'In house' Pre-implantation Oxygenated Hypothermic Machine Perfusion Reconditioning after Cold Storage versus Cold Storage alone in ECD Kidneys from Brain Dead Donors

Acronym: COPE-POMP trial

Internal Reference No: COPE:305934:WP3

Ethics Ref: *TBA*

ISRCTN Number: ISRCTN63852508

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Roles and Responsibilities:

Chief Investigator and Principal Investigator will have oversight of:
Design and conduct of the study
Preparation of protocol and revisions
Preparation of standard operating procedures
Organising trial management committee meetings
Publication of study reports

Local Lead investigators:
In each participating centre a lead investigator will be identified, to be responsible for obtaining local ethics committee and research governance approval; the identification, recruitment, data collection and completion of CRF's, along with follow up of study participants and adherence to

study protocol. Lead investigators will be trial management committee members.

Local Coordinators
The local coordinators will aid the lead investigators.

Surgical Intervention Trials Unit (SITU):
Will be responsible for:

- Participant randomisation
- Database design
- Management of data collection
- Statistical analysis of trial data
- Providing data for regular DMC meetings
- Monitoring the trial
- Site initiation and close-out

To provide effective statistical support for clinical and pre-clinical studies, to ensure successful implementation of organ protection strategies.
To perform health economic evaluations of the proposed novel technologies to demonstrate cost-effectiveness.

COPE Trials Central Manager:
The Central Manager will be based in SITU and ensure that regulatory standards are maintained and the trial is conducted according to the principles of GCP. The Central Manager will also be responsible for:

- Organisation of trial management committee meetings
- Regular reporting to the sponsor on the progress of the clinical investigation.

Trial statistician:
Statistical design and analysis will be managed by the Surgical Intervention Trials Unit (SITU) statistician, Ms. Virginia Chiocchia, and supervised by Mrs. Susan Dutton.
The trial statistician is responsible for the statistical design of the trial and analysis of trial data. The trial statistician will also prepare documents for review by the DMC

Consortium for Organ Preservation in Europe
The Consortium for Organ Preservation in Europe (COPE) is the official organ task force of the European Society for Organ Transplantation (ESOT) and is funded by a European Commission

FP7 Award. The Consortium brings together academic institutions, clinical and scientific experts and SMEs from across Europe to work together on advancing organ preservation techniques.
There are currently a range of clinical and translational studies, known as Work Packages (WP), being funded by COPE with the aim of advancing and developing organ preservation technology.

Committees:

Trial Management Committee:

- Agreement of final protocol
- Reviewing progress of study and if necessary agreeing changes to the protocol to facilitate the smooth running of the study.
- The trial management committee for WP3 will report directly to the COPE Management Board.

The Trial management Committee (TMC) will provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of GCP and the relevant regulations. The TMC will provide guidance on any protocol amendments and decisions about continuation or termination of the trial or substantial amendments to the protocol.

- Andreas Paul, Essen (Chair)
- Rutger Ploeg, Oxford (Coordinator)
- Zeeshan Akhtar, Oxford
- Catherine Boffa, Oxford
- Ally Bradley
- Richéal Burns (SITU), Oxford
- Virginia Chiocchia (SITU), Oxford
- Peri Kocabayoglu, Essen
- Christina Krikke, Groningen
- Martin Kuizenga, Organ Assist
- Thomas Minor, Bonn
- Liset Pengel, Centre for Evidence in Transplantation
- Jacques Pirenne, Leuven
- Ina Jochmans, Leuven

Data Monitoring Committee
An independent Data Monitoring Committee

(DMC) will be formed with the help of the Centre for Evidence in Transplantation. All the members of the committee will be independent of the study. At each DMC review, the DMC will receive a report from the trial statistician.
The roles and responsibilities of the DMC will be detailed in the charter

- Chair: Chris Watson (Professor of Transplantation, University of Cambridge,UK)
- Vice Chair: Josep Grinyo (Professor of Nephrology, Bellvitge Hospital, Barcelona, Spain)
- Patrizia Burra (Professor of Hepatology, Padua, Italy)
- Susan Charman (Statistician, London School of Hygiene and Tropical Medicine, UK)
- Gabriel Oniscu (Consultant Transplant Surgeon, Edinburgh, UK)

In the event of technical issues being discussed an industry expert will be invited to attend these meetings.

COPE Management Board
The COPE Management Board will consist of the COPE coordinator, administrators, Work Package leaders and representatives from the Small and Medium Enterprises (SMEs) involved in the various COPE trials, including Organ Assist. The TMC will report to the Management Board periodically with updates on trial progress. If the COPE Management Board has concerns about the progress of the trial, the TMC will be informed of this with information about specific issues that need to be addressed. If required, the COPE Management Board can receive advice from the External Advisory Board, as described below.
The COPE coordinator and administrators are also required to periodically provide updates to the EU Scientific Officer on the progress of all Work Packages within the COPE consortium.

WP8
Work Package 8 (WP8) of the COPE project is concerned with study design, statistical analysis and cost effectiveness. Its objectives are:
• To deliver clinical and pre-clinical trials that are conform to the highest standard of methodological quality.

• To ensure patient safety is paramount during clinical trials, through developing sound methodologies and establishing data monitoring committees.

The WP8 will be comprised of Centre of Evidence in Transplantation (CET) members and SITU members. It will act as a source of advice to the TMC and Management Board should they request it.

CET members:

- Peter Morris
- Simon Knight
- Liset Pengel
- John O'Callaghan

SITU members:

- Susan Dutton
- Victoria Rush
- Virginia Chiocchia
- Rajeev Kumar
- Allyson Bradley
- Richéal Burns

External Advisory Board

The external advisory board (EAB) will act as a source of independent, impartial advice to the TMC should they request it. This may be in the context of concerns or conflicts arising from recommendations by the DMC, or regarding unforeseen circumstances that arise during the course of the trial.

- Axel Rahmel
- Mike Bos
- James Neuberger

An organogram of the committee structure for WP3 can be found in Appendix C.

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(Grant number 305934 – Work Package 3, under the auspices of COPE www.cope-eu.org)
The funding agency had no role in the design of this study and will not have any role during its execution, analysis, interpretation of data or decision to submit results for publication.

Protocol sign-off

Chief investigator:	Signature…… Name…… Date……..
Principal Investigator:	Signature…… Name…… Date……..
Local **Lead Investigators:**	Signature…… Name…… Date…….. Signature…… Name…… Date…….. Signature…… Name…… Date…….. Signature…… Name…… Date……..
Senior statistician:	Signature…… Name…… Date……..

Investigator Signature page

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

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

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

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

I will ensure that the requirements relating to Research Ethics Committee (REC) and regulatory authority review and approval are met. I will provide the University of Essen with any material that is provided to the REC or regulatory authority for ethical approval.

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

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

Signature……

Name…… Date…….

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, regulatory authorities, and members of the Research Ethics Committee, unless authorised to do so.

Conflicts of Interest

None

Contents

SYNOPSIS

Study Title	'In house' Pre-implantation Oxygenated Hypothermic Machine Perfusion Reconditioning after Cold Storage versus Cold Storage alone in ECD Kidneys from Brain Dead Donors (POMP Trial)
Internal ref. no.	COPE:305934:WP3
Clinical Phase	Phase III trial
Trial Design	A multi-centre, participant-blinded, randomized, 24-month, parallel-group, prospective superiority study to compare the efficacy of brief 'in house' oxygenated hypothermic machine perfusion prior to transplantation of extended criteria donor kidneys from brain dead donors versus static cold storage alone to enhance 1 year graft survival after transplantation
Trial Participants	Donation after brain death donors, fulfilling the UNOS ECD criteria for kidney donation; adult kidney-only transplant recipients
Planned Sample Size	262 kidneys (131 kidneys in each treatment arm)
Follow-up duration	12 months
Planned Trial Period	36 months (24 month recruitment, 12 month follow-up)
Primary Objective	To compare the effect of HRMP+O_2 versus SCS on 1-year graft survival in ECD-kidneys.
Secondary Objectives	To compare graft and patient survival between SCS and short-term HRMP+O_2. To compare the estimated GFR (eGFR) as a surrogate of kidney function between oxygenated HRMP and SCS kidneys. To compare the incidence of delayed graft function (DGF), slow graft function (SGF) and primary non-function (PNF) between oxygenated HRMP and SCS kidneys. To compare the incidence of acute rejection between oxygenated HRMP and SCS kidneys. To assess health economic implications of oxygenated HRMP in comparison to SCS kidneys.
Primary Endpoint	Graft survival at 12 months after transplantation
Secondary Endpoints	Patient and (death censored) graft survival at day 7, 3, 6 and 12 months after transplantation. eGFR defined by the MDRD equation at day 7, 3, 6 and 12 months after transplantation. DGF defined as the need for dialysis in the first 7 days after transplantation and preceding the return of kidney function. SGF based on functional DGF defined as the absence of decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first 7 days after transplantation. PNF defined as the continued need for dialysis at 3 months after transplantation. Comparison of biopsy proven acute rejection between the 2 groups. Quality of life measures (EQ-5D-5L) at consent, 3 and 12 months, length of hospital stay (including ICU) and need/length for dialysis treatment.
Device Details	Kidney Assist (CE marked) Manufacturer- Organ Assist B.V The device has been in use for 4 years.

1 ABBREVIATIONS

AE	Adverse Event
CET	Centre of Evidence in Transplantation
CI	Chief Investigator
CIT	Cold Ischemic Time
CRF	Case Report Form
eCRF	Electronic Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
DBD	Donation after Brain Death
DCD	Donation after Cardiac Death
DGF	Delayed Graft Function
DMC	Data Monitoring Committee
EAB	External Advisory Board
eGFR	Estimated Glomerular Filtration Rate
ECD	Extended Criteria Donor
GCP	Good Clinical Practice
GP	General Practitioner
GS	Graft Survival
HMP	Hypothermic Machine Perfusion
HRMP	Hypothermic Reconditioning by Machine Perfusion
ICH	International Conference of Harmonisation
NHSBT	National Health Service Blood and Transplant
PI	Principal Investigator
PIL	Participant Information Leaflet
PNF	Primary Non-Function

R&D	NHS Trust Research & Development Department
REC	Research Ethics Committee
RR	Renal Resistance
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SITU	Surgical Intervention Trials Unit
SME	Small and Medium Enterprises
SOP	Standard Operating Procedure
TMC	Trial Management Committee
UNOS	United Network of Organ Sharing

2 BACKGROUND AND RATIONALE

Renal transplantation remains the therapy of choice for patients with end stage renal disease. However, the number of patients waiting for a kidney graft continues to increase and far exceeds the availability of donor grafts (1-3). A large number of deceased organ transplants manifest a degree of early dysfunction leading to the clinical syndrome of Delayed Graft Function (DGF) (4,5). DGF represents a significant problem in clinical kidney transplantation affecting up to 30% of all deceased donor graft recipients (6). This has an impact on short-term management, including the requirement of hemodialysis treatment and is associated with an increased risk of acute rejection. Moreover DGF has been shown in multivariate analyses to increase the incidence of chronic nephropathy and later graft loss (4). The shortage of donor organs has led the transplant community to accept an increasing number of older and more marginal donor grafts for transplantation (7). Kidneys from these donors are particularly vulnerable for the development of DGF and have decreased long term graft survival. One important modifiable risk factor of DGF is ischemia injury sustained during organ preservation. Optimizing the preservation of grafts during the preservation phase is essential to reduce this ischemic injury. In those older and more marginal donor kidneys, an optimized preservation is therefore of even greater importance.

An international randomized controlled trial comparing Hypothermic Machine Perfusion (HMP) versus Static Cold Storage (SCS) in deceased donor kidneys demonstrated a significant reduction in the incidence of DGF as well as an improvement in 1- and 3-year graft survival (GS) by use of machine preservation (8-10). The improvement in GS was most pronounced in kidneys from extended criteria donors (ECD). ECDs were defined according to the UNOS (United Network for Organ Sharing) criteria, which include: Donor age ≥ 60 years, or ≥ 50 years with at least two of the following: history of hypertension, cerebrovascular cause of death and serum creatinine > 1.5 mg/dl prior to retrieval. As shown in a subset cohort publication of this trial (5), the difference was most striking in ECD kidneys that developed DGF. Machine perfusion was clearly shown to have a protective impact on DGF and GS while Cold Ischemic Time (CIT) has been re-affirmed to be a major risk factor for DGF. During this trial, machine preservation was used during the entire preservation period; from procurement until time of transplantation and the preservation fluid was not actively oxygenated. From a logistical and technical perspective, this trial also demonstrated that it was perfectly feasible to use HMP for organ sharing within an international

organization like Eurotransplant. However it seems obvious that use of HMP only upon arrival of the kidney at the recipient center would facilitate logistics even further.

Whether HMP after initial SCS is effective remains subject of debate. Therefore, research from our group as well as others has been directed towards the possibility of reconditioning the marginal graft by short term in-house treatment immediately prior to the implantation of the graft. This novel strategy, that we termed *'Hypothermic Reconditioning by Machine Perfusion'* (HRMP), was based on the assumption that the ischemic period itself only pre-disposes the tissue to compromised resumption of cellular function upon warm reperfusion, but the major impact of actual preservation induced graft injury are only manifested after reperfusion of the kidney (11-13).

In experimental studies using isolated porcine kidneys, it has been shown that the outcome of potentially marginal grafts could be improved by post-hoc re-vitalization through HMP applied only at the end of the cold ischemia period (14). Here, transplanted kidneys showed improved kidney function after transplantation, when subjected to HRMP. An even higher, threefold increase in renal function could be measured, when HRMP kidneys had been perfused with oxygenated perfusion solution during machine perfusion rather than non-oxygenated perfusion solution. The benefit of a brief period of HMP just prior to renal transplantation was subsequently confirmed in a large animal auto-transplant model showing significantly improved renal function during one week after transplantation when compared to SCS alone (15). This study also demonstrated that HRMP after initial SCS preservation was equally effective at reducing early graft dysfunction after transplantation as the use of HMP during the entire preservation period. Various recent studies performed on either kidney or liver grafts give evidence for improved graft function after transplantation when oxygen is provided during machine perfusion (12, 17-18)

This study is a controlled, sufficiently powered, randomized multi-centre trial to evaluate the impact of end-ischemic reconditioning by oxygenated HMP after initial SCS on graft function of kidneys from extended criteria donors (ECD) after brain death (DBD).

While the overall study comparing HMP versus SCS as published by Moers et al. (8) had been statistically powered based on the assumption to detect a difference of at least 10% in

incidence of DGF; the subset analysis on ECD kidneys (as published by Treckmann et al. (5)) in itself had not been powered to detect one year graft survival differences. Nevertheless, the univariate analysis of this subset study on ECD kidneys revealed a significant impact of HMP on 1 year graft loss, 1-year graft function and incidence of PNF. Multivariate analysis demonstrated that HMP decreased DGF and one year graft loss.

The MP-Trial showed the potential impact of HMP in ECD kidneys. Moreover, pre-clinical studies using a large animal auto-transplant model indicate beneficial effects of short time HMP over SCS; therefore it is the goal of this study to validate these results in a clinical proof of concept study.

3 OBJECTIVES

While the overall study comparing HMP versus SCS as published by Moers et al. (8) had been statistically powered based on the assumption to detect a difference of at least 10% in incidence of DGF; the subset analysis on ECD kidneys (as published by Treckmann et al. (5)) in itself had not been powered to detect one year graft survival differences. Nevertheless, the univariate analysis of this subset study on ECD kidneys revealed a significant impact of HMP on 1 year graft loss, 1-year graft function and incidence of PNF. Multivariate analysis demonstrated that HMP decreased DGF and one year graft loss. This study will compare SCS with a short period of oxygenated HMP after initial cold static storage using an established and certified machine perfusion device.

3.1 PRIMARY OBJECTIVE

To compare 1-year graft survival after HRMP with oxygenated perfusion solution versus SCS of ECD kidneys from DBD donors.

3.2 SECONDARY OBJECTIVES

- To compare graft and patient survival between SCS and short-term HRMP+O_2.
- To compare the estimated GFR (eGFR) as a surrogate of kidney function between oxygenated HRMP and SCS kidneys.
- To compare the incidence of delayed graft function (DGF), slow graft function (SGF) and primary non-function (PNF) between oxygenated HRMP and SCS kidneys.
- To compare the incidence of acute rejection between oxygenated HRMP and SCS kidneys.

- To assess health economic implications of oxygenated HRMP in comparison to SCS kidneys.

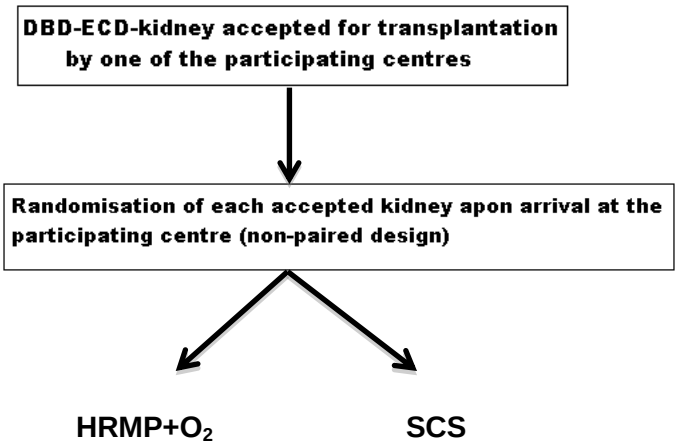
4 TRIAL DESIGN

4.1 SUMMARY OF TRIAL DESIGN

The study will be conducted as a prospective, randomized, parallel group, single blinded, controlled, multi-centre, non-paired superiority trial; allocation will be on a 1:1 basis and an intention-to-treat method will be used to analyze the results.

After accepting the potential ECD organ for donation, kidneys will be allocated following the standard Eurotransplant/ UK-transplant allocation rules.

In a non-paired-design, each ECD kidney that has been accepted for transplantation by one of the participating centres will be randomly assigned to SCS or to SCS followed by reconditioning by end-ischemic oxygenated HMP (HRMP+O_2). Randomizing kidneys after they have been accepted for transplantation avoids a potential bias that acceptance of the kidney is being influenced by the preservation modality.

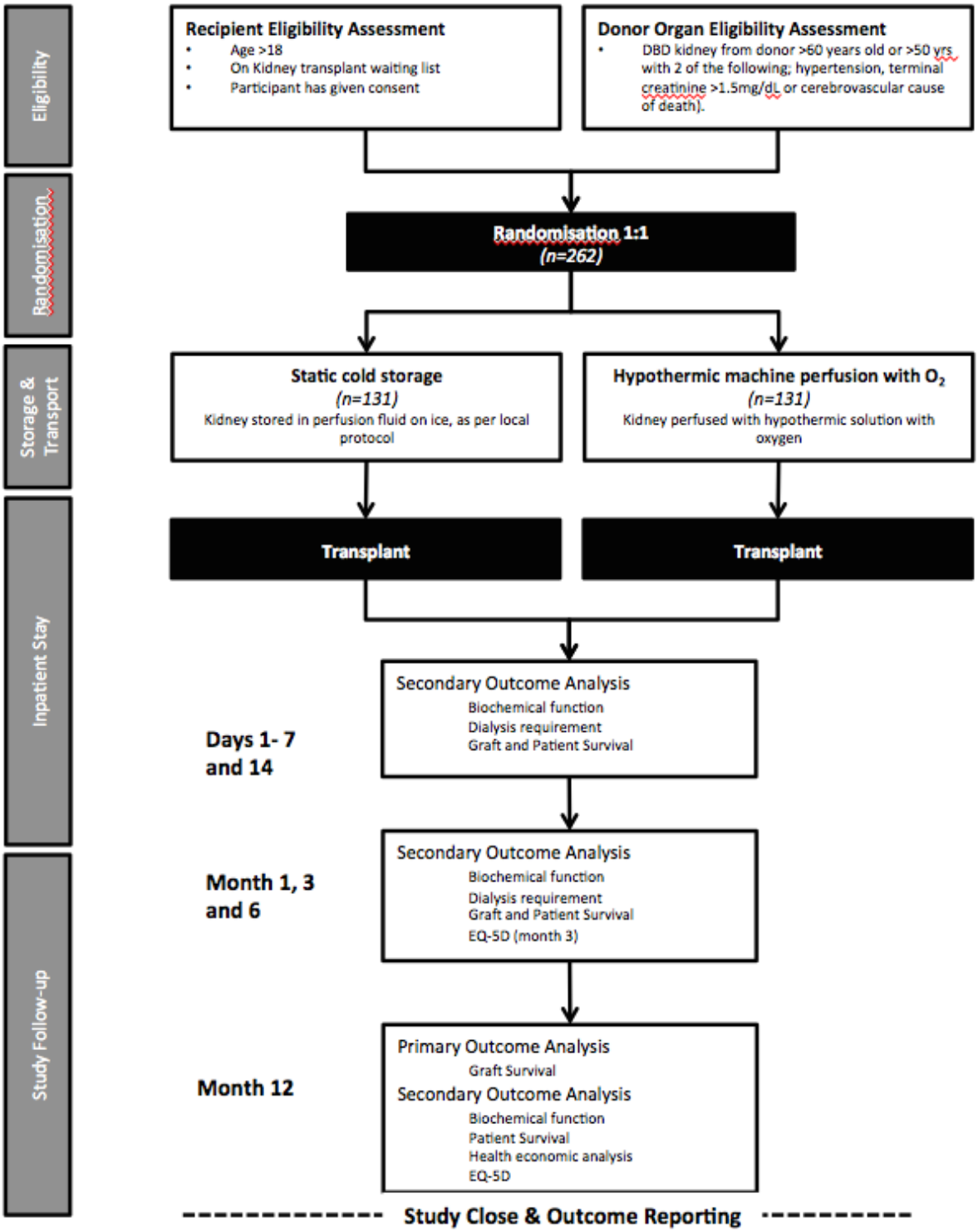


If two kidneys are accepted for transplantation by the same centre the surgeon will decide which kidney to transplant first according to their local protocol.

It is not essential for both kidneys from a donor to enter the trial. If only one of the kidneys is deemed transplantable, it can still be included in the trial analysis.

Exceptionally anatomical situations may not allow connection of a kidney to the perfusion circuit. In this situation the kidney should be preserved by cold storage alone even though it was randomized to HRMP+O_2.

Anticipated flow of patients through the trial is depicted below



4.2 PRIMARY and SECONDARY OUTCOME MEASURES

4.2.1 Primary outcome measures

The primary outcome for this study is Graft survival at 12 months after transplantation.

Reasons for choosing this primary end point are:

- Improvement of graft survival by HMP was most pronounced in kidneys from ECD in the previous publication by Treckmann et al (5).
- Definition of 1-year graft survival is clear and objective (in contrast to DGF): Is graft still functioning after one year: Y/N?
- The clinical and economic relevance of intermediate and long-term graft survival.

4.2.2 Secondary outcome measures

- Patient and (death censored) graft survival at day 7, 3, 6 and 12 months after transplantation. eGFR defined by the MDRD equation at day 7, 3, 6 and 12 months after transplantation.
- DGF defined as the need for dialysis in the first 7 days after transplantation and preceding the return of kidney function.
- SGF based on functional DGF defined as the absence of decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first 7 days after transplantation.
- PNF defined as the continued need for dialysis at 3 months after transplantation.
- Comparison of biopsy proven acute rejection between the 2 groups.
- Quality of life measures (EQ-5D-5L) at consent, 3 and 12 months, length of hospital stay (including ICU) and need/length for dialysis treatment

4.2.3 COPE WP7 Bioresource

Work Package 7 (WP7) of the COPE project aims to establish a bio-repository with biological specimens from clinical trials to support research work relevant to improving the outcomes of kidney and liver transplantation. Using the bio-repository, WP7 aims to identify bio-markers that can be used to predict the outcomes of transplantation. These include markers detected during and after machine preservation and as a consequence of ischemia reperfusion injury. Sample types include blood, perfusate and tissue specimens. Next generation high throughput -omics technology, including proteomics and metabolomics, will be used to evaluate and short-list candidate biomarkers that will be used in conjunction with clinical trial

data. WP7 will support further sub-studies and promote collaborations, through establishing an access policy for samples to be used in research projects outside of the funding scope of the FP7 grant.

The nature and timing of samples to be collected for this bioresource as part of the present study are detailed in appendix A.

4.3 TRIAL PARTICIPANTS

4.3.1 Overall description of trial participants

All ECD kidneys allocated to one of the participating study centres and complying with the inclusion criteria for participant and donor organ selection will be included in the study. Organ allocation and logistics will be unchanged by the study. Logistics will follow standard policies of the participating centres and the study should not change any procedure or the timing of transplantation in individual recipient centres. The participants in the study are all patients registered with EuroTransplant or NHSBT on the kidney transplant waiting list. Donor organs are allocated according to standard procedures.

4.3.2 Inclusion criteria donor kidneys

All kidneys from DBDs fulfilling the UNOS-ECD criteria; donors >60 years old, or >50 yrs with 2 of the following; hypertension, terminal creatinine >1.5mg/dL or cerebrovascular cause of death.

4.3.3 Inclusion criteria transplant participant

- Participant is 18 years old or older.
- Listed for renal transplantation due to end stage renal disease on the ET or NHSBT renal waiting list within one of the participating centers.
- Participant is willing to participate in the study and has provided written informed consent.
- This transplantation is the participant's first or re-transplantation.

4.3.4 Exclusion criteria donor kidney

- Kidneys used for a multi-organ transplant procedure (like Kidney-Pancreas, Kidney-Liver, etc.)
- Kidneys from standard criteria donors (SCD).

- Kidneys procured from DCD donors (i.e. donation after cardiac death) Kidneys used for a double kidney transplant within the same recipient.
- Kidneys procured from donors older than 85 years

Kidneys with multiple renal arteries or missing aortic patch should NOT automatically be excluded from the study. The group allocation should NOT be switched if one of the kidneys demonstrates a more challenging arterial anatomy than the other.

4.3.5 Exclusion criteria transplant participant

- Simultaneous participation in another perfusion trial
- Scheduled to undergo multi-organ transplantation
- Planned dual-kidney transplantation
- Is unable or unwilling to provide informed consent

4.3.6 Exclusion after randomisation

If a kidney is judged not transplantable after randomization this case will be documented with reasons for exclusion and will be excluded from the study.

4.4 STUDY PROCEDURES

4.4.1 Informed consent

The emergency nature of kidney transplantation means that once a potential participant is called in for a transplant there will be a 3-4 hour window for the consent and screening process to occur. This does not allow sufficient time for the potential participant to consider the full implications of participating in the study. For this reason, we will ask the potential participants at the moment of availability of the donor organ only the essential informed consent for that given moment (i.e. biopsy, drawing of blood during transplantation and a questionnaire). After transplantation the participant will receive further information and will be given a week to consider complete informed consent for use of clinical data on follow-up and the two remaining questionnaires.

At that moment we will inform them that we would want to take their consent for access to their data. Patients will have the opportunity to discuss this with a trained individual involved in the trial if they would like via a telephone number that will be included in the patient information letter, handed to them at the moment of arrival in the hospital.

If the patient is not willing to participate in the study then the kidney preservation will be carried out using conventional procedures. If the patient is willing to consent for access to their data, but not for extra biological samples to be taken, they will not be excluded from the study. If the patient is not willing to allow access to their data they will be excluded from the study and biological samples will be destroyed. Since the biopsy is taken routinely, it will not be destroyed, but only be used for the routine, clinical purposes.

The participant will also be required to complete a surgical consent form for the transplant procedure as per standard local policy.

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

A copy of the signed Patient Information Leaflet, as well as the signed and dated consent form will be given to the participant. The original signed form will be retained at the study site and a copy will be placed in the medical notes.

Subjects are free to withdraw consent at any time, irrespective of their initial consent. Subjects who withdraw consent will be replaced.

Each subject must also give permission for the sponsor's representatives to review their hospital records for the purpose of source document verification.

The subject's general practitioner/family doctor will be informed of their participation in the study.

4.4.2 Screening and Eligibility Assessment

Consecutive ECD kidneys that are offered to the participating transplant centres for transplantation will be included in the study. Potential recipients will be informed and consented.

Laboratory tests will include blood samples that will be taken as part of standard follow-up.

4.4.3 Baseline Assessments

The paper version of the EuroQol EQ-5D-5L questionnaire will be completed at the time of consenting the patient to the trial (Appendix D). Here, index-based values are recorded, in order to facilitate calculation of quality-adjusted life years or months of patient survival, that are used to make a health-economic evaluation. The questionnaire will be completed again at 3 and 12 months. Patients who are not willing to complete this questionnaire, will not be excluded from the study.

Donor and recipient demographics will be recorded as per CRF form.

4.4.4 Randomisation

4.4.4.1 Allocation: Sequence generation

Recruiting site personnel will be responsible for randomisation of individual kidneys. Kidneys will be randomly assigned to either cold storage or machine perfusion group with a 1:1 allocation as per a computer generated randomisation schedule held centrally by the trial office. The allocation will be stratified by centre.

4.4.4.2 Allocation: Concealment mechanism

Allocation concealment will be ensured by use of central computerised randomisation (with telephone backup). Allocation will not be revealed until the patient has been recruited to the trial and donor and recipient baseline characteristics have been recorded. Random permuted block length will be used; block sizes will not be disclosed

4.4.4.3 Implementation

Recruiting site personnel will log in to an online data collection and randomisation tool once all criteria for inclusion have been checked. The allocation sequence will be generated on a web-accessible server by the Surgical Intervention Trials Unit (SITU), University of Oxford.

4.4.4.4 Blinding

Transplant participants will be blinded.

Care providers will not be blinded for the treatment arm.

Routine laboratory parameters will be analysed in the central university laboratory not knowing about the allocation to the study groups

4.4.4.5 Emergency unblinding

No formal procedure will be established for the un-blinding of participants. None of the possible complications potentially related to the preservation method (e.g. discarding a kidney, primary non-function) require the absolute need for the participant to know the allocation arm in order to treat them in the best way. All adverse events will be collected and reported.

4.5 TRIAL INTERVENTIONS

4.5.1 Machine perfusion study group

Kidneys initially stored on ice and randomized to HRMP will be placed on the Kidney Assist device (Organ Assist, Groningen, The Netherlands) as soon as possible upon arrival in the transplant center. Arrival time of the kidney on SCS at the transplant center as well as start of perfusion time will be recorded.

The Kidney Assist –manufactured and dispersed by Organ Assist (Groningen, the Netherlands)– is a CE marked medical device designed for isolated hypothermic oxygenated perfusion of a donor kidney during storage. More information on the Organ Assist device can be found in the instruction manual, or on their website www.organ-assist.nl. The renal artery is connected to a cannula and a cold preservation solution (perfusate) is pumped through the kidney while adding oxygen to the perfusate. The Kidney Assist uses a 0.6 litre oxygen cylinder with a pressure of 300 bar. The flow will be set at 100 ml/min. The Kidney Assist pumps the perfusate through the kidney vasculature in a pulsatile way (60 bpm). The Kidney Assist pump is pressure controlled. The pressure can vary from 0 to 60 mmHg according to the chosen setting. The pressure will be set at 25 mmHg and cannot be modified during the preservation of the kidney unless the Kidney Assist gives a ‘flow alarm’. The ‘flow alarm’ is activated when the flow limit of the Kidney Assist is reached (500 mL/min). The set pressure

will then be automatically decreased to a lower value. At this point the connection of the kidney with the Kidney Assist will be checked to rule out any problems with the connection (e.g. leak of perfusion fluid) that can explain the high flow. If no reason for the high flow can be found, then the lower perfusion pressure will be accepted and noted in the eCRF.
Perfusate and kidney are cooled to 0-4°C using crushed ice outside the sterile container. The Kidney Assist continuously registers renal resistance and flow measures.
All kidneys will be perfused with Belzers Machine Perfusion Solution.
Organ Assist will provide training on the use of the machine in all participating centres.
For more details on the Organ Assist device please see Appendix E

In order to allow marginal kidneys to be connected to the perfusion pump as long as possible after arrival at the transplant center, kidneys will be completely prepared for transplantation prior to being placed on the device, if possible. This will allow keeping the kidney on the pump until the recipient vasculature has been completely prepared for implantation of the organ. If applicable, cross-match testing will be performed during machine perfusion time, which is expected to allow a minimum perfusion time of at least 2 hours. In case of a positive cross match or a medical reason due to which the patient cannot undergo transplantation, the kidney will be re-allocated. The accepting transplant center will be able to choose whether they would like the kidney to be transported while connected to the perfusion machine or prefer the kidney to be disconnected from machine perfusion, in which case, the kidney will be placed in cold storage solution.
Use of machine preservation should not prolong the total ischemia time. Total perfusion time as well as total cold ischemia time (CIT) will be recorded.

Perfusion measurements will not be used for accepting/ discarding kidneys. In order to verify whether the kidney is being adequately perfused, the study coordinator will record perfusion flow and renal resistance (RR) at 15 minutes after initiation of perfusion. Any necessary corrective actions will be recorded (check for leakages, obstructions, re-cannulation, etc). Also at the end of perfusion, final flow, RR and temperature will be recorded by the study coordinator. All machine perfusion data will be downloaded at the end of each case. Perfusion parameters such as flow and renal vascular resistance will be hidden to the transplant team. The study coordinator will not be directly involved with recipient care.

4.5.2 Static cold storage (control) group

Control kidneys will be treated according to usual procedures of cold preservation.

No specific preservation solution for SCS will be mandated; type of SCS preservation solution used will be recorded.

After flush out upon retrieval with ice-cold preservation solution the organs will be shipped on ice submerged in the respective solution and transplanted in the implantation center using established standard procedures.

Included participants will receive immunosuppressive regimens according to the standard treatment of their respective transplant centres.

4.6 ANCILLARY AND POST-TRIAL CARE

All participants will be monitored as per normal practice for the participating centres.
Patients will be followed up as per the local protocol.

A schedule of study procedures can be found in Appendix B.

4.7 DEFINITION OF END OF TRIAL

The end of the trial is the date of the last visit at one-year post transplant of the last enrolled recipient.

4.8 WITHDRAWAL OF PARTICIPANTS

The participant will be advised in the consent forms that they have the right to withdraw from the study at any time without prejudice, and may be withdrawn at the discretion of the Investigator at any time.

Reasonable effort should be made to contact any subject lost to follow up during the course of the trial in order to complete assessments and retrieve any outstanding data.

As this randomised controlled trial does not intervene with the patient, we would only expect withdrawals to be due to the patient withdrawing their consent for us to access their data, or if they are lost to follow-up.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised. In case the recipient gives consent, is included in the study but then loses capacity during the running of the trial, first degree relatives or the recipients medical proxy will be able to withdraw consent.

5 SAFETY REPORTING

Reporting of all Serious Adverse Events will be done in accordance with the European Commission Guidelines on Medical Devices Serious Adverse Event Reporting (MEDDEV 2.7/3; December 2010).

It is the responsibility of the local investigator to ensure that all adverse events which fall in to the category of Serious Adverse Events (SAEs) and any device deficiencies (including Serious Adverse Device Effects (SADEs)) are reported to the coordinating centre, chief investigator, principle investigator, national investigators as soon as possible after becoming aware of the event but no later than 24 hours.

Adverse event and serious adverse event reporting will be via the electronic data collection tool using the COPE SAE form, with SAEs being automatically forwarded to the Trial Coordinator and clinical reviewers by the reporting tool. The clinical reviewers are the Chief Investigator, Principle Investigator and Central Investigator and National Investigators. Reporting by Fax will provide a backup system (+32 (0)16 34 87 43) in the event that the online data collection tool is unavailable. The Fax machine is located in the central coordinating centre and is manned during normal office hours only.

Within the following 5 working days, the Local Investigator should provide any additional information on the initial SAE or device deficiency in the form of a written narrative using the same SAE form submitted initially – do not create a new form for follow up information. This should include a copy of the completed SAE form, and any other diagnostic or relevant information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the coordinating centre and clinical reviewers using the same electronic COPE SAE form.

On submission of an electronic SAE form, the co-ordinating centre and all of the clinical reviewers will be immediately notified by email. They will review SAEs and, if they feel they pose an immediate risk to patient health or safety, then they will report them to the DMC immediately and to the device manufacturer and research ethics committees within 2 calendar days of the principle Investigator becoming aware of the event.

All other reported SAEs will be reported to the DMC within 7 calendar days of notification, if appropriate. This will not include SAEs that may be expected as part of the risks of kidney transplant surgery. Adverse device events (SADEs, USADEs) and device deficiencies will also be reported to the device manufacturer. All SAEs will be followed up to resolution. The DMC will review the accumulating data at regular intervals.

SAE reporting will continue until completion of follow up at 1 year after transplantation. Participants transferred back for on-going care to referring centres will have their data including AEs related to the outcome measures collected by data collection forms sent to the participant's specialist. Participant cards will be provided to all participants of the study, with a contact telephone number (research nurse/ researcher) to inform regarding the occurrence of SAEs.

The Principle Investigator will also inform all investigators concerned and the device manufacturer of relevant information about USADEs that could adversely affect the safety of participants.

5.1 ADVERSE EVENT (AE)

According the GCP (Clinical Trial Directive 2001/20/EC), an adverse event (AE) is defined as any untoward medical occurrence in a participant of the treated group during an experiment, and which does not necessarily have a causal relationship with this treatment. This definition includes physical signs, symptoms and laboratory test values. At study enrolment, laboratory values that fall outside the relevant reference range will not be reported as AEs.

5.2 SERIOUS ADVERSE EVENT (SAE)

An adverse event that results in:

- Death
- Life threatening condition
- Persistent significant disability or incapacity
- Prolongation of initial hospitalisation or re-hospitalisation
- Requiring intervention to prevent one of above
- Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.

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

5.3 ADVERSE DEVICE EFFECT (ADE)

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

5.4 SERIOUS ADVERSE DEVICE EFFECT (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

5.5 ANTICIPATED SERIOUS ADVERSE DEVICE EFFECT (ASADE)

An effect which by its nature, incidence, severity or outcome has been identified as a risk associated with the procedure.

5.6 UNANTICIPATED SERIOUS ADVERSE DEVICE EFFECT (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk report.

5.7 DEVICE DEFICIENCY

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling.

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

5.8 USE ERROR

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

5.9 CAUSALITY OF AN AE IN RELATION TO THE INTERVENTION

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

5.10 GRADE OF SEVERITY

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

5.11 ANTICIPATED ADVERSE EVENTS

General

- Infection (chest, urine, blood, wound, abdominal)
- Cardiac failure
- Respiratory failure

Events related to the disease / condition /surgery

- Fluid collection (around transplanted kidney)
- Lymphocele
- Rejection
- Delayed graft function
- Primary non function
- Graft loss
- Admission for suspected rejection
- Occurrence and treatment of abdominal or wound infection
- Respiratory failure requiring appropriate treatment
- Hospitalisation for pre-existing condition that has not deteriorated
- Clinically significant abnormal laboratory finding or other abnormal assessments that is associated with the disease being studied (unless judged by the investigator as more severe than expected for the participant's condition)

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

5.12 PROCEDURES FOR RECORDING ADVERSE EVENTS

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

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

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

It is the responsibility of the local lead investigator to collect all directly observed adverse events and all adverse events spontaneously reported by the participant. In addition each participant should be questioned about adverse events at each visit.

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

5.13 Surgical Complications Classification (within 30 days of transplantation)

The Clavien-Dindo classification (19) will be used to rank surgical complications according to an objective, simple, reliable and reproducible way. This classification is based on the type of therapy required to treat the complication.

Table 1: Clavien-Dindo Classification of Surgical Complications

Grades	Definition
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Acceptable therapeutic regimens are: drugs as anti-emetics, antipyretics, analgesics, diuretics and electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complication. Blood transfusions and total parenteral nutrition are also included.
Grade III	Requiring surgical, endoscopic or radiological intervention.
Grade III-a	Intervention not under general anaesthesia.
Grade III-b	Intervention under general anaesthesia.
Grade IV	Life-threatening complication (including CNS complications) requiring intensive care treatment.
Grade IV-a	Single organ dysfunction (including dialysis).

Grade IV-b	Multi-organ dysfunction.
Grade V	Death of patient.
Suffix "d"	If the patient suffers from a complication at the time of discharge, the suffix "d" (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

5.14 STUDY SUSPENSION OR EARLY TERMINATION

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

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

If suspension or premature termination occurs, the terminating party shall justify its decision in writing and promptly inform the other parties with whom they are in direct communication. The principal investigator and sponsor shall keep each other informed of any communication received from either the ethics committee or the regulatory authority.

If, for any reason, the sponsor suspends or prematurely terminates the study at an individual investigation site, the sponsor shall inform the responsible regulatory authority as appropriate and ensure that the Ethics Committee is notified, either by the principal investigator or by the sponsor. If the suspension or premature termination was in the interest of safety, the sponsor shall inform all other investigators.

If suspension or premature termination occurs,

a) the sponsor shall remain responsible for providing resources to fulfil the obligations from the study protocol and existing agreements for following up the subjects enrolled in the study, and

b) the principal investigator or authorized designee shall promptly inform the enrolled subjects at his/her study site, if appropriate.

Device deficiencies and use errors not falling into the categories of ADEs or SADEs should be reported via the online data collection tool and will be collected by the study investigators for investigation by the manufacturer.

SAE reporting will continue until the last patient recruited has completed 12 months of follow-up. Patients transferred back for on-going care to referring centres will have their data including AEs related to the outcome measures collected by data collection forms sent to the patient's specialist. Patient cards will be provided to all participants of the study, with a contact telephone number (research nurse / researcher) to inform regarding the occurrence of SAEs.

6 STATISTICS

6.1 DESCRIPTION OF STATISTICAL METHODS

The primary analysis of the trial will be an intention-to-treat analysis comparing intervention (HRMP) against control (SCS) for all primary and secondary outcomes. The primary endpoint, one year graft survival, will be analysed using logistic regression with adjustment for stratification factors. Additional analyses adjusting for other important prognostic factors will also be undertaken. Binary outcomes will be assessed using chi-squared tests and logistic regression to adjust for prognostic factors. Continuous outcomes will be compared using the T-test if normally distributed, otherwise using the Mann-Whitney U test. Time-to-event outcomes will be analysed using survival analysis methods, including Kaplan-Meier plots and Cox proportional hazards regression model with calculation of hazard ratios. Outcomes will be reported with 95% confidence intervals and two-sided p-values to 3 decimal places.

A secondary analysis of the primary endpoint will be done as per protocol.

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

6.2 NUMBER OF PARTICIPANTS

Sample size calculation

Based on the previously published paper by Treckmann et al., the 1-year graft survival for ECD kidneys preserved by SCS is being estimated at 80%. The use of HRMP is being hypothesized to improve 1-year graft survival from 80% to 92% based upon the improvement in graft survival of ECD kidneys seen in the MP Trial.

For a power of 80% with Type I error (alpha) of 5%, in a two-sided statistical model, the required sample size to detect an improvement in 1-year graft survival from 80% to 92% is 131 kidneys in each treatment arm, 262 in total.

6.3 RECRUITMENT

Each participating centre has given an estimate of expected participant numbers, fulfilling the inclusion criteria for the study.

Essen: 40 per year
Leuven: 30-45 per year
Oxford: 35-40 per year
Groningen: 20 per year

Based on these data the required number of participants will be recruited in a 2-year study period.
If recruitment is slower than anticipated then additional centres will be approached to participate in the study. Once 262 participants are recruited to the study, recruitment will stop.

6.4 THE LEVEL OF STATISTICAL SIGNIFICANCE

A p value of less than 0.05 will be considered statistically significant.

6.5 PROCEDURE FOR ACCOUNTING FOR MISSING, UNUSED AND SPURIOUS DATA

The central database will be monitored for discrepancies and missing data. The SITU will be

responsible for managing the database, and if such discrepancies are identified the trial coordinator will be responsible for identifying the problem and contacting the local center to ensure resolution. The trial coordinator will be responsible for the production of weekly reports to each participating centre containing information and details of missing data or missed visits requiring completion.

6.6 PROCEDURES FOR REPORTING ANY DEVIATION(S) FROM THE ORIGINAL STATISTICAL PLAN

Procedures for reporting any deviation(s) from the original statistical plan: (any deviation(s) from the original statistical analysis plan (SAP) should be described and justified in a revised version of the SAP and/or in the final report, as appropriate).

6.7 INCLUSION IN ANALYSIS

The selection of participants to be included in the analyses will be detailed in the statistical analysis plan. e.g., all randomised participants, all dosed participants, all eligible participants, evaluable participants.

Only in rare cases when connection to the perfusion circuit is impossible, the kidney will be stored on ice. Data about the number of kidneys that could not be connected to perfusion circuit will be recorded; including reasons for not having connected the kidney to the perfusion circuit. These kidneys remain included in the study for Intention-to-treat analysis (i.e. in an Intention-to-treat analysis; these kidneys will be considered as HRMP kidneys despite the fact that these kidneys have actually been preserved by SCS).

6.8 HEALTH ECONOMICS ANALYSIS

An economic analysis will be performed, with the objective of estimating average costs and effectiveness in each arm of the study. This will inform a cost-effectiveness analysis using a health service perspective and incremental cost effectiveness ratios (ICER's) will be reported. Quality adjusted survival will be obtained by administration of the EuroQol EQ-5D-5L questionnaire. Quality of life data will be collected at baseline (at time of consent) and at two study follow-up visit following kidney transplantation (at 3 and 12 months).

Costs will be estimated based upon measured resource use and national unit costs; overall, average and incremental costs will be reported in Euro using purchasing power parity methods to adjust for variation in cost of living across different countries. Resources will include machine and disposables costs, immunosuppression and other drugs, inpatient hospital stays (including intensive care days), and dialysis. Data will be assessed at each follow-up visit as outlined in the CRF.

7 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

8 MONITORING AND QUALITY ASSURANCE

8.1 DATA MONITORING

A risk assessment will be carried out and the Monitoring Plan written as determined by the risk assessment.

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

8.2 QUALITY ASSURANCE

During the course of the study, the sponsor will appoint quality assurance personnel to provide audit of the administration and conduct of the study. The relevant competent authority could potentially conduct audits / inspections.

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

8.3 DATA MONITORING: INTERIM ANALYSIS

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

8.4 STUDY DOCUMENTATION

It is the responsibility of the local lead investigator to maintain complete, accurate and current study records. Such records will be maintained during the course of the study and for five years following the date on which the study is terminated or completed. Investigator records shall include, but not be limited to the following:

- A current copy of the trial protocol and any amendments
- A copy of the signed protocol agreement
- A copy of the local Competent Authority approval
- Evidence of indemnity insurance
- All information pertaining to Research Ethics Committee submission, review and approval of this study
- A blank copy of the approved patient consent form and patient information sheet
- Copies of current signed and dated curricula vitae of the local investigators and all relevant site personnel
- Delegation and signature logs
- Training records for use of the data entry and randomisation system and for device use
- Signed informed consent forms and supporting documents for online case reporting (laboratory reports, reports of diagnostic tests, medical records etc.)
- Records of all reports and information pertaining to adverse events
- Accountability records, use and disposition of all investigational devices and study materials.

8.5 PROTOCOL DEVIATIONS

A protocol deviation is a failure to adhere to the requirements specified in this study protocol without adequate justification. Examples may include the enrolment of a study patient who does not meet all of the inclusion/exclusion criteria specified in Section 4.3, or missed study procedures without documentation.

All protocol deviations must be recorded and reported to the data monitoring committee. The DMC will review all deviations and assess their impact on patient safety.

The investigators shall conduct this study in accordance with this protocol and any conditions of approval/notification imposed by the Research Ethics Committee and Competent Authority. Failure to comply with and/or inability to meet regulations may jeopardize further participation of the investigator of investigative site in this and future clinical studies.

9 ETHICS

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (2013), the principles of GCP and in accordance with all applicable local regulatory requirements including but not limited to the Research Governance Framework as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to a recognized REC appropriate for each participating centre.

Since machine perfusion is a well-established method for kidney preservation and

- Demonstrated no serious adverse events during the MP trial
- Will be executed with the CE certified Organ Assist device
- Will not prolong total ischemia time in this study

It is estimated that there will be no increased risk for the recipient by participating in this trial. On the contrary, based on previous findings, recipients might benefit from participating in this trial resulting in improved graft survival. The recipient will also be asked for consent to take blood samples and a biopsy of the kidney to be sent to and stored or archived at the bio-bank. If the recipient does not consent to this, it will not preclude the recipient from the trial.

9.1 DECLARATION OF HELSINKI

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki. The Declaration of Helsinki provides detail on what must be included in a protocols: funding, sponsorship, affiliations and potential conflicts of interest, incentives to participate, compensation for harm, post-study access to drugs and care

9.2 ICH GUIDELINES FOR GOOD CLINICAL PRACTICE

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

9.3 APPROVALS

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Trial Management Committee, the sponsor, the REC and receive local approvals prior to implementation.
The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate REC, regulatory authorities, and host institution(s) for written approval.

9.4 PARTICIPANT CONFIDENTIALITY

All study related information will be stored securely at the study site/ study centre. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Laboratory specimens, reports, study data collection, process and administrative forms will be indentified by ID number only to maintain participant confidentiality. Computer data entry will be done exclusively by ID number. Forms, lists and any other listings that link participant ID numbers to other identifying information will be stored in a separate locked file in an area with limited access.

To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

10 DATA HANDLING AND RECORD KEEPING

10.1SOURCE DATA

Source documents are where data are first recorded and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the eCRF), clinical and office charts, laboratory reports, pharmacy records, subject diaries or logs, microfiches, radiographs, correspondence, device accountability records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, and at medico-technical departments involved in the clinical investigation.
eCRF entries will be considered source data if the eCRF is the site of original recording (eg. there is no other written or electronic record of the data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code and not by name.

10.2DATA COLLECTION METHODS

Data collection will be achieved using secure internet-based forms. Data will be input by local lead investigators/coordinators trained in the use of the system prior to receiving log-in details. Data will be uploaded to a central database maintained by the database manager at the SITU. Demographic data, laboratory results and survival data will be recorded locally on the data collection system. Pre-transplantation, 3 and 12-month quality of life data from the EQ-5D-5L and details of resource utilisation will also be recorded.

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

Biopsies taken by centres as part of their protocol will be split, with a portion being analysed locally, and the other portion analysed centrally as part of the COPE bio-resource (WP7) and the biopsy scores recorded in the main database.

10.3DATA COLLECTION METHODS: RETENTION

As described in section 10.2, data will be entered onto online forms, which will be transmitted and stored in a database maintained on a central server at the University of Oxford.

Validation rules prior to submission will ensure that data are entered in the correct format, within valid ranges and minimise the chance of missing data. Data already entered will be retrievable for viewing through the data entry system. The extent of an individual user's activity in the data entry system will be limited by privileges associated with his/her login and password.

All electronic data will be stored in a secure fashion on password protected central servers, with data identified only by the unique participant study ID. A separate database will be maintained to allow identification of study participants, if required for safety reasons, from their unique ID – this will be kept on a different server to the main database.

10.4 DATA MANAGEMENT

All clinical data from each participating centre pertinent to the study will be collected in a specifically designed database at SITU, University of Oxford. Normal documentation for Eurotransplant/ UK-transplant purposes will be carried out in the standard fashion in each participating center. Collected data evaluation is based on pseudonymous registration. Every participant will be given a unique identifier for the duration of their participation and is represented by an individual ET/UK-ODT number, which is representative for the respective data set.

10.5 DATA SECURITY

The database will be developed using ASP.NET (.NET Framework 4.0/4.5) and the database server used will be MySQL (5.6.12). The database/web application will be hosted on an IIS (8.0) web server. The operating system on the server where the applications installed is Windows Server 2012 and it uses a RAID 6 configuration for replicating data across hard drives. The server is managed by the SITU Programmer and the only other person who has access to the server is the departmental (Nuffield Department of Surgical Sciences) IT officer.

The databases will be accessible only over https so that the connection between the server and client is encrypted. No access will be allowed from any computer on any network (including from within the department) to the database server. The port used by MySQL server will be blocked. The access to databases will be controlled by username and password. When the user logs on to the first time, he/she will have to change the password.

The DMC will review recruitment conduct of study and safety and will provide recommendations to trail management committee at regular intervals as per the DMC charter

Final data evaluation will be performed by the trial statistician and the results discussed with the trail management committee.

11 FINANCE AND INSURANCE

Patients will not be charged any additional costs related to the POMP Trial.

12 DISSEMINATION POLICY

12.1 DISSEMINATION POLICY: TRIAL RESULTS

The results of this study are intended for publication.
Publication may include any or all of the following: posting of a synopsis online, abstract and/ or presentation at a scientific meeting, or publication of a full manuscript.
Any publication must be agreed upon by the trial management committee.

12.2 DISSEMINATION POLICY: AUTHORSHIP

Any publication arising from data collected as part of this study will be subjected to the agreed publication policies of the COPE Management Board (as defined in the Consortium Agreement) and Consortium Agreement. Publications will reflect the input of every centre, details regarding this policy will be discussed within the Trial Management Committee and approved by the COPE Management Board. Reports relating to primary outcomes will be published in peer-reviewed journals of appropriate relevance. Participating centres and their Local Investigators Individual centres will not report any trial data independently. A final report on the primary clinical outcomes of the study will be completed by the Central Investigator and PI, discussed in the Trial Management Committee and confirmed by the COPE Management Board.

The Work Package leader at University Hospital Essen, as scientific lead of the Study shall in close cooperation with the COPE Coordinator (i.e. the Chief Investigator) and after discussion with the COPE Management Board initiate, coordinate the review and submission of abstracts, posters and publications. The Work Package leader at the University Hospital Essen will coordinate together with the COPE Coordinator and the representative of Work Package 9 responsible for Dissemination and Exploitation the dissemination of the final results of the Study. The choice of Lead and Senior author in the final clinical trial publication lies with the Work Package leader at the University Hospital Essen. Co-authorship will be advised upon by the Trial Management Committee (as defined in the Protocol) and confirmed by the COPE Management Board. For additional research proposals within the Work Package and between Work Packages, the process agreed by the COPE Management Board shall apply including the formulation of research proposals using the provided format, the submission of proposals to the Trial Management Committee for discussion and then to the COPE Management Board for confirmation and approval.

REFERENCES

(1) Cho YW, Terasaki PI, Cecka JM, Gjertson DW. Transplantation of kidneys from donors whose hearts have stopped beating. N Engl J Med 1998;338(4):221-5.

(2) Lindell SL, Compagnon P, Mangino MJ, Southard JH. UW solution for hypothermic machine perfusion of warm ischemic kidneys. Transplantation 2005 May 27;79(10):1358-61.

(3) Brasile L, Stubenitsky B, Booster M, Kootstra G. The cadaveric kidney and the organ shortage--a perspective review. Clin Transplant 2001 Dec;15(6):369-74.

(4) Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. Lancet 2004 Nov 13;364(9447):1814-27.

(5) Treckmann J, Moers C, Smits JM, Gallinat A, Maathuis MH, Kasterop-Kutz M, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. Transplant Int 2011;24:548-54.

(6) Szwarc I, Garrigue V, Delmas S, Deleuze S, Chong G, Mourad G. [Delayed graft function: a frequent but still unsolved problem in renal transplantation]. Nephrol Ther 2005 Dec;1(6):325-34.

(7) Frei U, Noeldeke J, Machold-Fabrizii V, Arbogast H, Margreiter R, Fricke L, et al. Prospective age-matching in elderly kidney transplant recipients--a 5-year analysis of the Eurotransplant Senior Program. Am J Transplant 2008 Jan;8(1):50-7.

(8) Moers C, Smits JM, Maathuis MH, Treckmann J, van Gelder F, Napieralski BP, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009 Jan 1;360(1):7-19.

(9) Gallinat A, Moers C, Smits JM, Strelniece A, Pirenne J, et al. Machine perfusion versus static cold storage in expanded criteria donor kidney transplantation: 3-year follow-up data. Transpl Int. 2013 Jun;26(6):E52-3.

(10) Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial Study Group. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med. 2012 Feb 23;366(8):770-1.

(11) Menger MD, Lehr HA, Messmer K. Role of oxygen radicals in the microcirculatory manifestations of postischemic injury. Klin Wochenschr 1991;69:1050-5.

(12) Minor T, Sitzia M, Dombrowski F. Kidney transplantation from NHBD after oxygenated low flow machine perfusion preservation with HTK. Transplant Int 2005;17(11):707-12.

(13) Lemasters JJ. V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. Am J Physiol 1999 Jan;276(1 Pt 1):G1-G6.

(14) Koetting M, Frotscher C, Minor T. Hypothermic reconditioning after cold storage improves postischemic graft function in isolated porcine kidneys. Transplant Int 2010;23:38-542.

(15) Gallinat A, Paul A, Efferz P, Luer B, Kaiser G, Wohlschlaeger J, et al. Hypothermic Reconditioning of Porcine Kidney Grafts by Short-Term Preimplantation Machine Perfusion. Transplantation 2012 Mar 21;93:787-93.

(16) White CA, Siegal D, Akbari A, Knoll GA. Use of kidney function end points in kidney transplant trials: a systematic review. Am J Kidney Dis 2010 Dec;56(6):1140-57.

(17) de Rougemont O, Breitenstein S, Leskosek B, Weber A, Graf R, Clavien PA, Dutkowski P. One hour hypothermic oxygenated perfusion (HOPE) protects nonviable liver allografts donated after cardiac death. Ann Surg 2009;250:674-683.

(18) Minor T, Koetting M, Kaiser G, Efferz P, Luer B, Paul A. Hypothermic reconditioning by gaseous oxygen improves survival after liver transplantation in the pig. Am J Transplant 2011;11:2627-2634.

(19) Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D.The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg. 2009 Aug;250(2):187-96.

APPENDIX A: BIOLOGICAL SPECIMENS

Rationale for Sampling to Validate Relationship Histopathology and Function in Different Modes of Preservation after ECD Donation and Kidney Transplantation

Background:

The purpose of the sampling is to underpin the clinical trial in kidney transplantation by assessing (immuno) histopathological criteria of ECD donor kidneys in both arms, with oxygenated end-perfusion vs SCS, and analysis of the relationship of histological markers with donor demographics, donor management, preservation and outcomes after transplantation. Assumed 'gold standard' markers will be validated, and compared to novel molecular signatures and degradation products, developed from identified pathophysiological pathways, that might have potential to predict function and outcomes after transplantation. In addition, this will allow mechanistic research into molecular mechanisms of injury and repair during organ preservation using serum, perfusate and tissue samples.

It is recognised that ECD kidneys are increasingly being utilized to address the organ deficit. However, no accepted, universal, risk stratification tool exists to identify the quality of these organs and predict the outcomes of transplantation. The Remuzzi criteria have been suggested as a histological tool to evaluate extended criteria donor kidneys based on pre-implantation biopsies evaluating the degree of glomerulosclerosis, peri-glomerular fibrosis, arteriosclerosis and interstitial fibrosis and is employed by some transplant centres (1, 2). Other scoring system include the Maryland Aggregate Pathology Index (MAPI) and the total chronic Banff score (4). In the USA, centres that use pre-implantation histological criteria for kidney assessment end up discarding between 20-50% of 'high risk' kidneys (5).

In addition, our understanding of the molecular mechanisms that lead to ECD kidney dysfunction and early graft lost is not fully developed, whilst the implications of age, warm ischaemia on cellular injury are well recognised (6). End-organ injury as a result of cerebral damage and warm ischaemia leads to donor kidneys being further susceptible to preservation injury and enhancement of detrimental effects during reperfusion. Perfusion parameters for standardised kidney assessment and validated quality markers during machine preservation of ECD kidneys have also not been universally or widely accepted (7).

Aims of sample collection:

- Use of the reperfusion biopsies to histologically evaluate ECD kidneys following hypothermic oxygenated machine perfusion compared with cold stored kidneys

- Relate pathology to demographics, function and outcomes, and evaluate current scoring systems
- Use of next generation mass-spectrometry to analyse relevant segments of the nephron with genomics, proteomics and metabolomics techniques to identify candidate biomarkers and validate both new and existing markers predicting outcome of transplantation.
- To establish a simple assay for measurement in routine practice.
- To perform multivariate analyses on perfusion parameters, combined with histological and molecular markers to develop a composite kidney grading scoring system.
- To identify novel pathways of injury and repair in donor organs.

Samples:

Samples will be procured throughout the transplant process, as depicted in Figure 1.
Perfusate samples will be collected during HMP. One 6ml sample will be taken at all time-points and stored at 4°C as soon as possible.

Sampling of blood in the recipient will be done during anaesthesia through central line catheters that are routinely placed.
At each time-point where blood is collected:

- 1x EDTA 6 ml separator tube will be obtained
- 1x Serum 6ml separator tube will be obtained

To ensure minimal sample degradation and pre analytical variability, whole blood is kept at room temperature prior to separation of plasma from cellular parts.
Separation of cells from plasma and serum will be achieved by centrifugation at 1500g for 10 min at room temperature as close as possible to the blood collection. SOPs reflect practical time points for the handling and processing of samples. These SOPs are available to the Research Ethics Committee upon request. After centrifugation plasma and serum samples will be kept at 4°C.

The reperfusion biopsy will be taken at the end of the implantation procedure under vision. 16G biopsy needles will be used to take one tangential upper pole kidney biopsy from the cortex. Samples will be stored in formalin and RNA later. The biopsy site is over-sewn with a

6x0 prolene stitch. Previous experience with this approach has shown, providing that appropriate training is delivered and this approach is used, that the complication rate is extremely low (<1%) which correlates with the published literature (8).

Hypotheses to validate and number of samples required:

Through histological assessment of reperfusion biopsies we will validate:

- The application of the histological criteria to reperfusion biopsies, and the applicability and reliability of histological assessment following hypothermic machine perfusion.
- Validation of markers published in the literature including those known to be associated with acute kidney injury (Figure 2):
- Identification of novel markers and pathways of injury including validation of the following hypotheses:
    - Oxygenated machine perfusion improves basal ATP levels in tissues and allows aerobic metabolism.
    - Autophagy, an energy dependent cell degradation process is promoted as an alternative to apoptosis in an oxygen rich environment and prevents cell necrosis; a process with significant inflammatory repercussions.
    - Less mitochondrial dysfunction, as assessed using mitochondrial functional assays, is seen in hypothermic oxygenated machine perfusion.
    - Less inflammatory cell infiltration and endothelial activation is observed following oxygenated hypothermic machine perfusion, this reduces organ dysfunction and improves post-transplant survival. The greater the number of samples per arm collected, the greater the depth and breadth of research which can be performed.

The standardised conditions and randomised control nature of the study offers a unique opportunity to work on the samples to answer important scientific questions and identify new hypotheses to feed in further technical innovation.

However, limitations surrounding sample collection is recognised. The minimum number of samples required to support the proposed research is approximately 80 biopsies per arm (10).

Below are details of the samples to be collected from WP3 for the bio-repository (Figure 1):

Preservation samples for HMP preserved kidneys

(this does not apply to cold stored kidneys)

Sample	Type	Time point
P1	Perfusate	15 min after HMP start
P2	Perfusate	at end of HMP

Recipient samples

Sample	Type	Time Point
RB1	Recipient blood	taken after induction of anaesthesia, prior to transplant
RB2	Recipient blood	taken after reperfusion, prior to closure
L/R KT1	Kidney tissue biopsy	taken after reperfusion, prior to closure

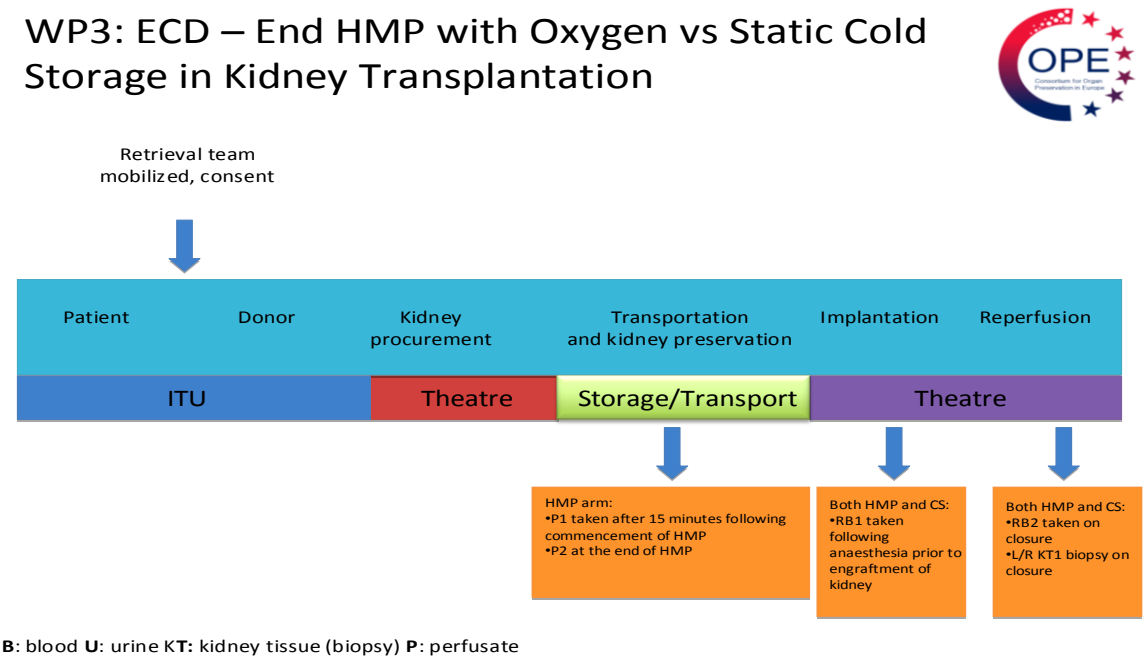


Figure 1. Sample collection during the clinical trial in WP3. P: Perfusate, RB: Recipient blood, L/R KT1: Left/right kidney biopsy

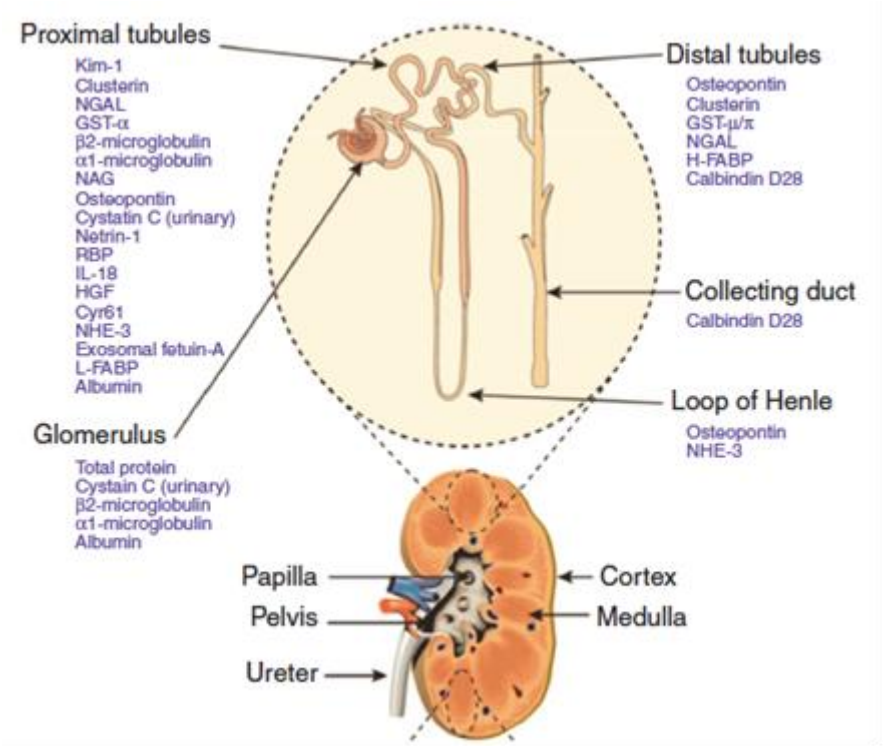


Figure 2. Biomarkers associated with acute kidney dysfunction (9). The biopsies will predominantly be used to assess dysfunction of the glomerulus, proximal and distal tubules.

References:

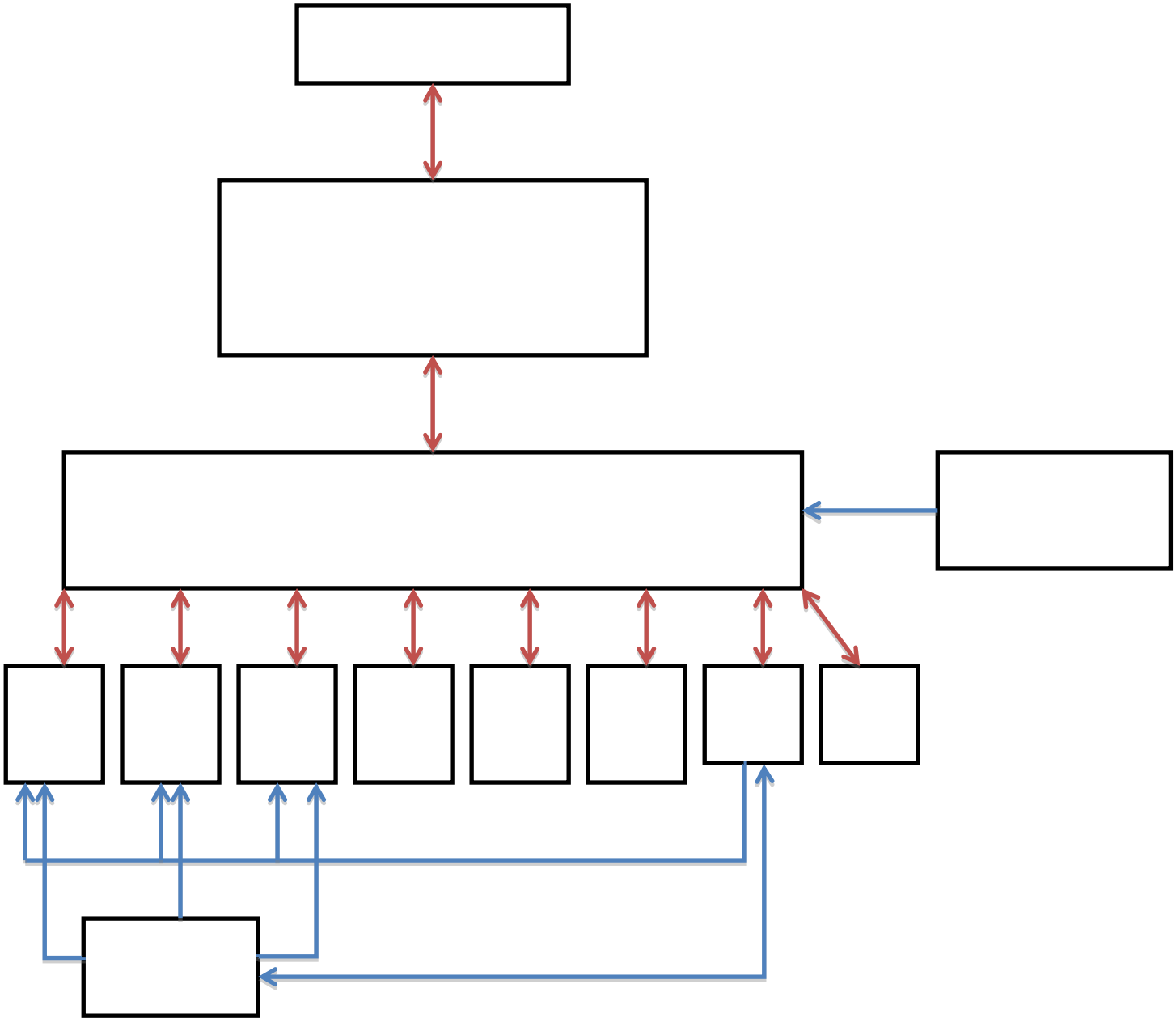
1. Remuzzi G, Cravedi P, Perna A, Dimitrov BD, Turturro M, Locatelli G, Rigotti P, Baldan N, Beatini M, Valente U, Scalamogna M, Ruggenenti P; Dual Kidney Transplant Group. Long-term outcome of renal transplantation from older donors. N Engl J Med. 2006 Jan 26;354(4):343-52.
2. Remuzzi G, Grinyò J, Ruggenenti P, Beatini M, Cole EH, Milford EL, Brenner BM. Early experience with dual kidney transplantation in adults using expanded donor criteria. Double Kidney Transplant Group (DKG). J Am Soc Nephrol. 1999 Dec;10(12):2591-8.
3. Munivenkatappa RB, Schweitzer EJ, Papadimitriou JC, Drachenberg CB, Thom KA, Perencevich EN, Haririan A, Rasetto F, Cooper M, Campos L, Barth RN, Bartlett ST, Philosophe B. The Maryland aggregate pathology index: a deceased donor kidney biopsy scoring system for predicting graft failure. Am J Transplant. 2008 Nov;8(11):2316-24.
4. Snoeijs MG, Buurman WA, Christiaans MH, van Hooff JP, Goldschmeding R, van Suylen RJ, Peutz-Kootstra CJ, van Heurn LW. Histological assessment of preimplantation biopsies may improve selection of kidneys from old donors after cardiac death. Am J Transplant. 2008 Sep;8(9):1844-51.

5. De Vusser K, Lerut E, Kuypers D, Vanrenterghem Y, Jochmans I, Monbaliu D, Pirenne J, Naesens M.The predictive value of kidney allograft baseline biopsies for long-term graft survival. J Am Soc Nephrol. 2013 Nov;24(11):1913-23.
6. Stratta RJ, Rohr MS, Sundberg AK, Armstrong G, Hairston G, Hartmann E, Farney AC, Roskopf J, Iskandar SS, Adams PL. Increased kidney transplantation utilizing expanded criteria deceased organ donors with results comparable to standard criteria donor transplant. Ann Surg. 2004 May;239(5):688-95; discussion 695-7.
7. Jochmans I, Moers C, Smits JM, Leuvenink HG, Treckmann J, Paul A, Rahmel A, Squifflet JP, van Heurn E, Monbaliu D, Ploeg RJ, Pirenne J. The prognostic value of renal resistance during hypothermic machine perfusion of deceased donor kidneys. Am J Transplant. 2011 Oct;11(10):2214-20.
8. Thomas JH, Pierce GE, Hermreck AS. Open renal biopsy. Surgical technique and results. Am Surg. 1983 Jul;49(7):400-2.
9. Murray PT, Mehta RL, Shaw A, Ronco C, Endre Z, Kellum JA, Chawla LS, Cruz D, Ince C, Okusa MD. Current use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. Kidney Int. 2013 Oct 9. doi: 10.1038/ki.2013.374.
10. Lai D, King TM, Moyé LA, Wei Q. Sample size for biomarker studies: more subjects or more measurements per subject? Ann Epidemiol. 2003 Mar;13(3):204-8.

APPENDIX B: SCHEDULE OF PROCEDURES

Activity	Pre-study Screening	Pre-study Baseline	During HRMP (at 15min)	During HRMP (at the end)	During surgery & before engraftment	During surgery & after engraftment (on closure)	D1	D2	D3	D4	D5	D6	D7	D14	M3	M6	M12
Informed Consent	x																
In-/Exclusion Criteria Recipient	x																
In-/Exclusion Criteria Donor		x															
Randomisation		x															
Donor Demographics		x															
Recipient Demographics		x															
Perfusion Parameters & Perfusate Samples			x	x													
Recipient Blood					x	x											
Graft biopsy						x											
Serum Creatinine							x	x	x	x	x	x	x	x	x	x	x
Dialysis requirement							x	x	x	x	x	x	x	x	x	x	x
Graft Survival							x	x	x	x	x	x	x	x	x	x	x
Recipient Survival							x	x	x	x	x	x	x	x	x	x	x
Quality of Life (EQ-5D-5L)	x														x		x

APPENDIX C: COPE COMMITTEE ORGANOGRAM



Advisory

Statutory

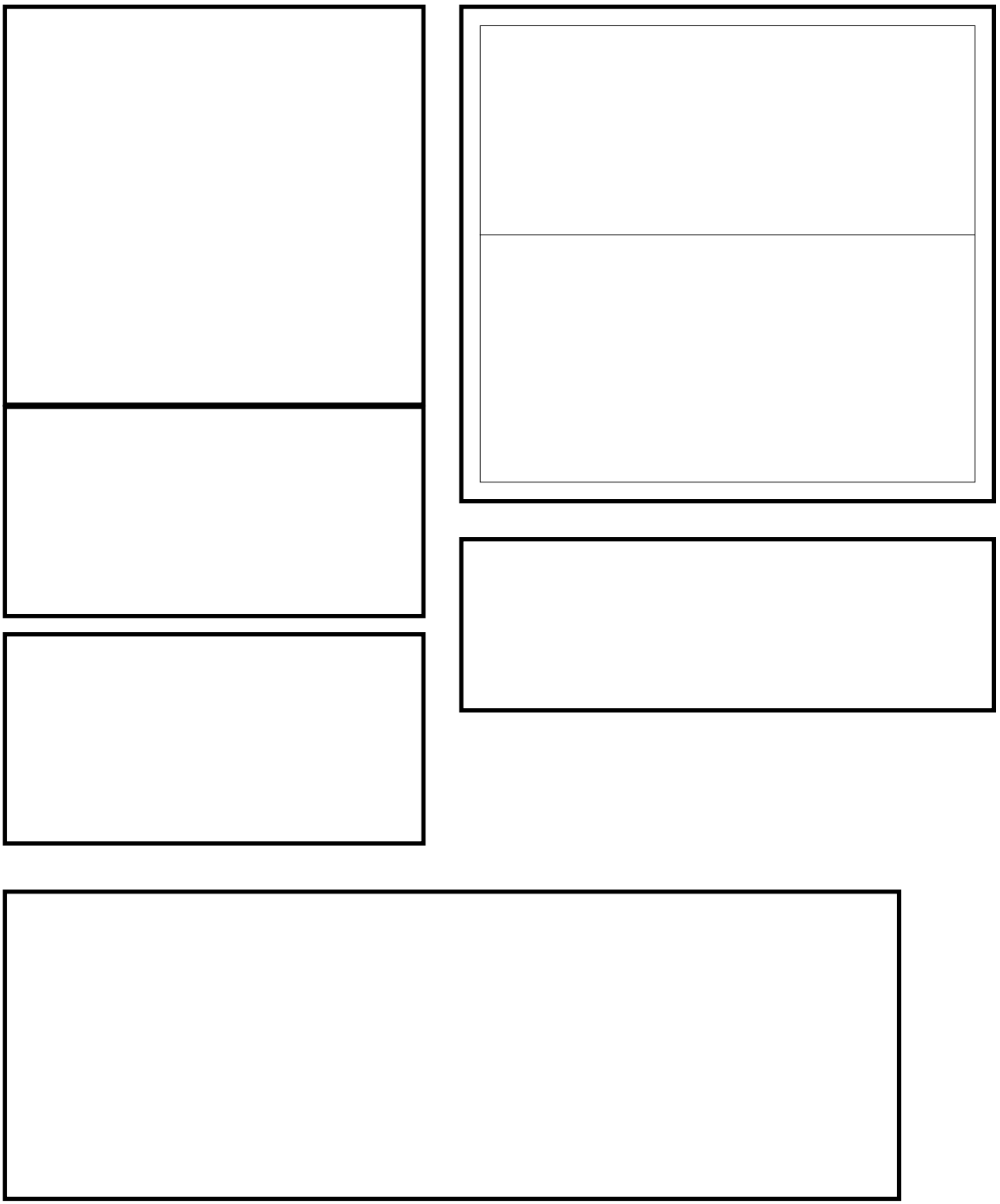
COPE Co-ordinator = Chief Investigator

Work Package (WP) Leader = Principal Investigator

TMC : Trial Management Committee (separate for each trial)

SMC : Scientific Management Committee

SME : Small or Medium Enterprise



APPENDIX D: EQ-5D-5L QUALITY OF LIFE QUESTIONNAIRE

Under each heading, please tick the ONE box that best describes your health TODAY

Mobility

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

Self-Care

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

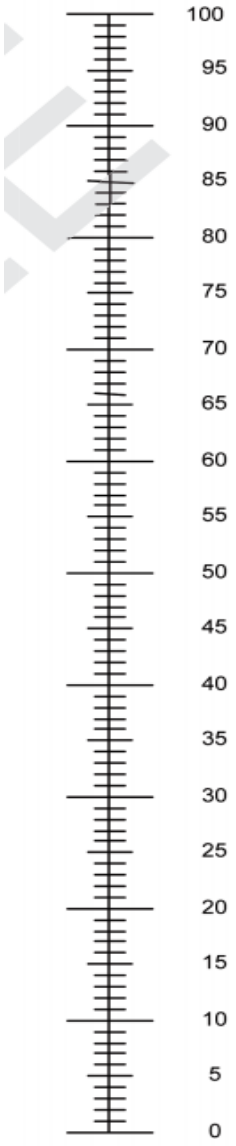
Anxiety/Depression

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

We would like to know how good or bad your health is TODAY.

- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

The best health you can imagine



YOUR HEALTH TODAY = ☐

The worst health you can imagine

APPENDIX E: ORGAN ASSIST- KIDNEY ASSIST DEVICE

Device description

The KIDNEY ASSIST enables isolated and oxygenated donor kidney perfusion during transport from donor to recipient in transplantation procedures.

The KIDNEY ASSIST consists of four main components:

- Pump unit (reusable), including sensors, batteries and oxygen cylinder
- Disposable set, including kidney chamber, perfusion pump head and oxygenator
- Cooling box in carrying bag

The KIDNEY ASSIST is a transportable pump system for controlled, oxygenated and isolated hypothermic perfusion of donor kidneys to bridge the timespan between procurement and transplantation.

Manufacturer:

Organ Assist Products B.V.
Aarhusweg 4 - 7
9723 JJ Groningen
The Netherlands
+31 50 3131905
info@organ-assist.nl
www.organ-assist.nl

Kidney Assist, model number 215C001 rev 02, firmware build 0.0.2.29. The accompanying disposable set is known as model number MEH132209 rev 09.

The Kidney Assist pump unit is traceable by its serial number (SN 10xx). The disposable set is traceable by its LOT number (LOT 201XxxxxMxxx).

In the COPE-POMP clinical study, the Kidney Assist is intended to be used for in-house end-ischemic oxygenated kidney perfusion after static cold storage of MP-assigned ECD kidneys.

The Kidney Assist incorporates a reusable pump unit in combination with a sterile disposable set. The disposable set includes a rotary pump head, organ chamber and reservoir, hollow fibre oxygenator and connecting tubing. The disposable components are manufactured of medical grade plastics (PE, PVC, PC, PU) that have been approved for compliance to EN ISO 10993.

The user group of the Kidney Assist is comprised of 1) surgeon and 2) device operator.

1) Surgeons are expected to have the necessary training and skills to prepare the kidney for canulation. Canulation itself will be demonstrated in an information session organised by Organ Assist. In addition, the device operator will have a Quick Start Manual to be used as instruction reference for the surgeon during canulation.
2) The device operator is expected to have (had) a (bio)medical education, and is familiar with a-septic techniques and OR etiquette. The device operator will be trained in operating and troubleshooting the Kidney Assist in a training session organised by Organ Assist.

In addition to the normal donor kidney procurement and/or back-table handling of the kidney, for the Kidney Assist procedure some extra handlings are involved:

- Clean the kidney of fatty tissue
- Cannulation of the renal artery by either aortic patch cannula or inserted straight cannula in combination with aortic patch cannula.
- Place kidney in cassette, close cassette.
- Insert cassette into chamber in Kidney Assist.

Device Safety

Storage conditions: 5 - 40°C, < 85%RH

Operating conditions: 5 - 25°C, < 85%RH