analysis of variance is applied. Postoperative complications are assessed by the Fischer's exact test. For comparisons between Kaplan-Meier curves of one-year graft and patient survival the log rank test is used.

Safety considerations

In the present study, solely certified medical products (CE certification) will be used. Blood draws and liver biopsies during the transplant procedure are performed according to the clinical routine at our institution. Thus, no relevant study related risks as well as no additional burden for the subjects are expected. Regular monitoring will be performed by the Clinical Trial Center Aachen (CTC-A, RWTH Aachen, Aachen, Germany) An interim analysis will be performed as soon as 12 patients per randomized group is reached. Data will be analyzed by an independent monitoring committee. The trial will be terminated immediately if one of the following criteria is fulfilled; Significantly higher serum ALT levels ($p<0.001$ using Student's t-test) in the HOPE group compared to the CCS group (Efficacy). The proportion of Grade ≥ III complications is significantly higher ($p<0.05$, Fischer's exact test) in the HOPE group when compared to the CCS group (Safety).

Ethics

This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, and good clinical practice guidelines (ICH-GCP) as well as all national legal and regulatory requirements. The institutional review board of the University RWTH Aachen has approved the study (EK 049/17). Members of the study team have completed a course in good clinical practice as certified by the German Medical Chamber. The trial was registered on clinicaltrial.gov on 20.03.2017 (NCT03124641).

Ischemia-reperfusion injury and inflammation

I/R-injury, depleted energy reserves, and oxidative stress are playing an important role in early graft dysfunction following liver transplantation of ECD liver grafts [3, 4, 13-17]. The translational research aim of the present study is to determine the effects of HOPE on I/R-injury, inflammation and energy household on human liver ECD-allografts. Blood and liver tissue samples will be used to measure various parameters of inflammation (Interleukins, Tumor necrosis factor-alpha, Macrophage Migration Inhibitory Factor) [18, 19], hepatocyte cell death (e.g., circulating cytokeratin 18 [CK18] fragments like M30 or M65) [20], energy- (Adenosine triphosphate levels) [21], and redox-household (Hemoxygenase-1, Malondialdehyde) [19]. Luminometry, Spectrophotometry, Luminex-assay, ELISA, RT-PCR and Western blot will be used for these analyses. Proteomics and metobolomics analysis will be performed on paired liver tissue samples to potentially identify early mediators of HOPE mediated organ protection.

DISCUSSION

Liver transplantation is the treatment of choice for patients with end-stage liver disease. The need to obtain the optimal benefit from a limited number of organs that are available has prompted the expansion of allograft selection criteria (ECD-allografts) aiming at increasing the donor pool and decrease overall waiting list mortality. ECD-allografts exhibit poor tolerance to I/R-injury, a syndrome initiated upon restoration of blood supply after cold and warm ischemia yielding in endothelial and Kupffer cell swelling, vasoconstriction, white blood cell infiltration, and sinusoidal platelet aggregation.

Technical innovations have been introduced to overcome these difficulties of ECD-allograft usage in OLT [17]. The implementation of machine perfusion for liver allografts was one of the most promising innovations in organ preservation over the last decade [17]. *In vivo* and *ex vivo* machine perfusion of liver allografts has been tested intensively in pre-clinical studies [3], and the beneficial effects of HOPE have been demonstrated among others to reduce the incidence of biliary complications, the degree of mitochondrial damage and the level of cellular energy-status [3]. In a recent international-matched case analysis, Dutkowski et al. demonstrated for the first time that HOPE treatment of DCD-allografts significantly decreased graft injury compared to matched CCS-livers regarding peak ALT levels (1239 vs. 2065 U/l, p = 0.02), intrahepatic cholangiopathy (0% vs. 22%, p = 0.015) and overall biliary complications (20% vs. 46%, p = 0.042), thus concluding that HOPE seems to offer beneficial effects in DCD-allograft preservation [4]. In DBD, the only legally accepted approach for organ donation in Germany and many other countries, HOPE and its effect on graft function and postoperative complications has not been reported yet [2]. Currently, we identified four active clinical trials on clinicaltrials.gov. Two trials are non-randomized observational studies both with the enrolment of 10 patients for machine perfusion, using different perfusion systems (NCT03098043; NCT03031067). One trial (NCT02584283) is a multi-center RCT investigating the effects of portal vein perfusion only versus portal- and arterial perfusion during HOPE and its effects on biliary complications in DCD transplantation (Table 1). The second RCT by Dutkowski et al. (NCT01317342) is recruiting patients in DBD transplantation, however, also including non-ECD organ thus appearing less suitable to investigate the specific effects of HOPE in ECD-allografts (Table 1).

Although we have designed our trial carefully, non-blinding of the transplant

team for the treatment groups is a limitation. This may be accounted to the nature of the surgical procedure and to pragmatic reasons, as HOPE and back-table preparation of the allograft are usually performed in the same operating room as the OLT procedure itself. The present trial has also some specific strengths; Firstly, HOPE ECD-DBD focuses on patients solely receiving ECD-allografts, a population we anticipate the best cost/benefit ratio from the utilization of HOPE. Secondly, donation after brain death is the most frequent source of ECD-allografts in Europe. Thirdly, we use a stratified randomization model that allows us to achieve a homogenous distribution based on prognostic variables of patients between the groups. Lastly, the results of the translational part of this study may deliver novel insights on the underlying subcellular effects of HOPE in human allografts.

DISSEMINATION PLAN

Results of the HOPE ECD-DBD trial will be presented at national and international scientific meetings, and published in international peer-reviewed medical journals. First results of the trial are expected in 2018.

AUTHORS' CONTRIBUTION

The initial study concept was derived from the initiating investigator study group (GL, ZC, UPN, RT, IA, JB). GL and ZC drafted the manuscript. ZC and GL performed the sample size estimation. All other authors participated in designing the study and are local investigators at the University Hospital RWTH Aachen. All authors were involved in revising the manuscript and approved the final version.

FUNDING STATEMENT

This research project is supported by the START-Program of the Faculty of Medicine, RWTH Aachen (691739, ID: 136/17).

COMPETING INTEREST STATEMENT

The authors have no competing interest to declare.

ETHICS APROVAL

Institutional review board of the RWTH Aachen, Aachen, Germany. Approval number: EK 049/17.

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FIGURE LEGENDS

Figure 1: Study flowchart

ECD, extended criteria donor; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; CRF, case report file; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BR, bilirubin; INR, international normalized ratio; GFR, glomerular filtration rate

Figure 2: Hypothermic oxygenated machine perfusion

First the donor organ is retrieved and transported to the transplant center on conventional ways. In the transplant center the liver is connected to the Liver Assist device and HOPE is performed. Hypothetically, organ reconditioning with HOPE triggers multiple protective responses leading to decreased oxidative stress, improved energy reserves, reduced cell-death.
ET, Eurotransplant; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; ATP, Adenosine triphosphate; ROS, reactive oxygen species. Adopted from: Schlegel et al. [14]

Figure 3: Interventions and study visits

Arabic number are representing the single study visits. Visit 1: screening, enrolment; Visit 2: admission; Visits 3, 4, 5: post-operative days 1st, 2nd, 3rd; Visit 6: 7th post-operative day; Visit 7: discharge; Visit 8: 6 months follow-up; Visit 9: 12 months follow-up, final visit
HOPE, hypothermic oxygenated machine perfusion

Table 1: Active RCTs on HOPE in OLT on clinicaltrials.gov (search date: 28th of April 2017)

Trial number	Study center	Study type	Enrolment	Donor group	Primary endpoint	Comment
NCT03124641*	RWTH Aachen University, Aachen, Germany	RCT	46	ECD-DBD	Early graft function (peak ALT level)	Recruiting
NCT01317342	University of Zurich, Zurich, Switzerland	RCT	70	DBD (ECD subgroup analysis only)	Early graft function (peak ALT level)	Recruiting
NCT02584283	University of Groningen, Groningen, Netherlands	RCT	156	DCD (Maastricht category III)	Incidence of symptomatic non-anastomotic biliary strictures (NAS)	Recruiting

ALT, alanine aminotransferase; ECD, extended criteria donation; DBD, donation of the brain death; DCD, donation of the cardiac death; HOPE, hypothermic oxygenated machine perfusion; NAS, non-anastomotic biliary strictures; OLT, orthotopic liver transplantation; RCT, randomized controlled trial; RWTH, Rheinisch-Westfälische Technische Hochschule

*the present trial

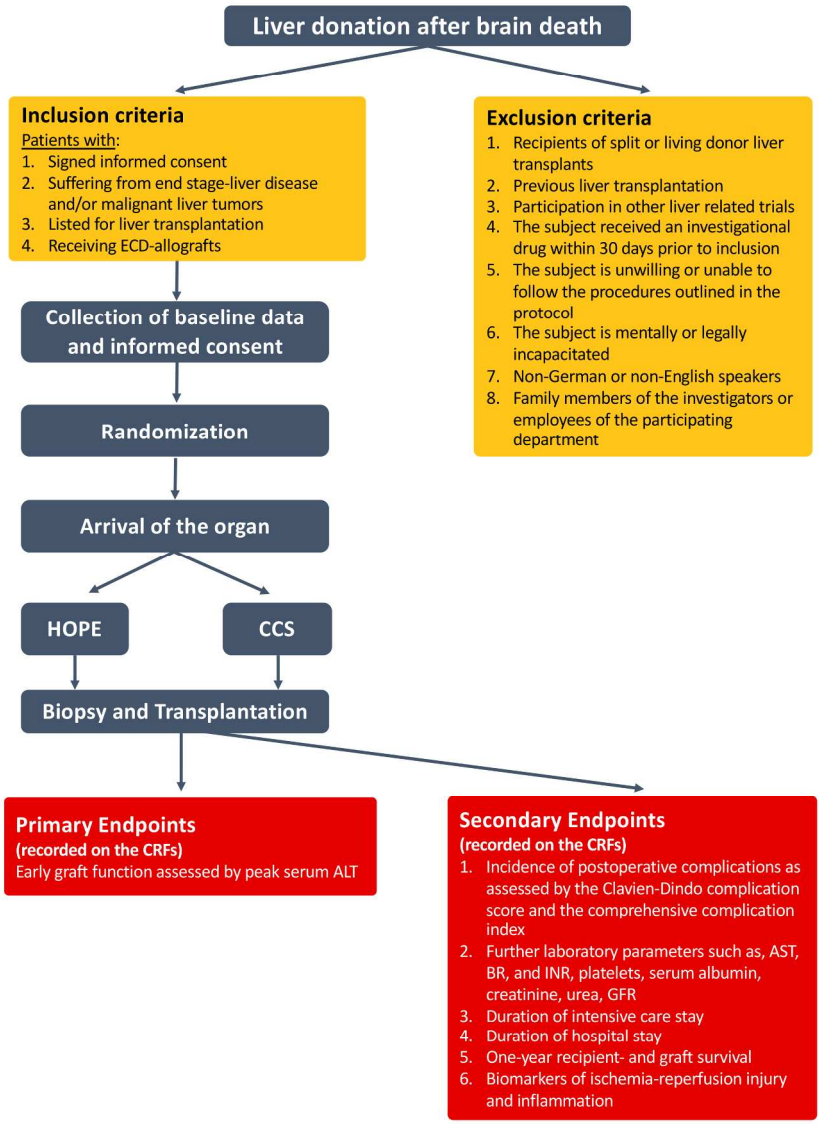

Figure 1

201x274mm (300 x 300 DPI)

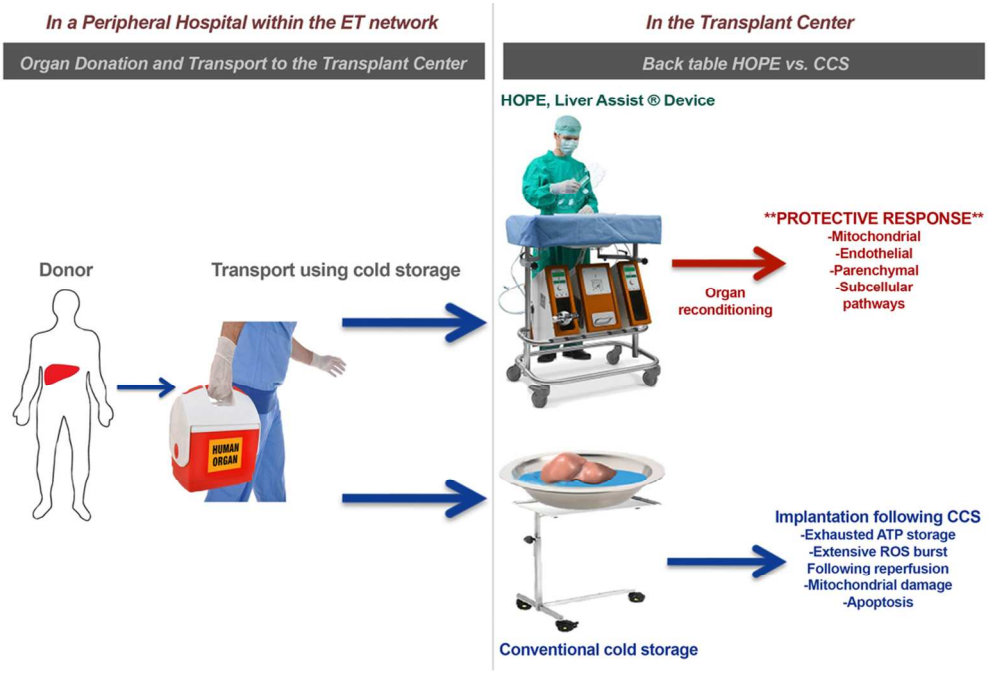

Figure 2

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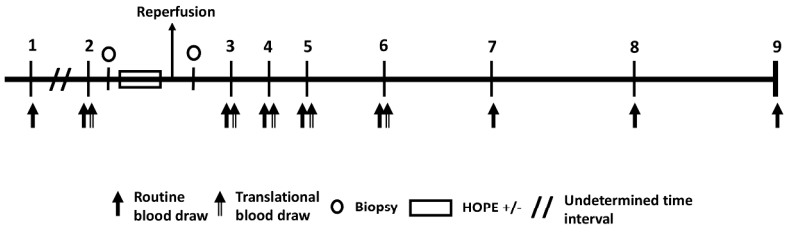

Figure 3

270x99mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	P1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	P3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	P4
	2b	Specific objectives or hypotheses	P4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	P5, P9
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	P5-6
	4b	Settings and locations where the data were collected	P8, P10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	P7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	P9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n.a.
Sample size	7a	How sample size was determined	P9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	P10
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	P6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	P6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	P6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	P6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	n.a.

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	n.a.
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	P10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	n.a.
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	n.a.
	13b	For each group, losses and exclusions after randomisation, together with reasons	n.a.
Recruitment	14a	Dates defining the periods of recruitment and follow-up	n.a.
	14b	Why the trial ended or was stopped	n.a.
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	n.a.
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	n.a.
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	n.a.
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n.a.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	n.a.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	n.a.
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	P12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	n.a.
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	n.a.
Other information			
Registration	23	Registration number and name of trial registry	P3
Protocol	24	Where the full trial protocol can be accessed, if available	n.a.
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	P13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective multicenter randomized controlled trial (HOPE ECD-DBD)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017558.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Jul-2017
Complete List of Authors:	Czigany, Zoltan; University Hospital RWTH Aachen, Department of Surgery and Transplantation Schöning, Wenzel; University Hospital RWTH Aachen, Department of Surgery and Transplantation Ulmer, Tom Florian; University Hospital RWTH Aachen, Department of Surgery and Transplantation Bednarsch, Jan; University Hospital RWTH Aachen, Department of Surgery and Transplantation Amygdalos, Iakovos; University Hospital RWTH Aachen, Department of Surgery and Transplantation Cramer, Thorsten; University Hospital RWTH Aachen, Department of Surgery and Transplantation Rogiers, Xavier; Ghent University Hospital and Medical School Popescu, I; Fundeni Clinical Institute, Bucharest - Romania; Fundeni Clinical Institute Botea, Florin; Institutul Clinic Fundeni, Department of General Surgery and Liver transplantation Froněk, Jiří ; Institute for Clinical and Experimental Medicine, Department of Transplantation Surgery Kroy, Daniela; University Hospital RWTH Aachen, Department of Medicine III Koch, Alexander; University Hospital RWTH Aachen, Department of Medicine III Tacke, Frank; University Hospital RWTH Aachen, Department of Medicine III Trautwein, Christian; University Hospital RWTH Aachen, Department of Medicine III Tolba, Rene; University Hospital RWTH Aachen, Institute for Laboratory Animal Science and Experimental Surgery Hein, Marc; University Hospital RWTH Aachen, Department of Anesthesiology Dejong, Cornelis; University Hospital RWTH Aachen, Department of Surgery and Transplantation; Maastricht University Medical Centre (MUMC) Neumann, Ulf Peter; University Hospital RWTH Aachen, Department of Surgery and Transplantation; Maastricht University Medical Centre (MUMC), Department of Surgery Lurje, Georg; University Hospital RWTH Aachen, Department of Surgery

	and Transplantation
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Intensive care
Keywords:	hypothermic oxygenated machine perfusion, donation after brain death, extended criteria donor

Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective multicenter randomized controlled trial (HOPE ECD-DBD)

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Running title: Hypothermic Oxygenated Perfusion (HOPE) of Human Liver Allografts from Extended Criteria Donors (ECD)

Keywords: Hypothermic oxygenated machine perfusion (HOPE), Donation After Brain Death (DBD), Extended Criteria Donor (ECD)

Trial Registration: clinicaltrials.gov (NCT03124641)

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ABREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BR	Bilirubin
CCI	Comprehensive complication index
CCS	Conventional cold storage
CE	Conformité européene (European conformity)
CK18	Circulating cytokeratine 18
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRF	Case report file
DBD	Donation after brain death
DCD	Donation after cardiac death
DGF	Delayed graft function
DSO	Deutsche Stiftung Organtransplantation (German Foundation for Organ Transplantation)
ECD	Extended criteria donor
ELISA	Enzyme-linked immunosorbent assay
ET	Eurotransplant
GST	Gluthation S-Transferase
HOPE	Hypothermic oxygenated machine perfusion
HTK	Histidine-tryptophan-ketoglutarate solution
ICH-GCP	International conference on harmonisation-Good clinical practice
ICU	Intensive care unit
INR	International normalized ratio
I/R	Ischemia-reperfusion
MP	Machine perfusion
MPS	Machine perfusion solution
MWU	Mann-Whitney-U test
OLT	Orthotopic liver transplantation
PNF	Primary graft non-function
RCT	Randomized controlled trial
RT-PCR	Reverse transcription polymerase chain reaction
RWTH	Rheinisch-Westfälische Technische Hochschule
SD	Standard deviation
USA	United States of America
UW	University of Wisconsin

ABSTRACT

Introduction: Orthotopic liver transplantation (OLT) has emerged as the mainstay of treatment for end-stage liver disease. In an attempt to improve the availability of donor allografts and reduce waiting list mortality, graft acceptance criteria were extended increasingly over the decades. The use of extended criteria donor (ECD) allografts is associated with a higher incidence of primary graft non-function (PNF) and/or delayed graft function (DGF). As such, several strategies have been developed aiming at reconditioning poor quality ECD liver allografts. Hypothermic oxygenated machine perfusion (HOPE) has been successfully tested in pre-clinical experiments and in few clinical series of donation after cardiac death (DCD) OLT.

Methods and analysis: HOPE ECD-DBD is an investigator-initiated, open-label, phase-II, prospective multicenter randomized controlled trial on the effects of HOPE on ECD-allografts in donation after brain death (DBD) OLT (HOPE ECD-DBD). Human whole organ liver grafts will be submitted to 1-2 hours of HOPE (n = 23) via the portal vein before implantation and are going to be compared to a control-group (n = 23) of patients transplanted after conventional cold storage (CCS). Primary (peak and Δ-peak alanine aminotransferase-ALT within 7 days) and secondary (aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, post-operative complications, early allograft dysfunction, duration of hospital and intensive care unit stay, 1-year patient- and graft survival) endpoints will be analyzed within a 12-month follow up. Extent of ischemia-reperfusion (I/R) injury will be assessed using liver tissue, perfusate, bile, and serum samples taken during the perioperative phase of OLT.

Ethics and dissemination: The study was approved by the institutional review board of the RWTH Aachen University, Aachen, Germany (EK 049/17). First results are expected in 2018.

Trial registration number: NCT03124641 (clinicaltrials.gov)

ARTICLE SUMMARY

Strengths and limitations of this study:

- HOPE ECD-DBD is a multicenter randomized controlled trial investigating the specific effects of HOPE on ECD organs in DBD transplantation.
- Tissue-, blood-, perfusate-, and bile-samples, retrieved for translational research, will enable an in-depth analysis of the effects of HOPE on ischemia-reperfusion injury and inflammation.
- The stratified randomization model will allow us to achieve a homogenous distribution of patients between treatment groups based on prognostic variables.
- Open-label design is considered a limitation of the study. Owing to the nature of the surgical procedure, it is not possible to blind the surgical team for the group allocation.

INTRODUCTION

Since Thomas Starzl`s pioneering efforts in 1963, orthotopic liver transplantation (OLT) has evolved as the standard therapy for patients with end-stage liver disease [1]. In 2015, approximately 1500 patients were listed for liver transplantation in Germany with only 894 transplants performed due to organ shortage [2]. Several strategies for donor pool expansion are being pursued concurrently. These include the use of living donors, splitting of cadaveric livers for two recipients, and the use of extended criteria donor (ECD) allografts for OLT. These ECD-allografts, however, exhibit poor tolerance to ischemia-reperfusion (I/R) injury, an important cause of liver damage. As such, I/R-injury is the underlying cause of graft dysfunction in ECD-allografts and negatively affects the process of liver regeneration in surgical conditions including hepatic resections and OLT [2].

Accordingly, several strategies have been developed, aiming at reconditioning poor quality ECD-allografts [3-7]. Hypothermic oxygenated machine perfusion (HOPE) has been tested intensively in pre-clinical animal experiments [3]. The positive effects of HOPE in this setting have been demonstrated among others to reduce the incidence of biliary complications, mitochondrial damage and level of cellular energy-status [3]. In the clinical setting, hypothermic oxygenated organ perfusion is performed in the transplant center shortly before the actual implantation using an extra-corporal organ perfusion system with full oxygen saturation over the portal vein [3]. The first clinical study with HOPE was recently published by Dutkowski et al. in a cohort of patients undergoing OLT with donation after cardiac death (DCD) allografts [8]. In donation after brain death (DBD), the only legally accepted approach for organ donation in Germany and many other European countries, HOPE and its effect on early graft function and post-operative complications has not been reported yet [2].

The purpose of this study is to test the effects of HOPE in a prospective multicenter randomized clinical trial (RCT) on ECD liver allografts in DBD liver-transplantation compared to conventional cold storage (HOPE ECD-DBD). As such, we hypothesize, that HOPE might have an organ reconditioning effect by substantially reducing early allograft injury in pre-damaged ECD organs. Primary (peak and Δ-peak alanine aminotransferase-ALT within 7 days) and secondary (e.g. peak aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, lactate, post-operative complications, hospital stay, graft- and patient

survival) endpoints are going to be analyzed. I/R-injury and biomarkers of HOPE induced subcellular responses will be assessed using liver tissue, perfusate, bile, and serum samples during the perioperative phase of OLT. Follow-up is set at 12 months.

METHODS AND ANALYSIS

Study type

HOPE ECD-DBD is an investigator initiated, open-label, phase-II, prospective multicenter randomized controlled trial on ECD-allografts in DBD OLT. Figure 1 summarizes the trial design. The study protocol was written in accordance to the CONSORT- and SPIRIT-recommendations.

Eligibility

Patients above 18 years of age, suffering from end stage-liver disease and/or malignant liver tumors, listed for OLT and receiving ECD organs at the Department of Surgery and Transplantation, University Hospital RWTH Aachen, Aachen, Germany and at other participating centers are eligible for the study. Informed consent is obtained from all subjects participating in the trial by a qualified member of the study team in the outpatient clinic. In each center, potential study participants on the OLT-waiting list will be screened for defined in- and exclusion criteria and will be enrolled accordingly (Figure 1). Allocation of liver allografts follows national (Deutsche Stiftung Organtransplantation; DSO, The Belgian Transplantation Society; BTS, Romanian National Transplant Agency; ANT) and/or European (Eurotransplant; ET) policies. The study will not influence the allocation procedure or cause any delay in the actual implantation procedure.

Inclusion Criteria

Inclusion criteria are:

- Signed informed consent
- Patients 18 years or older
- Patients suffering from end stage-liver disease and/or malignant liver tumors
- Listed for OLT
- Receiving ECD-allografts

Exclusion Criteria

Exclusion criteria are:

- Recipients of split or living donor OLT
- Previous OLT
- Combined transplantations (liver-kidney, liver-lung, etc.)
- Participation in other liver related trials
- The subject received an investigational drug within 30 days prior to inclusion
- The subject is unwilling or unable to follow the procedures outlined in the protocol
- The subject is mentally or legally incapacitated
- An inability to understand the procedures due to language barriers
- Family members of the investigators or employees of the participating department

Randomization

Randomization is performed at arrival of the allograft at the transplant center and after acceptance for transplantation, using an online randomizing tool for clinical trials (www.randomizer.at), either by the principal investigator or, in case of absence, by a trained member of the study team [9, 10]. A stratified randomization model will be used to ensure a balance of prognostic variables between the treatment groups. Expected cold ischemia time and graft steatosis were selected for stratification. A median cold ischemic time of 8 hours is based on our local OLT database (2011-2017) containing more than 320 OLTs. Therefore, 8 hours of expected cold ischemia time has been selected as a cut-off value for the present study. For steatosis, we use 30% macrovesicular steatosis as a cut-off value for the stratification process as defined by the NASH Clinical Research Network Scoring System and validated as prognostic marker in liver transplantation by several authors [11-14].

Organ procurement, ECD criteria

All ECD liver-allografts will be retrieved by ET and/or national procurement teams. Following cross-clamping (*in situ* flushing of the abdominal organs and begin of cold ischemia time), allografts will be removed and transported in a standardized

fashion of conventional cold storage (CCS) on packed ice (4-6° C). Required data of the donor and the organ will be registered in a standard manner and will be transferred to a designated case report file (CRF).

Definition of ECD based on the recommendations of the German Medical Chamber (Bundesaerztekammer) [15].

- Donors 65 years of age and older
- Intensive care therapy of the donor was required before donation for at least 7 days
- Obesity of the donor with a Body Mass Index > 30 kg/m²
- Fatty liver (with histology) > 40 %
- Serum-Sodium > 165 mmol/l
- Serum AST or ALT > 3 x upper limits of normal
- Serum-Bilirubin > 2 mg/dl

HOPE versus CCS

The present RCT comprises two groups, a perfusion (group 1; HOPE) and a control conventional cold storage (group 2; CCS) group. Patients on the waiting list for OLT with proven written consent will be recruited.

In case of randomization to group 1, HOPE will be applied to the allograft in the operating room after regular organ procurement, transport and back table preparation (Figure 2) according to a protocol previously described by Dutkowski et al. for DCD liver transplantation [8]. HOPE (Liver Assist®; Organ Assist b.v., Groningen, The Netherlands) will be applied through the portal vein for 1-(2) hour(s) (if recipient hepatectomy is prolonged, HOPE will be continued to avoid repeated static cold storage), with a perfusion rate of 0.1 ml/g liver /min in a pressured control system with 2-3 mm Hg. The 10 °C perfusate (3-4 L) will be re-circulated (Belzer MPS®, Bridge to Life, London, United Kingdom). Perfusate will be oxygenized with a pO2 of 60-80 kPa by an oxygenator included as disposable in the setup. Perfusion parameters registered by the device will be stored automatically and evaluated for possible patterns. Immediately prior to perfusion, grafts are flushed with machine perfusion solution to wash out the residual HTK perfusate. Storage, management and use of the above mentioned medical products will be carried out according to the manufacturer's guidelines. Patients in the CCS control group will undergo the

clinically routine procedure with CCS using HTK solution (Custodiol®, Dr. Franz Köhler Chemie, Bernsheim, Germany) according to institutional protocols.

Orthotopic liver transplantation (OLT)

In center 1, OLT will be performed using the total-cava replacement technique with veno-venous bypass including the porto-mesenteric vascular bed using a roller pump system with an adjustable flow as previously described [16, 17]. Biliary reconstruction will be performed as a side-to-side common bile duct anastomosis with T-tube insertion or primary Roux-en-Y hepaticojejunostomy in cases of primary sclerosing cholangitis as previously described [18]. External centers will use their local standard techniques for OLT.

Sample collection and storage

I/R-injury will be assessed using liver tissue samples taken upon arrival of the organ (before HOPE or corresponding cold-storage) and at the end of implantation before closure of the abdomen, to evaluate the amount of I/R-injury (Figure 3). In total, two excision biopsies (2 cm^3) will be harvested from the ECD liver allograft (segment III). Perfusate samples will be collected repeatedly during machine perfusion. Blood samples are taken as part of the daily routine during the peri- and postoperative course of OLT (Figure 3). Blood parameters of liver tissue damage and/or function (serum AST, ALT, BR, albumin, INR, alpha-GST, lactate) as well as kidney damage and/or function (creatinine, urea, glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation) will be monitored. An additional 20 ml blood will be drawn on hospital-admission and on post-operative days 1, 2, 3, and 7 (POD 1, POD 2, POD 3, and POD 7) and will be used for translational research (Figure 3). Bile samples can be easily collected from the T-Drain during the first 3 PODs (only applicable for center 1). All liver tissue, serum, perfusate and bile samples will be directly snap-frozen in liquid nitrogen (-80℃) and stored for 12 months after completion of the trial.

Post-operative care and immunosuppression

All patients are treated in accordance to our institution´s routine clinical algorithm for OLT recipients. Apart for the *ex vivo* allograft perfusion (group 1, HOPE), patients will be treated according to standard operating procedures for perioperative medical, interventional and surgical OLT management. Patients after OLT will be monitored at the intensive care unit (ICU) during the early postoperative phase and later, depending on their individual recovery, transferred to regular ward.

The used immunosuppressive regimen is based on induction therapy with intravenous basiliximab and methylprednisolone followed by corresponding oral doses of prednisolone, tacrolimus, and mycophenolate mofetil [16, 17].

Study endpoints

Primary endpoints

Early graft injury in DBD-OLT using ECD allografts undergoing HOPE or CCS, as assessed by peak alanine aminotransferase-ALT during the first 7-days post-OLT and by Δ Peak-ALT* [19].

Secondary endpoints

1. Incidence of post-operative complications as assessed by the Clavien-Dindo complication score and the comprehensive complication index (CCI) [20]
2. Further laboratory parameters such as serum AST, BR, INR, platelet count, albumin, creatinine, urea, lactate, glomerular filtration rate using the CKD-EPI equation, alpha gluthation S-Transferase (GST)
3. Early allograft dysfunction as defined by the Olthoff criteria (bilirubin >=10mg/dL on day 7, international normalized ratio >= 1.6 on day 7, and alanine or aspartate aminotransferases >2000 IU/L within the first 7 days) [21]
4. Duration of intensive care stay
5. Duration of hospital stay
6. One-year recipient- and graft survival
7. Analysis of serum, tissue, bile, and perfusate biomarkers as translational aspects of the project

*Δ Peak-ALT: To correct for an assumed washout effect of machine perfusion, besides the absolute values, relative changes of serum peak-ALT will be assessed [19]. Peak-ALT will be corrected to the values measured in the routine blood analysis after reperfusion at the time point of admission to the ICU.

Power of the study

A sample size of 23 patients per group (Σn=46) was calculated with the G*Power software (Heinrich-Heine-University, Düsseldorf, Germany) using the following settings: α=0.05; 1-β(power)=0.8; 2-sided t-test, including 15% drop-out and invalid data; peak AST and ALT levels 48 hours post-transplantation. Power calculation was performed based on the previous data from Guarrera et al. [22]. A reduction of 65% (AST) – 59% (ALT) is expected in the mean peak transaminase levels following machine perfusion treatment (peak AST: MP, 1154 ± 355.5 SD IU/mL, CCS, 3339 ± 3376.9.1 SD IU/mL; peak ALT: MP, 560.0 ± 355.5 SD IU/mL, CCS, 1358 ± 1208.4 SD IU/mL) [22].

Data collection and statistics

All collected data are documented on CRFs and considered as source data. Members of the study team document the required information into the CRF system following previous training. A study database will be created based on the CRFs and data correction, record keeping, archiving and subsequently the destruction of study documents will be performed according to the ICH-GCP guidelines. Subjects will be informed about data protection and that data will be pseudonymized. Furthermore, data will be handed out to third party only anonymized. Encoded data will only be provided to authorized persons (clinical monitor, authorized study staff, authorities, institutional review board). The study will be prematurely terminated for an individual subject in case of study related complications or if the subject withdraws informed consent. Values of p-value less than 0.05 will be considered significant. Unpaired t-test and Mann-Whitney-U test are going to be applied in case of normally and non-normally distributed data, respectively. For time course analysis of laboratory parameters two-way analysis of variance is applied. Postoperative complications are

assessed by the Fischer's exact test. For comparisons between Kaplan-Meier curves of one-year graft and patient survival the log rank test is used.

Analysis of endpoints will be performed by an independent committee in a blinded fashion (Institute for Medical Statistics, RWTH Aachen).

Safety considerations

In the present study, solely certified medical products (CE certification) will be used. Blood draws and liver biopsies during the transplant procedure are performed according to the clinical routine. Thus, no relevant study-related risks and no additional burden for the subjects are expected. Independent monitoring of data will be performed by the Clinical Trial Center Aachen (CTC-A, RWTH Aachen, Aachen, Germany) An interim analysis will be performed as soon as 12 patients are enrolled in each randomized group. The trial will be terminated immediately if one of the following criteria is fulfilled; Significantly higher serum ALT levels ($p<0.001$ using Student's t-test) in the HOPE group compared to the CCS group (Efficacy). The proportion of Grade ≥ III complications is significantly higher ($p<0.05$, Fischer's exact test) in the HOPE group when compared to the CCS group (Safety).

Ethics

This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, and good clinical practice guidelines (ICH-GCP) as well as all national legal and regulatory requirements. The institutional review board of the University RWTH Aachen has approved the study protocol, including consent form and patient information (EK 049/17). Members of the local study team have completed a course in good clinical practice as certified by the German Medical Chamber. The trial was registered on clinicaltrial.gov on 20.03.2017 (NCT03124641).

Study group

The study group of the HOPE ECD-DBD trial comprises the trial sponsor (GL, UPN, ZC) and the PI (GL) of the University Hospital RWTH Aachen (center 1), the local investigators in Ghent (center 2; XR), Bucharest (center 3; IP, FB) and Prague (center 4; JF). Each local investigator is in charge of the execution of the study and

collection of data. The trial sponsor is responsible for randomization, trial database, storage, statistical analysis and scientific writing. The trial will expectedly include the first patients (applicable for center 1) in Q3 of 2017. External centers are expected to start the recruitment phase until the end of 2017.

Ischemia-reperfusion injury and inflammation

I/R-injury, depleted energy reserves, and oxidative stress play an important role in early graft dysfunction following liver transplantation of ECD liver grafts [3, 4, 8, 23-26]. The translational research aim of the present study is to determine the effects of HOPE on I/R-injury, inflammation and energy household on human liver ECD-allografts. Blood and liver tissue samples will be used to measure various parameters of inflammation (Interleukins, Tumor necrosis factor-alpha, Macrophage Migration Inhibitory Factor) [27, 28], hepatocyte cell death (e.g., circulating cytokeratin 18 [CK18] fragments like M30 or M65) [29], energy- (Adenosine triphosphate levels) [30], and redox-household (Hemoxygenase-1, Malondialdehyde) [28]. Luminometry, Spectrophotometry, Luminex-assay, ELISA, RT-PCR and Western blot will be used for these analyses. Proteomics and metobolomics analysis will be performed on paired liver tissue samples to potentially identify early mediators of HOPE mediated organ protection.

Biochemical parameters of biliary epithelial cell function and injury (bilirubin, biliary pH, bicarbonate, biliary glucose, lactate dehydrogenase, alcalic phosphatase, gamma-GT) will be assessed using standard laboratory methods [19, 31].

Due to the scarcity of clinical data regarding the utilization of HOPE for quality assessment of liver grafts, it is still unclear, whether this method is applicable for such quality predictions. Therefore, we will use the perfusion fluid to assess certain parameters which might have a predictive value in evaluating graft quality under hyporthermic conditions (pH, lactate, PO2, PCO2, AST, ALT, LDH, GST liver fatty acid binding protein/L-FABP levels) [31-33].

DISCUSSION

Liver transplantation is the treatment of choice for patients with end-stage liver disease. The need to obtain the optimal benefit from a limited number of organs that are available has prompted the expansion of allograft selection criteria (ECD-

allografts) aiming at increasing the donor pool and decrease overall waiting list mortality. ECD-allografts exhibit poor tolerance to I/R-injury, a syndrome initiated upon restoration of blood supply after cold and warm ischemia yielding in endothelial and Kupffer cell activation, vasoconstriction, neutrophil infiltration, and sinusoidal platelet aggregation [34, 35].

Technical innovations have been introduced to overcome these difficulties of ECD-allograft usage in OLT [4]. The implementation of machine perfusion for liver allografts was one of the most promising innovations in organ preservation over the last decade [4]. *In vivo* and *ex vivo* machine perfusion of liver allografts has been tested intensively in pre-clinical studies [3], and the beneficial effects of HOPE have been demonstrated among others to reduce the incidence of biliary complications, the degree of mitochondrial damage and the level of cellular energy-status [3]. In a recent international-matched case analysis, Dutkowski et al. demonstrated for the first time that HOPE treatment of DCD-allografts significantly decreased graft injury compared to matched CCS-livers regarding peak ALT levels (1239 vs. 2065 U/l, p = 0.02), intrahepatic cholangiopathy (0% vs. 22%, p = 0.015) and overall biliary complications (20% vs. 46%, p = 0.042), thus concluding that HOPE seems to offer beneficial effects in DCD-allograft preservation [8]. In DBD, the only legally accepted approach for organ donation in Germany and many other countries, HOPE and its effect on graft function and postoperative complications has not been reported yet [2]. Currently, we identified four active clinical trials on clinicaltrials.gov using hypothermic oxygenated machine perfusion in liver transplantation. Two trials are non-randomized observational studies both with the enrolment of 10 patients for machine perfusion, using different perfusion systems (NCT03098043; NCT03031067). One trial (NCT02584283) is a multi-center RCT investigating the effects of portal- and arterial perfusion HOPE (dual-HOPE) versus cold storage on biliary complications in DCD transplantation (Table 1). The second RCT by Dutkowski et al. (NCT01317342) is recruiting patients in DBD transplantation, however, also including non-ECD organs thus appearing less suitable to investigate the specific effects of HOPE in ECD-allografts (Table 1).

Although we have designed our trial carefully, non-blinding of the transplant team for the treatment groups is a limitation. This may be accounted to the nature of the surgical procedure and to pragmatic reasons, as HOPE and back-table preparation of the allograft are usually performed in the same operating room as the

OLT procedure itself. The present trial has also some specific strengths; Firstly, HOPE ECD-DBD focuses on patients solely receiving ECD-allografts, a population we anticipate the best cost/benefit ratio from the utilization of HOPE. Secondly, donation after brain death is the most frequent source of ECD-allografts in Europe. Thirdly, we use a stratified randomization model that allows us to achieve a homogenous distribution based on prognostic variables of patients between the groups. Lastly, the results of the translational part of this study may deliver novel insights on the underlying subcellular effects of HOPE in human allografts.

DISSEMINATION PLAN

Results of the HOPE ECD-DBD trial will be presented at national and international scientific meetings, and published in international peer-reviewed medical journals. First results of the trial are expected in 2018.

AUTHORS' CONTRIBUTION

The initial study concept was derived from the initiating investigator study group (GL, ZC, UPN, RHT, IA, JB). GL and ZC drafted the manuscript. ZC and GL performed the sample size estimation. WS, TFU, TC, XR, IP, FB, JF, DK, AK, FT, CT, MH, CHCD participated in designing the study, preparing the revised protocol and are investigators at the University Hospital RWTH Aachen or at the external centers. All authors were involved in revising the manuscript and approved the final version.

FUNDING STATEMENT

This research project is supported by the START-Program of the Faculty of Medicine, RWTH Aachen (691739, ID: 136/17).

COMPETING INTEREST STATEMENT

The authors have no competing interest to declare.

ETHICS APROVAL

Institutional review board of the RWTH Aachen, Aachen, Germany. Approval number: EK 049/17.

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FIGURE LEGENDS

Figure 1: Study flowchart

ECD, extended criteria donor; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; CRF, case report file; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BR, bilirubin; INR, international normalized ratio; GFR, glomerular filtration rate; alphaGST, alpha gluthation S-Transferase

Figure 2: Hypothermic oxygenated machine perfusion

First the donor organ is retrieved and transported to the transplant center in a conventional way. In the transplant center the liver is connected to the Liver Assist device and HOPE is performed. Hypothetically, organ reconditioning with HOPE triggers multiple protective responses leading to decreased oxidative stress, improved energy reserves, reduced cell-death.
ET, Eurotransplant; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; ATP, Adenosine triphosphate; ROS, reactive oxygen species. Adopted from: Schlegel et al. [24]

Figure 3: Interventions and study visits

Arabic numbers represent the single study visits. Visit 1: screening, enrolment; Visit 2: admission; Visits 3, 4, 5: post-operative days 1st, 2nd, 3rd; Visit 6: 7th post-operative day; Visit 7: discharge; Visit 8: 6 months follow-up; Visit 9: 12 months follow-up, final visit
HOPE, hypothermic oxygenated machine perfusion

Table 1: Active RCTs on HOPE in OLT on clinicaltrials.gov (search date: 03rd of July 2017)

Trial number	Study center	Study type	Enrolment	Donor group	Primary endpoint	Comment
NCT03124641 (present trial)	RWTH Aachen University, Aachen, Germany	RCT	46	ECD-DBD	Early graft function (peak ALT level)	Recruiting
NCT01317342	University of Zurich, Zurich, Switzerland	RCT	70* 170§	DBD (ECD subgroup analysis only)	Early graft function (peak ALT level)* Major postoperative complications (Clavien Grade ≥III) and CCI§	Recruiting
NCT02584283	University of Groningen, Groningen, Netherlands	RCT	156	DCD (Maastricht category III)	Incidence of symptomatic non-anastomotic biliary strictures (NAS)	Recruiting

ALT, alanine aminotransferase; CCI, comprehensive complication index; DBD, donation of the brain death; DCD, donation of the cardiac death; ECD, extended criteria donation; HOPE, hypothermic oxygenated machine perfusion; NAS, non-anastomotic biliary strictures; OLT, orthotopic liver transplantation; RCT, randomized controlled trial; RWTH, Rheinisch-Westfälische Technische Hochschule

*based on clinicaltrials.gov
§information based on personal communication

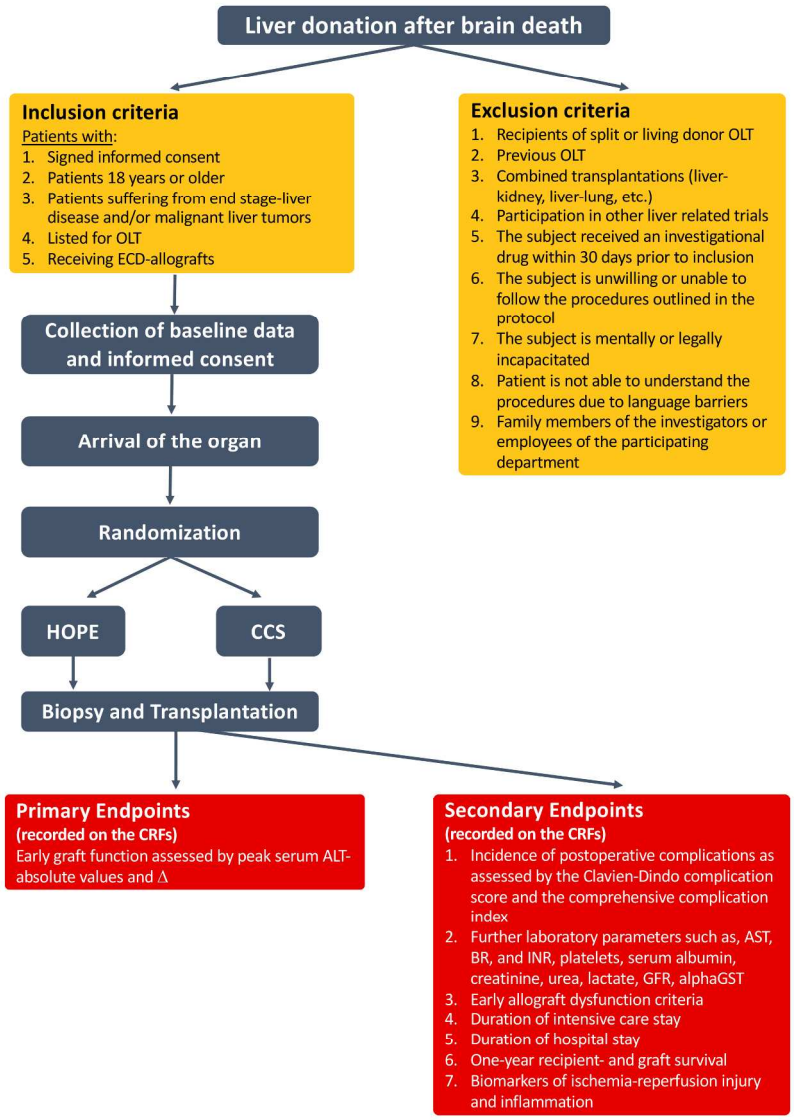

Figure 1

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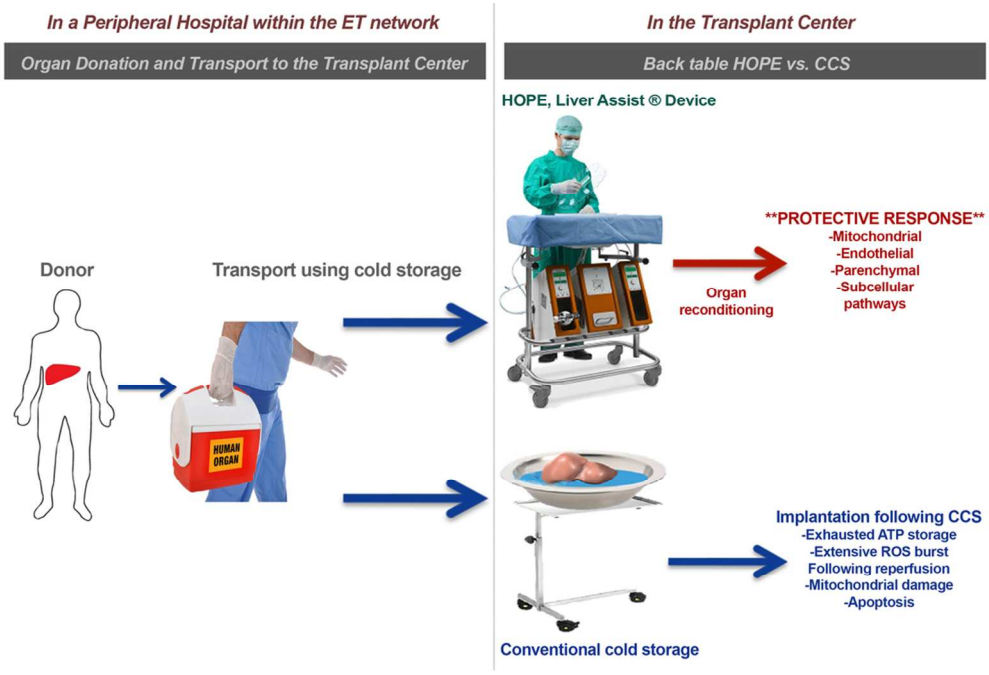

Figure 2

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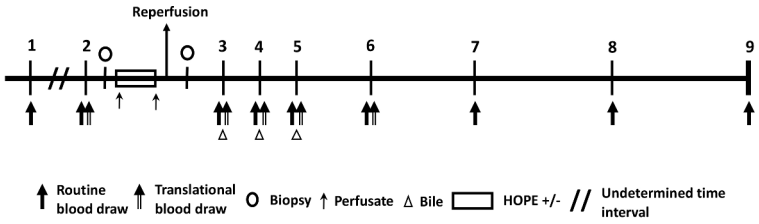

Figure 3

279x119mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P1
	2b	All items from the World Health Organization Trial Registration Data Set	P1-15, and Study protocol (SP)
Protocol version	3	Date and version identifier	P1 of SP
Funding	4	Sources and types of financial, material, and other support	P15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P1, P15
	5b	Name and contact information for the trial sponsor	P1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P12-13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including a summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P5
	6b	Explanation for choice of comparators	P5
Objectives	7	Specific objectives or hypotheses	P5, P6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P5, P6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P12
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P7-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n.a.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6-7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P10-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P9 and Fig3

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P7
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P4, P6, P12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n.a.
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P12 and SP
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	P6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P11-12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P12-13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	P12 and SP
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P15
	31b	Authorship eligibility guidelines and any intended use of professional writers	P15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	P12
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	P11, 13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective multicenter randomized controlled trial (HOPE ECD-DBD)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017558.R2
Article Type:	Protocol
Date Submitted by the Author:	26-Aug-2017
Complete List of Authors:	Czigany, Zoltan; University Hospital RWTH Aachen, Department of Surgery and Transplantation Schöning, Wenzel; University Hospital RWTH Aachen, Department of Surgery and Transplantation Ulmer, Tom Florian; University Hospital RWTH Aachen, Department of Surgery and Transplantation Bednarsch, Jan; University Hospital RWTH Aachen, Department of Surgery and Transplantation Amygdalos, Iakovos; University Hospital RWTH Aachen, Department of Surgery and Transplantation Cramer, Thorsten; University Hospital RWTH Aachen, Department of Surgery and Transplantation Rogiers, Xavier; Ghent University Hospital and Medical School Popescu, I; Fundeni Clinical Institute, Bucharest - Romania; Fundeni Clinical Institute Botea, Florin; Institutul Clinic Fundeni, Department of General Surgery and Liver transplantation Froněk, Jiří ; Institute for Clinical and Experimental Medicine, Department of Transplantation Surgery Kroy, Daniela; University Hospital RWTH Aachen, Department of Medicine III Koch, Alexander; University Hospital RWTH Aachen, Department of Medicine III Tacke, Frank; University Hospital RWTH Aachen, Department of Medicine III Trautwein, Christian; University Hospital RWTH Aachen, Department of Medicine III Tolba, Rene; University Hospital RWTH Aachen, Institute for Laboratory Animal Science and Experimental Surgery Hein, Marc; University Hospital RWTH Aachen, Department of Anesthesiology Dejong, Cornelis; University Hospital RWTH Aachen, Department of Surgery and Transplantation; Maastricht University Medical Centre (MUMC) Neumann, Ulf Peter; University Hospital RWTH Aachen, Department of Surgery and Transplantation; Maastricht University Medical Centre (MUMC), Department of Surgery Lurje, Georg; University Hospital RWTH Aachen, Department of Surgery

	and Transplantation
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Intensive care
Keywords:	hypothermic oxygenated machine perfusion, donation after brain death, extended criteria donor

Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective multicenter randomized controlled trial (HOPE ECD-DBD)

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Running title: Hypothermic Oxygenated Perfusion (HOPE) of Human Liver Allografts from Extended Criteria Donors (ECD)

Keywords: Hypothermic oxygenated machine perfusion (HOPE), Donation After Brain Death (DBD), Extended Criteria Donor (ECD)

Trial Registration: clinicaltrials.gov (NCT03124641)

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ABREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BR	Bilirubin
CCI	Comprehensive complication index
CCS	Conventional cold storage
CE	Conformité européene (European conformity)
CK18	Circulating cytokeratine 18
CKD-EPI	Chronic kidney disease epidemiology collaboration
CRF	Case report file
DBD	Donation after brain death
DCD	Donation after cardiac death
DGF	Delayed graft function
DSO	Deutsche Stiftung Organtransplantation (German Foundation for Organ Transplantation)
ECD	Extended criteria donor
ELISA	Enzyme-linked immunosorbent assay
ET	Eurotransplant
GST	Gluthation S-Transferase
HOPE	Hypothermic oxygenated machine perfusion
HTK	Histidine-tryptophan-ketoglutarate solution
ICH-GCP	International conference on harmonisation-Good clinical practice
ICU	Intensive care unit
INR	International normalized ratio
I/R	Ischemia-reperfusion
MP	Machine perfusion
MPS	Machine perfusion solution
MWU	Mann-Whitney-U test
OLT	Orthotopic liver transplantation
PNF	Primary graft non-function
RCT	Randomized controlled trial
RT-PCR	Reverse transcription polymerase chain reaction
RWTH	Rheinisch-Westfälische Technische Hochschule
SD	Standard deviation
USA	United States of America
UW	University of Wisconsin

ABSTRACT

Introduction: Orthotopic liver transplantation (OLT) has emerged as the mainstay of treatment for end-stage liver disease. In an attempt to improve the availability of donor allografts and reduce waiting list mortality, graft acceptance criteria were extended increasingly over the decades. The use of extended criteria donor (ECD) allografts is associated with a higher incidence of primary graft non-function (PNF) and/or delayed graft function (DGF). As such, several strategies have been developed aiming at reconditioning poor quality ECD liver allografts. Hypothermic oxygenated machine perfusion (HOPE) has been successfully tested in pre-clinical experiments and in few clinical series of donation after cardiac death (DCD) OLT.

Methods and analysis: HOPE ECD-DBD is an investigator-initiated, open-label, phase-II, prospective multicenter randomized controlled trial on the effects of HOPE on ECD-allografts in donation after brain death (DBD) OLT (HOPE ECD-DBD). Human whole organ liver grafts will be submitted to 1-2 hours of HOPE (n = 23) via the portal vein before implantation and are going to be compared to a control-group (n = 23) of patients transplanted after conventional cold storage (CCS). Primary (peak and Δ-peak alanine aminotransferase-ALT within 7 days) and secondary (aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, post-operative complications, early allograft dysfunction, duration of hospital and intensive care unit stay, 1-year patient- and graft survival) endpoints will be analyzed within a 12-month follow up. Extent of ischemia-reperfusion (I/R) injury will be assessed using liver tissue, perfusate, bile, and serum samples taken during the perioperative phase of OLT.

Ethics and dissemination: The study was approved by the institutional review board of the RWTH Aachen University, Aachen, Germany (EK 049/17). First results are expected in 2018.

Trial registration number: NCT03124641 (clinicaltrials.gov)

ARTICLE SUMMARY

Strengths and limitations of this study:

- HOPE ECD-DBD is a multicenter randomized controlled trial investigating the specific effects of HOPE on ECD organs in DBD transplantation.
- Tissue-, blood-, perfusate-, and bile-samples, retrieved for translational research, will enable an in-depth analysis of the effects of HOPE on ischemia-reperfusion injury and inflammation.
- The stratified randomization model will allow us to achieve a homogenous distribution of patients between treatment groups based on prognostic variables.
- Open-label design is considered a limitation of the study. Owing to the nature of the surgical procedure, it is not possible to blind the surgical team for the group allocation.

INTRODUCTION

Since Thomas Starzl`s pioneering efforts in 1963, orthotopic liver transplantation (OLT) has evolved as the standard therapy for patients with end-stage liver disease [1]. In 2015, approximately 1500 patients were listed for liver transplantation in Germany with only 894 transplants performed due to organ shortage [2]. Several strategies for donor pool expansion are being pursued concurrently. These include the use of living donors, splitting of cadaveric livers for two recipients, and the use of extended criteria donor (ECD) allografts for OLT. These ECD-allografts, however, exhibit poor tolerance to ischemia-reperfusion (I/R) injury, an important cause of liver damage. As such, I/R-injury is the underlying cause of graft dysfunction in ECD-allografts and negatively affects the process of liver regeneration in surgical conditions including hepatic resections and OLT [2].

Accordingly, several strategies have been developed, aiming at reconditioning poor quality ECD-allografts [3-7]. Hypothermic oxygenated machine perfusion (HOPE) has been tested intensively in pre-clinical animal experiments [3]. The positive effects of HOPE in this setting have been demonstrated among others to reduce the incidence of biliary complications, mitochondrial damage and level of cellular energy-status [3]. In the clinical setting, hypothermic oxygenated organ perfusion is performed in the transplant center shortly before the actual implantation using an extra-corporal organ perfusion system with full oxygen saturation over the portal vein [3]. The first clinical study with HOPE was recently published by Dutkowski et al. in a cohort of patients undergoing OLT with donation after cardiac death (DCD) allografts [8]. In donation after brain death (DBD), the only legally accepted approach for organ donation in Germany and many other European countries, HOPE and its effect on early graft function and post-operative complications has not been reported yet [2].

The purpose of this study is to test the effects of HOPE in a prospective multicenter randomized clinical trial (RCT) on ECD liver allografts in DBD liver-transplantation compared to conventional cold storage (HOPE ECD-DBD). As such, we hypothesize, that HOPE might have an organ reconditioning effect by substantially reducing early allograft injury in pre-damaged ECD organs. Primary (peak and Δ-peak alanine aminotransferase-ALT within 7 days) and secondary (e.g. peak aspartate aminotransferase-AST, bilirubin-BR, and international normalized ratio-INR, lactate, post-operative complications, hospital stay, graft- and patient

survival) endpoints are going to be analyzed. I/R-injury and biomarkers of HOPE induced subcellular responses will be assessed using liver tissue, perfusate, bile, and serum samples during the perioperative phase of OLT. Follow-up is set at 12 months.

METHODS AND ANALYSIS

Study type

HOPE ECD-DBD is an investigator initiated, open-label, phase-II, prospective multicenter randomized controlled trial on ECD-allografts in DBD OLT. Figure 1 summarizes the trial design. The study protocol was written in accordance to the CONSORT- and SPIRIT-recommendations.

Eligibility

Patients above 18 years of age, suffering from end stage-liver disease and/or malignant liver tumors, listed for OLT and receiving ECD organs at the Department of Surgery and Transplantation, University Hospital RWTH Aachen, Aachen, Germany and at other participating centers are eligible for the study. Informed consent is obtained from all subjects participating in the trial by a qualified member of the study team in the outpatient clinic. In each center, potential study participants on the OLT-waiting list will be screened for defined in- and exclusion criteria and will be enrolled accordingly (Figure 1). Allocation of liver allografts follows national (German Foundation for Organ Transplantation; The Belgian Transplantation Society; Romanian National Transplant Agency) and/or European (Eurotransplant; ET) policies. The study will not influence the allocation procedure or cause any delay in the actual implantation procedure.

Inclusion Criteria

Inclusion criteria are:

- Signed informed consent
- Patients 18 years or older
- Patients suffering from end stage-liver disease and/or malignant liver tumors
- Listed for OLT
- Receiving ECD-allografts

Exclusion Criteria

Exclusion criteria are:

- Recipients of split or living donor OLT
- Previous OLT
- Combined transplantations (liver-kidney, liver-lung, etc.)
- Participation in other liver related trials
- The subject received an investigational drug within 30 days prior to inclusion
- The subject is unwilling or unable to follow the procedures outlined in the protocol
- The subject is mentally or legally incapacitated
- An inability to understand the procedures due to language barriers
- Family members of the investigators or employees of the participating department

Randomization

Randomization is performed at arrival of the allograft at the transplant center and after acceptance for transplantation, using an online randomizing tool for clinical trials (www.randomizer.at), either by the principal investigator or, in case of absence, by a trained member of the study team [9, 10]. A stratified randomization model will be used to ensure a balance of prognostic variables between the treatment groups. Expected cold ischemia time and graft steatosis were selected for stratification. A median cold ischemic time of 8 hours is based on our local OLT database (2011-2017) containing more than 320 OLTs. Therefore, 8 hours of expected cold ischemia time has been selected as a cut-off value for the present study. For steatosis, we use 30% macrovesicular steatosis as a cut-off value for the stratification process as defined by the NASH Clinical Research Network Scoring System and validated as prognostic marker in liver transplantation by several authors [11-14].

Organ procurement, ECD criteria

All ECD liver-allografts will be retrieved by ET and/or national procurement teams. Following cross-clamping (*in situ* flushing of the abdominal organs and begin of cold ischemia time), allografts will be removed and transported in a standardized

fashion of conventional cold storage (CCS) on packed ice (4-6° C). Required data of the donor and the organ will be registered in a standard manner and will be transferred to a designated case report file (CRF).

Definition of ECD based on the recommendations of the German Medical Chamber (Bundesaerztekammer) [15].

- Donors 65 years of age and older
- Intensive care therapy of the donor was required before donation for at least 7 days
- Obesity of the donor with a Body Mass Index > 30 kg/m²
- Fatty liver (with histology) > 40 %
- Serum-Sodium > 165 mmol/l
- Serum AST or ALT > 3 x upper limits of normal
- Serum-Bilirubin > 2 mg/dl

HOPE versus CCS

The present RCT comprises two groups, a perfusion (group 1; HOPE) and a control conventional cold storage (group 2; CCS) group. Patients on the waiting list for OLT with proven written consent will be recruited.

In case of randomization to group 1, HOPE will be applied to the allograft in the operating room after regular organ procurement, transport and back table preparation (Figure 2) according to a protocol previously described by Dutkowski et al. for DCD liver transplantation [8]. HOPE (Liver Assist®; Organ Assist b.v., Groningen, The Netherlands) will be applied through the portal vein for 1-(2) hour(s) (if recipient hepatectomy is prolonged, HOPE will be continued to avoid repeated static cold storage), with a perfusion rate of 0.1 ml/g liver /min in a pressured control system with 2-3 mm Hg. The 10 °C perfusate (3-4 L) will be re-circulated (Belzer MPS®, Bridge to Life, London, United Kingdom). Perfusate will be oxygenized with a pO2 of 60-80 kPa by an oxygenator included as disposable in the setup. Perfusion parameters registered by the device will be stored automatically and evaluated for possible patterns. Immediately prior to perfusion, grafts are flushed with machine perfusion solution to wash out the residual HTK perfusate. Storage, management and use of the above mentioned medical products will be carried out according to the manufacturer's guidelines. Patients in the CCS control group will undergo the

clinically routine procedure with CCS using HTK solution (Custodiol®, Dr. Franz Köhler Chemie, Bernsheim, Germany) according to institutional protocols.

Orthotopic liver transplantation (OLT)

In center 1, OLT will be performed using the total-cava replacement technique with veno-venous bypass including the porto-mesenteric vascular bed using a roller pump system with an adjustable flow as previously described [16, 17]. Biliary reconstruction will be performed as a side-to-side common bile duct anastomosis with T-tube insertion or primary Roux-en-Y hepaticojejunostomy in cases of primary sclerosing cholangitis as previously described [18]. External centers will use their local standard techniques for OLT.

Sample collection and storage

I/R-injury will be assessed using liver tissue samples taken upon arrival of the organ (before HOPE or corresponding cold-storage) and at the end of implantation before closure of the abdomen, to evaluate the amount of I/R-injury (Figure 3). In total, two excision biopsies (2 cm^3) will be harvested from the ECD liver allograft (segment III). Perfusate samples will be collected repeatedly during machine perfusion. Blood samples are taken as part of the daily routine during the peri- and postoperative course of OLT (Figure 3). Blood parameters of liver tissue damage and/or function (serum AST, ALT, BR, albumin, INR, alpha-GST, lactate) as well as kidney damage and/or function (creatinine, urea, glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation) will be monitored. An additional 20 ml blood will be drawn on hospital-admission and on post-operative days 1, 2, 3, and 7 (POD 1, POD 2, POD 3, and POD 7) and will be used for translational research (Figure 3). Bile samples can be easily collected from the T-Drain during the first 3 PODs (only applicable for center 1). All liver tissue, serum, perfusate and bile samples will be directly snap-frozen in liquid nitrogen (-80°C) and stored for 12 months after completion of the trial.

Post-operative care and immunosuppression

All patients are treated in accordance to our institution´s routine clinical algorithm for OLT recipients. Apart for the *ex vivo* allograft perfusion (group 1, HOPE), patients will be treated according to standard operating procedures for perioperative medical, interventional and surgical OLT management. Patients after OLT will be monitored at the intensive care unit (ICU) during the early postoperative phase and later, depending on their individual recovery, transferred to regular ward.

The used immunosuppressive regimen is based on induction therapy with intravenous basiliximab and methylprednisolone followed by corresponding oral doses of prednisolone, tacrolimus, and mycophenolate mofetil [16, 17].

Study endpoints

Primary endpoints

Early graft injury in DBD-OLT using ECD allografts undergoing HOPE or CCS, as assessed by peak alanine aminotransferase-ALT during the first 7-days post-OLT and by Δ Peak-ALT* [19].

Secondary endpoints

1. Incidence of post-operative complications as assessed by the Clavien-Dindo complication score and the comprehensive complication index (CCI) [20]
2. Further laboratory parameters such as serum AST, BR, INR, platelet count, albumin, creatinine, urea, lactate, glomerular filtration rate using the CKD-EPI equation, alpha gluthation S-Transferase (GST)
3. Early allograft dysfunction as defined by the Olthoff criteria (bilirubin >=10mg/dL on day 7, international normalized ratio >= 1.6 on day 7, and alanine or aspartate aminotransferases >2000 IU/L within the first 7 days) [21]
4. Duration of intensive care stay
5. Duration of hospital stay
6. One-year recipient- and graft survival
7. Analysis of serum, tissue, bile, and perfusate biomarkers as translational aspects of the project

*Δ Peak-ALT: To correct for an assumed washout effect of machine perfusion, besides the absolute values, relative changes of serum peak-ALT will be assessed [19]. Peak-ALT will be corrected to the values measured in the routine blood analysis after reperfusion at the time point of admission to the ICU.

Power of the study

A sample size of 23 patients per group (Σn=46) was calculated with the G*Power software (Heinrich-Heine-University, Düsseldorf, Germany) using the following settings: α=0.05; 1-β(power)=0.8; 2-sided t-test, including 15% drop-out and invalid data; peak AST and ALT levels 48 hours post-transplantation. Power calculation was performed based on the previous data from Guarrera et al. [22]. A reduction of 65% (AST) – 59% (ALT) is expected in the mean peak transaminase levels following machine perfusion treatment (peak AST: MP, 1154 ± 355.5 SD IU/mL, CCS, 3339 ± 3376.9.1 SD IU/mL; peak ALT: MP, 560.0 ± 355.5 SD IU/mL, CCS, 1358 ± 1208.4 SD IU/mL) [22].

Data collection and statistics

All collected data are documented on CRFs and considered as source data. Members of the study team document the required information into the CRF system following previous training. A study database will be created based on the CRFs and data correction, record keeping, archiving and subsequently the destruction of study documents will be performed according to the ICH-GCP guidelines. Subjects will be informed about data protection and that data will be pseudonymized. Furthermore, data will be handed out to third party only anonymized. Encoded data will only be provided to authorized persons (clinical monitor, authorized study staff, authorities, institutional review board). The study will be prematurely terminated for an individual subject in case of study related complications or if the subject withdraws informed consent. Values of p-value less than 0.05 will be considered significant. Unpaired t-test and Mann-Whitney-U test are going to be applied in case of normally and non-normally distributed data, respectively. For time course analysis of laboratory parameters two-way analysis of variance is applied. Postoperative complications are

assessed by the Fischer's exact test. For comparisons between Kaplan-Meier curves of one-year graft and patient survival the log rank test is used.

Analysis of endpoints will be performed by an independent committee in a blinded fashion (Institute for Medical Statistics, RWTH Aachen).

Safety considerations

In the present study, solely certified medical products (CE certification) will be used. Blood draws and liver biopsies during the transplant procedure are performed according to the clinical routine. Thus, no relevant study-related risks and no additional burden for the subjects are expected. Independent monitoring of data will be performed by the Clinical Trial Center Aachen (CTC-A, RWTH Aachen, Aachen, Germany) An interim analysis will be performed as soon as 12 patients are enrolled in each randomized group. The trial will be terminated immediately if one of the following criteria is fulfilled; Significantly higher serum ALT levels ($p<0.001$ using Student's t-test) in the HOPE group compared to the CCS group (Efficacy). The proportion of Grade ≥ III complications is significantly higher ($p<0.05$, Fischer's exact test) in the HOPE group when compared to the CCS group (Safety).

Ethics

This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, and good clinical practice guidelines (ICH-GCP) as well as all national legal and regulatory requirements. The institutional review board of the University RWTH Aachen has approved the study protocol, including consent form and patient information (EK 049/17). Members of the local study team have completed a course in good clinical practice as certified by the German Medical Chamber. The trial was registered on clinicaltrial.gov on 20.03.2017 (NCT03124641).

Study group

The study group of the HOPE ECD-DBD trial comprises the trial sponsor (GL, UPN, ZC) and the PI (GL) of the University Hospital RWTH Aachen (center 1), the local investigators in Ghent (center 2; XR), Bucharest (center 3; IP, FB) and Prague (center 4; JF). Each local investigator is in charge of the execution of the study and

collection of data. The trial sponsor is responsible for randomization, trial database, storage, statistical analysis and scientific writing. The trial will expectedly include the first patients (applicable for center 1) in Q3 of 2017. External centers are expected to start the recruitment phase until the end of 2017.

Ischemia-reperfusion injury and inflammation

I/R-injury, depleted energy reserves, and oxidative stress play an important role in early graft dysfunction following liver transplantation of ECD liver grafts [3, 4, 8, 23-26]. The translational research aim of the present study is to determine the effects of HOPE on I/R-injury, inflammation and energy household on human liver ECD-allografts. Blood and liver tissue samples will be used to measure various parameters of inflammation (Interleukins, Tumor necrosis factor-alpha, Macrophage Migration Inhibitory Factor) [27, 28], hepatocyte cell death (e.g., circulating cytokeratin 18 [CK18] fragments like M30 or M65) [29], energy- (Adenosine triphosphate levels) [30], and redox-household (Hemoxygenase-1, Malondialdehyde) [28]. Luminometry, Spectrophotometry, Luminex-assay, ELISA, RT-PCR and Western blot will be used for these analyses. Proteomics and metobolomics analysis will be performed on paired liver tissue samples to potentially identify early mediators of HOPE mediated organ protection.

Biochemical parameters of biliary epithelial cell function and injury (bilirubin, biliary pH, bicarbonate, biliary glucose, lactate dehydrogenase, alkaline phosphatase, gamma-GT) will be assessed using standard laboratory methods [19, 31].

Due to the scarcity of clinical data regarding the utilization of HOPE for quality assessment of liver grafts, it is still unclear, whether this method is applicable for such quality predictions. Therefore, we will use the perfusion fluid to assess certain parameters which might have a predictive value in evaluating graft quality under hypothermic conditions (pH, lactate, PO2, PCO2, AST, ALT, LDH, GST liver fatty acid binding protein/L-FABP levels) [31-33].

DISCUSSION

Liver transplantation is the treatment of choice for patients with end-stage liver disease. The need to obtain the optimal benefit from a limited number of organs that are available has prompted the expansion of allograft selection criteria (ECD-

allografts) aiming at increasing the donor pool and decrease overall waiting list mortality. ECD-allografts exhibit poor tolerance to I/R-injury, a syndrome initiated upon restoration of blood supply after cold and warm ischemia yielding in endothelial and Kupffer cell activation, vasoconstriction, neutrophil infiltration, and sinusoidal platelet aggregation [34, 35].

Technical innovations have been introduced to overcome these difficulties of ECD-allograft usage in OLT [4]. The implementation of machine perfusion for liver allografts was one of the most promising innovations in organ preservation over the last decade [4]. *In vivo* and *ex vivo* machine perfusion of liver allografts has been tested intensively in pre-clinical studies [3], and the beneficial effects of HOPE have been demonstrated among others to reduce the incidence of biliary complications, the degree of mitochondrial damage and the level of cellular energy-status [3]. In a recent international-matched case analysis, Dutkowski et al. demonstrated for the first time that HOPE treatment of DCD-allografts significantly decreased graft injury compared to matched CCS-livers regarding peak ALT levels (1239 vs. 2065 U/l, p = 0.02), intrahepatic cholangiopathy (0% vs. 22%, p = 0.015) and overall biliary complications (20% vs. 46%, p = 0.042), thus concluding that HOPE seems to offer beneficial effects in DCD-allograft preservation [8]. In DBD, the only legally accepted approach for organ donation in Germany and many other countries, HOPE and its effect on graft function and postoperative complications has not been reported yet [2]. Currently, we identified four active clinical trials on clinicaltrials.gov using hypothermic oxygenated machine perfusion in liver transplantation. Two trials are non-randomized observational studies both with the enrolment of 10 patients for machine perfusion, using different perfusion systems (NCT03098043; NCT03031067). One trial (NCT02584283) is a multi-center RCT investigating the effects of portal- and arterial perfusion HOPE (dual-HOPE) versus cold storage on biliary complications in DCD transplantation (Table 1). Preliminary data with dual-HOPE has been reported recently in a case-control feasibility and safety study by the same group [36]. The second RCT by Dutkowski et al. (NCT01317342) is recruiting patients in DBD transplantation, however, also including non-ECD organs thus appearing less suitable to investigate the specific effects of HOPE in ECD-allografts (Table 1).

HOPE using single (portal vein only) or dual (portal vein and hepatic artery) perfusion is subject of ongoing debate [24, 36-40]. Arguments for the use of dual

perfusion are based predominantly on the better perfusion and preservation of the biliary system and reduced incidence of biliary complications in DCD transplantation [36, 39]. However, according to some experimental data a complete end effective perfusion of the graft and the biliary system can also be achieved by using portal vein only perfusion [38]. Due to the lack of any *in vivo* clinical evidence or convincing comparative preclinical data which would demonstrate the superiority of any of the two approaches, we decided to use the pragmatic approach and perfuse the liver grafts via the portal vein only. The favorable effects of portal vein only HOPE has already been successfully demonstrated in clinical liver transplantation by Dutkowski et al. [8]. Nevertheless, the comparison of single vs. dual HOPE is of upmost clinical importance and should be addressed in future trials.

Although we have designed our trial carefully, non-blinding of the transplant team for the treatment groups is a limitation. This may be accounted to the nature of the surgical procedure and to pragmatic reasons, as HOPE and back-table preparation of the allograft are usually performed in the same operating room as the OLT procedure itself. The present trial has also some specific strengths; Firstly, HOPE ECD-DBD focuses on patients solely receiving ECD-allografts, a population we anticipate the best cost/benefit ratio from the utilization of HOPE. Secondly, donation after brain death is the most frequent source of ECD-allografts in Europe. Thirdly, we use a stratified randomization model that allows us to achieve a homogenous distribution based on prognostic variables of patients between the groups. Lastly, the results of the translational part of this study may deliver novel insights on the underlying subcellular effects of HOPE in human allografts.

DISSEMINATION PLAN

Results of the HOPE ECD-DBD trial will be presented at national and international scientific meetings, and published in international peer-reviewed medical journals. First results of the trial are expected in 2018.

AUTHORS' CONTRIBUTION

The initial study concept was derived from the initiating investigator study group (GL, ZC, UPN, RT, IA, JB). GL and ZC drafted the manuscript. ZC and GL performed the sample size estimation. WS, TFU, TC, XR, IP, FB, JF, DK, AK, FT, CT, MH, CHCD participated in designing the study, preparing the revised protocol

and are investigators at the University Hospital RWTH Aachen or at the external centers. All authors were involved in revising the manuscript and approved the final version.

FUNDING STATEMENT

This research project is supported by the START-Program of the Faculty of Medicine, RWTH Aachen (691739, ID: 136/17).

COMPETING INTEREST STATEMENT

The authors have no competing interest to declare.

ETHICS APROVAL

Institutional review board of the RWTH Aachen, Aachen, Germany. Approval number: EK 049/17.

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37. Schlegel, A., et al., *Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation.* J Hepatol, 2013. **59**(5): p. 984-91.
38. Schlegel, A., et al., *Is single portal vein approach sufficient for hypothermic machine perfusion of DCD liver grafts?* J Hepatol, 2016. **64**(1): p. 239-41.
39. Bruggenwirth, I.M.A., et al., *Is single portal vein perfusion the best approach for machine preservation of liver grafts?* J Hepatol, 2016. **64**(5): p. 1194-1195.
40. Schlegel, A., et al., *Reply to 'Is single portal vein perfusion the best approach for machine preservation of liver grafts?'.* J Hepatol, 2016. **64**(5): p. 1195-1196.

FIGURE LEGENDS

Figure 1: Study flowchart

ECD, extended criteria donor; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; CRF, case report file; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BR, bilirubin; INR, international normalized ratio; GFR, glomerular filtration rate; alphaGST, alpha gluthation S-Transferase

Figure 2: Hypothermic oxygenated machine perfusion

First the donor organ is retrieved and transported to the transplant center in a conventional way. In the transplant center the liver is connected to the Liver Assist device and HOPE is performed. Hypothetically, organ reconditioning with HOPE triggers multiple protective responses leading to decreased oxidative stress, improved energy reserves, reduced cell-death.
ET, Eurotransplant; HOPE, hypothermic oxygenated machine perfusion; CCS, conventional cold storage; ATP, Adenosine triphosphate; ROS, reactive oxygen species. Adopted from: Schlegel et al. [24]

Figure 3: Interventions and study visits

Arabic numbers represent the single study visits. Visit 1: screening, enrolment; Visit 2: admission; Visits 3, 4, 5: post-operative days 1^{st}, 2^{nd}, 3^{rd}; Visit 6: 7^{th} post-operative day; Visit 7: discharge; Visit 8: 6 months follow-up; Visit 9: 12 months follow-up, final visit
HOPE, hypothermic oxygenated machine perfusion

Table 1: Active RCTs on HOPE in OLT on clinicaltrials.gov (search date: 26th of August 2017)

Trial number	Study center	Study type	Enrolment	Donor group	Primary endpoint	Comment
NCT03124641 (present trial)	RWTH Aachen University, Aachen, Germany	RCT	46	ECD-DBD	Early graft function (peak ALT level)	Recruiting
NCT01317342	University of Zurich, Zurich, Switzerland	RCT	170§	DBD (ECD subgroup analysis only)	Major postoperative complications (Clavien Grade ≥III) and CCI§	Recruiting
NCT02584283	University of Groningen, Groningen, Netherlands	RCT	156	DCD (Maastricht category III)	Incidence of symptomatic non-anastomotic biliary strictures (NAS)	Recruiting

ALT, alanine aminotransferase; CCI, comprehensive complication index; DBD, donation of the brain death; DCD, donation of the cardiac death; ECD, extended criteria donation; HOPE, hypothermic oxygenated machine perfusion; NAS, non-anastomotic biliary strictures; OLT, orthotopic liver transplantation; RCT, randomized controlled trial; RWTH, Rheinisch-Westfälische Technische Hochschule

§information based on personal communication

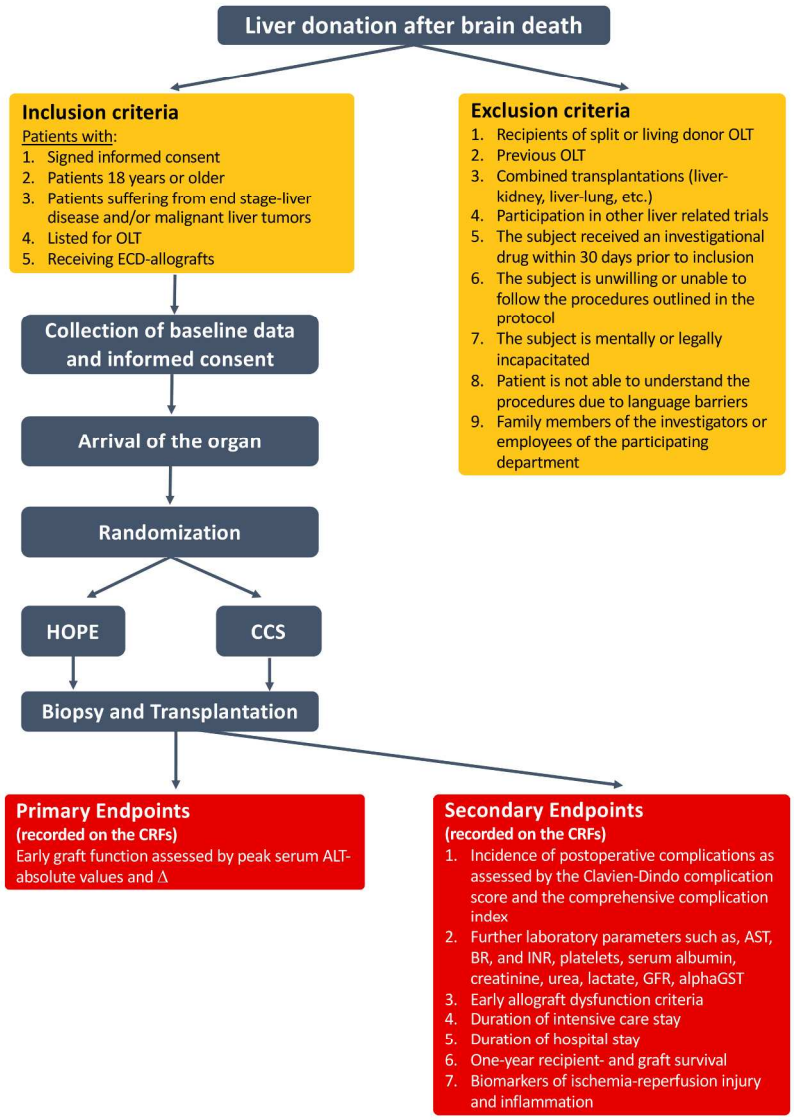

Figure 1

201x284mm (300 x 300 DPI)

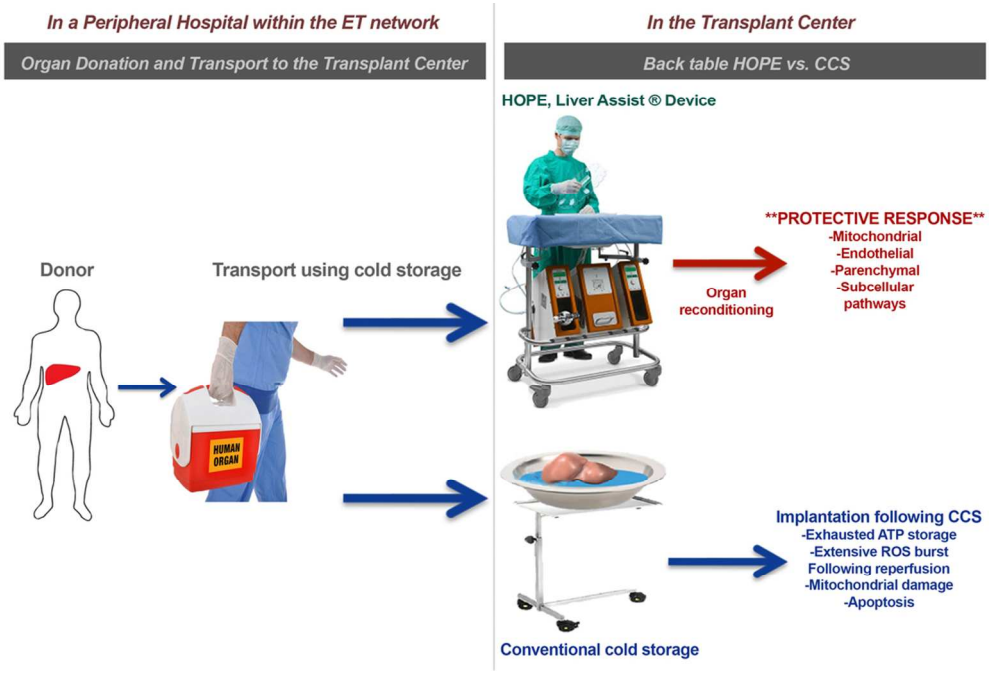

Figure 2

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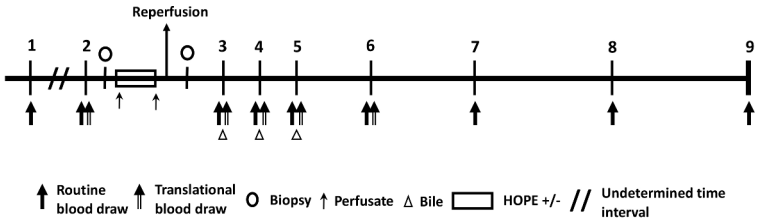

Figure 3

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P1
	2b	All items from the World Health Organization Trial Registration Data Set	P1-15, and Study protocol (SP)
Protocol version	3	Date and version identifier	P1 of SP
Funding	4	Sources and types of financial, material, and other support	P15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P1, P15
	5b	Name and contact information for the trial sponsor	P1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P12-13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P12

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including a summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P5
	6b	Explanation for choice of comparators	P5
Objectives	7	Specific objectives or hypotheses	P5, P6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P5, P6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P12
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P7-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n.a.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6-7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P10-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P9 and Fig3

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P7
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P4, P6, P12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P11-12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P11-12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n.a.
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P12 and SP
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	P6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P11-12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P12-13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	P12 and SP
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P15
	31b	Authorship eligibility guidelines and any intended use of professional writers	P15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	P12
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	P11, 13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.