Consortium for Organ Preservation in Europe

# COPE - COMPARE Trial

A multicentre randomised controlled trial to compare the efficacy of ex-vivo oxygenated hypothermic machine perfusion with non-oxygenated hypothermic machine perfusion of kidneys older than 50 years of age and donated after circulatory death

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| **ISRCTN:** | 32967929 |
| **Protocol Version:** | 4.0 (30th April, 2015) |
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| **National Investigators:** | Prof. Ina Jochmans (Leuven, Belgium)<br>Dr. Isabel Quiroga (Oxford, UK)<br>Dr. Sijbrand Hofker (Groningen, the Netherlands) |
| **Primary Sponsor:** | University of Oxford |
| **Funder:** | European Union Seventh Framework Programme (FP7)<br>Grant agreement 305934 |
| **Countries of recruitment:** | Belgium, the Netherlands, the United Kingdom |
| **Confidentiality Statement:** | This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisations and members of the Research Ethics Committee unless authorised to do so |

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| **Conflicts of Interest:** | None |

# Study synopsis

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| **Trial Title** | A multicentre, double blind, randomised, parallel-group, paired trial to compare the effect of hypothermic machine perfusion preservation with and without the addition of oxygen in transplantation of Maastricht category III kidneys donated after circulatory death from donors aged 50 years or older | |
| **Short Title** | COPE -COMPARE | |
| **Clinical Phase** | Phase III | |
| **Trial Design** | Multicentre randomised controlled trial | |
| **Trial Participants** | Donation after circulatory death donors aged 50 years or older, adult kidney-only transplant recipients | |
| **Planned sample size** | 108 donors, 216 kidneys recipients (108 per arm) | |
| **Follow-up duration** | 12 months | |
| **Planned trial period** | 72 months | |
| | **Objectives** | **Outcome measures/endpoints** |
| **Primary** | To compare the effect of oxygenated versus non-oxygenated HMP of grafts of DCD category III (awaiting circulatory death – controlled), kidneys aged 50 years or older on kidney graft function. | Glomerular filtration rate at 1 year after transplantation (time window of 30 days) as determined by 24-hour creatinine clearance. |
| **Secondary** | To compare graft and patient survival between HMP+$O_2$ and HMP. | 7 day, 3, 6, and 12 month graft and patient survival. |
| | To compare the incidence of delayed graft function between HMP+$O_2$ and HMP kidneys. | Delayed graft function (DGF), defined as the need for dialysis in the first 7 days after transplantation and preceding the return of kidney function |
| | To compare biochemical kidney function between HMP+$O_2$ and HMP kidneys. | Functional DGF, defined as the absence of a 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. |
| | To compare estimated GFR as a surrogate of kidney function between HMP+$O_2$ and HMP kidneys. | eGFR defined by the 4-variable MDRD equation and EPI-CKD equation at 3, 6, and 12 months after transplantation. |
| | To compare the incidence of primary non function between HMP+$O_2$ and HMP kidneys. | Primary non function (PNF), defined as the continued need for dialysis at 3 months after transplantation. |
| | To compare the incidence of acute rejection between HMP+$O_2$ and HMP kidneys. | Comparison of biopsy proven acute rejection between the 2 groups. |
| | To assess the feasibility and safety of HMP+$O_2$ as a method of organ preservation | Adverse events, transplantation and organ discard rates. |
| | To assess the health economic implications of HMP+$O_2$ and HMP in kidney preservation | Quality of life measures (EQ-5D-5L) at 3, and 12 months. Logistical and healthcare costs and resource use. |
| **Device Name** | Kidney Assist, CE approved | |
| **Device Manufacturer** | Organ Assist, Groningen, the Netherlands | |
| **Classification** | 2a | |

**Background**

Kidney transplantation is the best treatment for end-stage renal disease. Because of the ongoing organ shortage, higher-risk kidneys are being transplanted more often and especially the use of kidneys donated after circulatory death (DCD) from older donors (aged 50 years or more) is increasing exponentially. These higher-risk kidneys are especially vulnerable to ischaemia-reperfusion injury and optimal preservation to avoid delayed graft function and improve long term outcomes are vital. The standard method of storing and transporting a kidney for transplantation is to perfuse it with a cold perfusion solution and store the kidney in an ice box. However, it has already been shown that hypothermic machine perfusion preservation, where the cold preservation solution is continuously perfused through the kidney, improves short term graft function. Nevertheless, this preservation method still needs improvement to also increase long term graft function.

**Hypothermic machine perfusion with the addition of oxygen**

During cold storage, kidney metabolism – albeit decreased – continuous in an anaerobic environment, setting the stage for ischaemia-reperfusion injury at the time of transplantation. The addition of oxygen during hypothermic machine perfusion has the potential to reduce the damage that occurs, decrease ischaemia-reperfusion injury and ameliorate graft function.

**Study methods**

In an international randomised multicentre superiority trial with two parallel groups oxygenated hypothermic machine perfusion will be compared with non-oxygenated hypothermic machine perfusion. One kidney from all consecutive DCD III donors aged 50 years or older within the study region will be allocated to hypothermic machine perfusion (control group) and the other to hypothermic machine perfusion with oxygen (study group). Recipients will then undergo kidney transplantation and be managed according to standard local protocols. The two groups will be compared to investigate the potential benefit of the addition of oxygen during hypothermic machine perfusion preservation.

**Outcome measures**

The primary outcome measure will be the measured glomerular filtration rate – the best surrogate of graft function – at one year after transplantation, which is considered to be a predictor of long term kidney graft survival as well. Recipients will be followed for 1 year, with secondary endpoints to include delayed graft function, primary non-function, and graft and patient survival. A health economic analysis will also be performed.

# Protocol sign-off

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| **Chief investigator:** | Signature……<br>Name……Date…… |
| **Principal Investigator:** | Signature……<br>Name……Date…… |
| **Sponsor's representative:** | Signature……<br>Name……Date…… |
| **National Investigators:** | Signature……<br>Name……Date……<br>Signature……<br>Name……Date……<br>Signature……<br>Name……Date…… |
| **Senior statistician:** | Signature……<br>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 to the protocol after notifying the sponsor.

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

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

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

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

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

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

**Signature……** **Name……** **Date…….**

## Contents

# Abbreviations

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| ASADE | Anticipated Serious Adverse Device Event |
| CNS | Central Nervous System |
| COPE | Consortium for Organ Preservation in Europe |
| CRF | Case Report Form |
| CTA | Clinical Trial Assistant |
| DBMS | Database Management System |
| DCD | Donation after Circulatory Death |
| DGF | Delayed Graft Function |
| DMC | Data Monitoring Committee |
| eCRF | electronic case report form |
| ESOT | European Society for Organ Transplantation |
| EC | Ethics Committee |
| ET | Eurotransplant |
| FP 7 | 7th framework programme |
| GFR | Glomerular Filtration Rate |
| HMP | Hypothermic Machine Perfusion |
| IC/ICU | Intensive care/intensive care unit |
| IDMS | Isotope Dilution Mass Spectrometry |
| NHSBT | National Health Services / Blood and Tissue |
| PI | Principle Investigator |
| PNF | Primary Non Function |
| SADE | Serious Adverse Device Event |
| SAP | Statistical Analysis Plan |
| SME | Small and Medium Enterprise |
| SOP | Standard Operating Procedure |
| TMC | Trial Management Committee |

# 1. Administrative information

## 1.1.Funding

The trial is funded within the Seventh Framework Programme (FP7) of the European Union.
http://cordis.europa.eu/home_en.html
Grant number 305934 – Work Package 4
Time of support: 01-01-2013 to 30-06-2017
The funding agency will not take part in, nor has the ultimate authority over the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication.

## 1.2.Trial Sponsor representative

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## 1.3.Trial personnel

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### 1.3.2. SME Partners

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### 1.3.3. Database Programmer

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### 1.3.4. Trial Statistician

Statistical design and analysis will be managed by the Surgical Interventions Trials Unit (SITU) statistician, Virginia Chiocchia, and supervised by Dr. Susan Dutton.

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## 1.4.Roles and Responsibilities

*Chief Investigator, Principle investigator (PI) and Central Investigator*

The CI, PI and Central Investigator will have oversight of:

- Design and conduct of COMPARE
- Preparation of protocols and revisions
- Preparation of standard operating procedures (SOP)

- Preparation of case report forms (CRF)
- Organising Trial Management committee meetings
- Publication of study reports
- Appoint members of the Trial Management Committee

*National Investigators*

In each country a National Investigator (also a Trial Management Committee member) will be identified, to be responsible for follow-up of recruitment, data collection and completeness of eCRFs in his/her country. The National Investigator is the liaison between the Local Investigators (see below) and the Trial Management Committee (see 1.5).

Responsibilities will include:

- Obtaining local Ethics Committee and Research Governance approval (aided by the Central Investigator)
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants

The National Investigators are listed under 1.3.1.

All National Investigators are members of the Trial Management Committee.

*Local Investigators*

In each participating centre a Local Investigator (transplant surgeon or nephrologist) will be identified. Responsibilities include

- Obtaining local Ethics Committee and Research Governance approval (aided by the National Coordinator)
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection
- Follow-up of study participants and completion of CRF during follow-up

A list of the Local Investigators can be found in Appendix 2: Local Investigators

*Central Logistics Coordinator*

The Central Logistics Coordinator is

- Responsible for organisation of the logistics in Belgium and the Netherlands
- Management and conduct of logistics of the trial in Belgium and the Netherlands
- Liaise and communicate with the donor and transplantation hospitals in Belgium and the Netherlands

- Distribution of the Kidney Assist and disposables to National Logistics Centre and Satellite Centres
- Report to the Trial Management Committee on regular basis
- Recruitment, training and responsibility for Transplant Technicians in Belgium and the Netherlands

*National Logistics Coordinators*

The National Logistics Coordinators are

- Responsible for the organisation and management of the National Logistics Centres
- Coordinate formation of Satellite Centres where needed
- Liaise with Central Logistics Coordinator and Regional Logistics Coordinator
- Recruitment, training and responsibility for Transplant Technicians
- Responsible for day to day logistics work
- Responsible for sample collection and processing, eCRF

*Regional Logistics Coordinators*

The Regional Logistics Coordinators are

- Responsible for the organisation and management of the Satellite Centres
- Recruitment, training and for Transplant Technicians
- Responsible for day to day logistics work
- Responsible for sample collection and processing, CRF

*Transplant Technicians*

The Transplant Technicians are responsible for the logistics of the COMPARE trial. They are responsible for randomisation of the donor kidney, delivery of the Kidney Assist to the donor centre in time, assisting the donor surgeon with connection of the kidney to the Kidney Assist, collecting donor data and filling in these data in the eCRF, donor sample collection and processing, assisting the transplant surgeon with removal of the kidney from the Kidney Assist at time of transplantation, collecting recipient data and filling in these data in the eCRF, recipient sample collection and processing, cleaning and storage of the Kidney Assist, and all administration involved with these tasks. The Transplant Technicians will be properly trained in performing these tasks.

*Trial Database Programmer*

The Trial Database Programmer is responsible for the design of the trial database, the online eCRFs and the tutorial for the eCFR. He will design the IT system providing the randomisation procedure, data entry and verification.

*Trial Statistician*

The Trial Statistician is responsible for the development of the statistical analysis plan, providing statistical advice during the set-up, recruitment, follow-up of the trial, analysis and

reporting of trial data. The Trial Statistician will also prepare documentation for and attend the Data Monitoring Committee (DMC) meetings as per the DMC charter.

*Organ Assist*

Organ Assist is responsible for the production and delivery of the Kidney Assist machine perfusion devices. They are responsible for the training of the Transplant Technicians and all other individuals that need to operate the device. They are responsible for repairs and are obligated to deliver a replacement device within reasonable time (24 hours) after a defect device has been reported to Organ Assist by the Logistics Team.

*Med Assist*

Med Assist is responsible for the logistics of the COMPARE Trial in Belgium and the Netherlands. Their role in the UK will be modified to the local logistics organisation. The logistics process includes the following:

- Making contact with the transplant coordinators, Eurotransplant, the Nederlandse Transplantatie Stichting in the Netherlands, the Kidney Pancreas Committee in Belgium, to keep them updated on the progress of the COMPARE trial and discuss with them how we can work together
- Writing SOPS and information material for all users
- Employing and supervising the Transplant Technicians (TT)
- Make sure that the TT is trained to work with the Kidney Assist
- Ensure that the TT is present in time at donor and recipient procedures
- Facilitate the collection of survey data and samples, and enter it in the appropriate format
- Transport and storage of machines and consumables
- Provide assistance during unexpected events during the trial (e.g. broken car, machine that does not work, etc)

*Surgical Interventions Trial Unit (SITU), Oxford UK*

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

Central Trial Manager

The Central Trial 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 Trial Manager will also be responsible for:

- Organisation of trial management committee meetings

- Regular reporting to the sponsor on the progress of the clinical investigation.
- Regional coordination in collaboration with the National Investigator for the UK remit.

## 1.5.Trial Management Committee

The Trial Management Committee is responsible for:

- Agreement of the final protocol
- Reviewing progress of the study and, if necessary, agreeing to changes to the study protocol and/or SOPs to facilitate the success of the study
- Reviewing new studies that may be of relevance to the current protocol

The Trial Management Committee members are:

- Jacques Pirenne (JP) (University of Leuven, Belgium)
- Ina Jochmans (IJ) (University of Leuven, Belgium)
- Rutger J Ploeg (RJP) (University of Oxford, United Kingdom)
- Maria Kaisar (MKa) (University of Oxford, United Kingdom)
- Dirk Ysebaert (DY) (University of Antwerp, Belgium)
- Henri Leuvenink (HL) (University of Groningen, the Netherlands)
- Cyril Moers (CM) (University of Groningen, the Netherlands)
- Sijbrand Hofker (SH) (University of Groningen, the Netherlands)
- Jan Ringers (JR) (University of Leiden, the Netherlands)
- Olivier Detry (OD) (University of Liège, Belgium)
- Ernest van Heurn (EvH) (University of Maastricht, the Netherlands)
- John O'Callaghan (JOC) (Centre for Evidence in Transplantation, United Kingdom)
- Martin Kuizenga (MKu) (Organ Assist, the Netherlands)
- Victoria Rush (VR) (Surgical Intervention Trial Unit, United Kingdom)
- Virginia Chiocchia (VC) (Surgical Intervention Trial Unit, United Kingdom)
- Richéal Burns (RB) (Surgical Intervention Trial Unit, United Kingdom)
- Allyson Bradley (AB) (Surgical Intervention Trial Unit, United Kingdom)

## 1.6.Data Monitoring Committee

The Data Monitoring Committee (DMC) is independent from the trial and is responsible for:

- Agreeing a charter for the conduct of the DMC
- Reviewing data from the study according to the schedule set out in the charter
- Reviewing serious adverse events (device related or not) and any device deficiencies

The DMC consist out of:

- Chair: Christopher Watson (professor of Transplantation, University of Cambridge, United Kingdom)
- Vice chair: Josep Grinyó (professor of Nephrology, Bellvitge hospital Barcelona, Spain)
- Gabriel Oniscu (Consultant Transplant Surgeon and Honorary Clinical Senior Lecturer, Royal Infirmary of Edinburgh, United Kingdom)

- Susan Charman (Statistician, London School of Hygiene and Tropical Medicine, United Kingdom)
- Patrizia Burra (Hepatologist, Liver Transplantation, Padua, Italy)

# 2. Background and rationale

## 2.1. The donor shortage and the changing donor population

Kidney transplantation is the preferred treatment for end-stage renal disease because it results in improved patient survival, improved quality of life and cost reduction compared to chronic dialysis.[1, 2]

Over the last decade however, the demand for organs has far exceeded organ availability. This has, in part, been attributed to an ageing population with increased incidence of diseases that lead to end stage organ failure, such as diabetes. In the past the most important problem in transplantation was to achieve better immunosuppression to prevent rejection and subsequent graft failure. Due to major improvements in the therapeutic regimes and tailored immunosuppression, the focus has now shifted to achieving immunosuppression minimisation. To date the key problem and Achilles heel in transplantation is the persistent donor organ shortage in absolute numbers.

In addition, the past decades have seen a relative reduction in the number of organ donors, due to welcome improvements in neurosurgical treatment, intensive care management and road safety. In the Eurotransplant zone for example, the relative proportion of cerebral trauma donors has declined from 43% in 1990 to 35% in 2005.

The organ shortage is predicted to worsen over the course of the next decade, making it one of the most important issues facing the medical community today. As a result, the transplant community has been turning to alternative organ sources to bridge the current shortage. This includes older organ brain dead donors, who have additional co-morbidities, such as diabetes, renal impairment, cardiac disease and hypertension. Organs obtained from these expanded criteria high risk donors have poorer short- and long-term outcomes when compared to organs obtained from “optimal” brain dead organ donors or living donors.

In addition to expanded criteria donors, donors who have been declared dead by circulatory rather than neurological criteria, referred to as donation after circulatory death (DCD), are being used to bridge the organ shortage. In 1995, the first international workshop on DCDs established a consensus on categories of DCD organ donation (Table 1~~Table 1~~). Hereafter DCD will refer to DCD Maastricht category III donors.

**Table 1** Maastricht classification of Donors after Circulatory Death[3]

| Maastricht Classification | Description | |
|---|---|---|
| I | Dead on arrival into hospital | Uncontrolled |
| II | Unsuccessful resuscitation | Uncontrolled |
| III | Awaiting circulatory arrest | Controlled |
| IV | Cardiac arrest after brain stem death | |

The use of DCD donors is rapidly increasing, and among them the percentage of DCD donors older than 50 years of age has risen to up to 60% of DCD donors in Belgium, the Netherlands and the United Kingdom (Fig. 1~~Fig. 1~~). "Older" DCDs are likely more susceptible to preservation-induced injury resulting in poorer short and long term outcome (as has been shown in expanded criteria donors). Therefore, research is now focussing on improving preservation of these grafts to improve short and long term outcomes. These kidneys may benefit more from improved preservation strategies, i.e. the use of machines to perfuse and preserve the kidney from time of removal in the donor until transplantation in the recipient.


| | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 |
|---|---|---|---|---|---|---|---|---|---|---|
| B | 36% | 60% | 20% | 41% | 53% | 51% | 33% | 63% | 47% | 60% |
| Ndl | 46% | 37% | 46% | 45% | 63% | 58% | 55% | 62% | 55% | 64% |
| UK | 43% | 44% | 39% | 47% | 48% | 46% | 50% | 63% | 63% | 66% |

**Fig. 1** Percentage of effective DCD III donors ≥ 50 y compared to total DCD donors in Belgium (B), the Netherlands (Ndl), United Kingdom (UK).[4, 5] Of note: UK: effective kidney donors, B / Ndl DCDs not specified whether kidneys were donated; however liver only in DCD is uncommon.

## 2.2.Machine perfusion preservation

Hypothermic machine perfusion (HMP) preservation is a well-established standard method of kidney preservation. A recent randomised controlled trial showed that continuous HMP – started immediately after procurement until transplantation – is superior to static cold storage (SCS) for the preservation of DCD kidney grafts.[6, 7] Pumped DCD category III kidneys[8] suffered less delayed graft function (DGF) and functioned better early post-transplant. In this trial, the preservation solution used to perfuse the kidney was not actively oxygenated. At present, HMP is indeed not supplemented with oxygen based on the presumption that air equilibrated perfusion solutions are

sufficient to support energy metabolism (oxygen consumption at 4°C is approximately 5% of that found at body temperature).[9, 10] However, several experimental studies have reported that with non-oxygenated HMP levels of oxidative stress, namely lipid peroxidation, are elevated despite improved function compared with static storage conditions, possibly as a result of the hypoxic conditions.[11, 12] Therefore, the addition of oxygen under these conditions may be of particular importance.

There are currently no reported clinical studies directly assessing the effect of continuous oxygenation during HMP in kidney transplantation. Nevertheless, animal experiments have shown that addition of oxygen during HMP may be beneficial, particularly for DCD organs that have suffered warm ischaemic injury, by increasing/restoring ATP levels.[13, 14] Furthermore, a preclinical porcine study showed that the addition of oxygen during HMP for DCD kidneys improved initial graft function and reduced interstitial fibrosis at 3 months after transplantation.[15]

# 3. Objectives and Endpoints

**Hypothesis:**

Null hypothesis is that there is no difference between oxygenated HMP and non-oxygenated HMP for storage of kidneys of DCD category III donors 50 years of age or older.

## 3.1. Objectives

**Primary objective:**

To compare the effect of oxygenated versus non-oxygenated HMP of grafts of DCD category III (awaiting circulatory death – controlled), kidneys aged 50 years or older on kidney graft function.

**Secondary objectives**

1. To compare graft and patient survival between HMP+$O_2$ and HMP-$O_2$.
2. To compare the incidence of delayed graft function between HMP+$O_2$ and HMP-$O_2$ kidneys
3. To compare biochemical kidney function between HMP+$O_2$ and HMP-$O_2$ kidneys
4. To compare estimated GFR as a surrogate of kidney function between HMP+$O_2$ and HMP-$O_2$ kidneys
5. To compare the incidence of primary non-function between HMP+$O_2$ and HMP-$O_2$ kidneys.
6. To compare the incidence of acute rejection between HMP+$O_2$ and HMP-$O_2$ kidneys.
7. To assess the feasibility and safety of HMP+$O_2$ as a method of organ preservation

**Tertiary objective**

To assess the health economic implications of HMP+$O_2$ and HMP-$O_2$ in kidney preservation.

## 3.2. Endpoints

### *3.2.1. Primary endpoint*

Glomerular filtration rate at 1 year after transplantation (time window of 30 days) as determined by 24-hour creatinine clearance.

This endpoint was selected as it is considered (1) to better reflect true glomerular filtration rate of the transplanted kidney compared to estimated glomerular filtration rate or serum creatinine values,[16-25] (2) be reproducibly attainable at all study sites, (3) be of clinical importance and already integrated in daily practice. Furthermore, glomerular filtration rate is an independent predictor of graft survival at the medium to long-term follow-up in all donor types.[26, 27]

### *3.2.2. Secondary endpoints*

1. 7 day, 3, 6 and 12 month graft (censored and uncensored for recipient death) and patient survival
2. DGF defined as the need for dialysis within the first 7 days after kidney transplantation and preceding the return of kidney function.
3. Functional DGF defined as the absence of a decrease in the serum creatinine level of at least 10% per day for at least 3 consecutive days in the first week after transplantation, not including patients in whom acute rejection or calcineurin inhibitor toxicity is proven on biopsy.[28]
4. Estimated glomerular filtration rate according to the 4-variable MDRD equation[29] and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation at 3 months, 6 months and 1 year after transplantation.
5. Primary non function (PNF) defined as the permanent lack of function of the graft from time of transplantation until months post-transplant. This endpoint is determined post-hoc at 3 months post-transplant.
6. Biopsy proven acute rejection.
7. Incidence of kidney discard at the recipient centre (so after the kidney has already been placed on the Kidney Assist)

### *3.2.3. Tertiary endpoints*

Resource use, logistical and healthcare costs to include length of recipient hospital stay and number of post-transplant dialysis sessions up until 1 year. A Quality of Life questionnaire (EQ5D-5L) will also be used and data collected at 3 and 12 month follow ups.

# 4. Trial Design

## 4.1.Trial design

The COMPARE Trial is designed as a surgeon, treating physician and patient blinded, randomised, controlled, multicentre superiority trial with two parallel groups and a primary endpoint of 24-hour creatinine clearance at one year after kidney transplantation. The COMPARE Trial will be carried out in academic hospitals with an active adult kidney transplant programme in Belgium, the Netherlands and the South region of the United Kingdom (UK) and their donor hospitals. A list of participating centres can be found in Appendix 2: Local Investigators.

**Kidney Donors**

When a donor kidney becomes available, transplant centres involved in the trial will be informed by the European Transplant register (ET) in Belgium and The Netherlands and by the National Health

Service Blood and Transplant in the UK. It is important to note that absolutely no changes will be made to national and international kidney allocation rules. The standard kidney allocation rules of ET and NHSBT will be followed. Although Belgian, Dutch and UK centres will be recruiting recipients into the COMPARE Trial, allocation will remain within the regions of the nationally approved allocation agencies (i.e. currently ET and NHSBT).

**Randomisation**

The retrieval surgeon will assess the organ's suitability for transplantation. If both kidneys of the same donor are suitable for transplantation and all inclusion and exclusion criteria are met, the kidney pair will be randomised to either HMP+$O_2$ or HMP-$O_2$. The kidney pair will be randomised by a Transplant Technician using a bespoke online randomisation tool and baseline data will be captured. The kidney will then be connected to the Kidney Assist machine. The Transplant Technician will set up the Kidney Assist to either oxygenated or non-oxygenated perfusion depending on the randomised allocation (in Belgium and The Netherlands this will be a trained Transplant technician from Organ Assist/Med Assist and in the UK will be a trained Transplant technician from a separate Logistics Team). Blinding of study allocation to all other members of the surgical team and researchers will be maintained as far as possible.

**Kidney Recipients**

ET (Belgium and The Netherlands) or NHSBT will identify suitable participants awaiting kidney transplantation. The study does not interfere or change the process of accepting or declining a kidney offered to a certain recipient in any way. As it is the organ itself being randomised and not the recipient, consent to use data collected, blood and tissue samples for research purposes will be sought. If possible, initial consent to use data, blood and tissue from recipients will be obtained prior to an organ becoming available. Once a suitable recipient for the randomised kidney is identified, the recipient will be offered the kidney and invited to the relevant transplant centre for the surgical procedure as per routine procedure. Standard care at individual transplant centres will apply. Prior to the kidney transplantation, informed consent to use their data, blood and tissue for the research study will be confirmed.

### 4.1.1. Concomitant care

*Donor:* Donors will be managed by local standard of care and ET/NHSBT protocols. No changes in donor management will be made for the sake of the COMPARE Trial. In situ flushing of the kidney during organ retrieval will be done according to local standard of care and ET/NHSBT protocols.

*Recipient:* Concomitant care of the recipient, including the implantation procedure, postoperative care, immunosuppression and other medications will reflect the current standard of care at the recipient hospital. Local immunosuppression protocols will not be altered for the sake of the COMPARE Trial.

### 4.1.2. Interventions

Eligible kidneys will be randomised in equal proportions between control and intervention.

**Group 1 – control group**: the kidney will be placed on the Kidney Assist HMP device and perfused with Belzers Machine Preservation Solution at a pulsatile pressure of 25 mmHg starting immediately after retrieval until back-table preparation immediately before kidney transplantation.

**Group 2 – intervention group**: the kidney will be placed on the Kidney Assist HMP device and perfused with oxygenated Belzers Machine Preservation Solution at a pulsatile pressure of 25 mmHg starting immediately after retrieval until back-table preparation immediately before kidney transplantation. Oxygen will be delivered at a rate of 100 ml/min via an oxygenator provided with oxygen from a portable oxygen cylinder, resulting in a partial oxygen tension in the perfusate of 90 kPa (for reference, +/- 675 mmHg).

#### 4.1.3. Modifications

- Since the intervention is a one-time event occurring prior to transplantation, the assigned study intervention will not need to be modified or discontinued once the kidney is transplanted.
- In the event that the machine fails, the kidney will be cold stored and preserved to allow transplantation (see earlier). The kidney does not need to be disconnected from the machine to allow proper cold storage. In cases where the cold-stored kidney is transplanted, the recipient will still be included in the intention-to-treat analysis.

#### 4.1.4. Adherence

Since the intervention occurs prior to kidney transplantation, there are no study specific changes to standard follow-up care (24-hour creatinine clearance at 12 months is standard of care), no additional strategies – outside general measures to ensure regular recipient follow-up - for monitoring and improving adherence are necessary.

### 4.2.Participant timeline

The timeline for interventions, data and sample collections, and measurements is shown in Table 2.

#### 4.2.1. Follow Up Visits

All follow-up visits and endpoint measures during follow-up are of standard care after kidney transplantation. The only study specific items are the EQ-5D-5L and resource use questionnaire that needs to be filled in.

The time window for follow up appointments is
Day 1 through 7 as stated
Month 3 +/- 7 days
Month 6 +/- 14 days
Month 12 +/- 30 days

During the course of the trial the following data will be retrieved:

- Donor demographics
- Recipient demographics
- Preservation parameters
- Surgical variables
- Graft and patient survival
- Follow-up data

- Quality of Life and Resource Use data

A non-exhaustive list can be found in Appendix 1: Data collection parameters

Additional biological samples will be obtained to be stored centrally for use in future studies of the pathobiology of kidney transplantation (COPE WP7, as described in Appendix 3). A materials consent from the recipient will be obtained to specifically address the collection of these histological specimens and plasma and serum samples. Refusal to consent for the storage of these samples will not preclude inclusion in the trial.

All samples will be collected in accordance with national regulations and requirements including standard operating procedures for logistics and infrastructure. In the UK, samples will be taken in appropriately licensed premises, stored and transported in accordance with the HTA guidelines and local trust policies. Samples for long-term storage will be kept in the Oxford Radcliffe bio resource. The stored tissues will be held under an extension of the University of Oxford's HTA license (12217). Analyses on these samples will be carried out according to protocols for Work Package 7 of the overall COPE project as described in the technical annex of the COPE grant agreement.

During the course of the trial the following research samples will be collected: (further information in Appendix 3: Samples)

- Donor samples: blood sample in the donor
- Preservation samples: perfusate samples of the kidney during preservation
- Recipient samples:
    - Blood immediately after induction of anaesthesia through central venous catheter
    - Blood 1 hour after reperfusion of the kidney (this is during anaesthesia, before closure) through the central venous catheter
    - Kidney biopsy 45 minutes to 1 hour after reperfusion, in essence immediately before closure (this is during anaesthesia, before closure)

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

### 4.2.2. Flow chart with estimated drop out

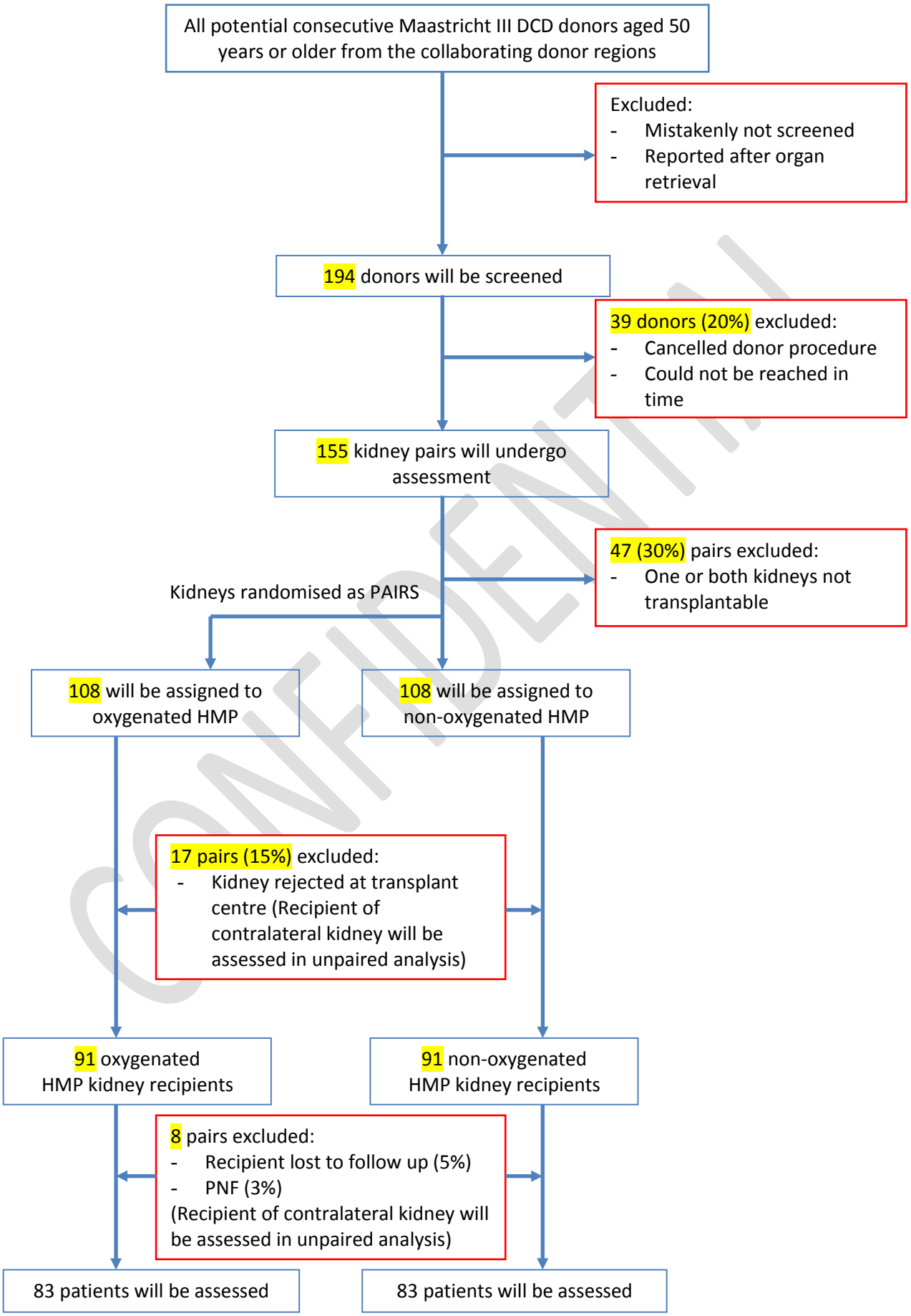


**Table 2** Timeline for interventions and assessments during the study

| Activity | Pre-study | Pre-study baseline | Pre-storage | Pre-surgery | Perioperative | Postoperative | | | | | | | Follow-up | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | D1 | D2 | D3 | D4 | D5 | D6 | D7 | M3 | M6 | M12 |
| Donor screening | X | | | | | | | | | | | | | | |
| Team to donor | X | | | | | | | | | | | | | | |
| Randomisation | | X | | | | | | | | | | | | | |
| Donor demographics | | X | | | | | | | | | | | | | |
| **Donor** blood* | | X | X | | | | | | | | | | | | |
| Preservation parameters | | | | X | | | | | | | | | | | |
| Perfusate **samples*** | | | | X | | | | | | | | | | | |
| Recipient screening | | | | X | | | | | | | | | | | |
| Informed consent | | | | X | | | | | | | | | | | |
| Recipient demographics | | | | X | X | | | | | | | | | | |
| Recipient blood* | | | | | X | | | | | | | | | | |
| Kidney biopsy* | | | | | X | | | | | | | | | | |
| Surgical variables | | | | | X | | | | | | | | | | |
| Dialysis requirement | | | | | | X | X | X | X | X | X | X | X | X | X |
| Graft survival | | | | | | X | X | X | X | X | X | X | X | X | X |
| Recipient survival | | | | | | X | X | X | X | X | X | X | X | X | X |
| Serum creatinine | | | | | | X | X | X | X | X | X | X | X | X | X |
| 24-h creatinine clearance | | | | | | | | | | | | | | | X |
| Quality of Life (EQ-5D) | X | | | X | | | | | | | | | X | | X |

*these are all research samples; for further information see Appendix 3: Samples

The time window for follow up appointments at M3, M6 and M12 is as follows: M3 +/- 1 week, M6 +/- 2 weeks, M12 +/- 1 month

# 5. Participants and interventions

## 5.1.Eligibility criteria

All donor and donor kidney eligibility criteria must be met at the time of randomisation.

### 5.1.1. Eligibility criteria for the donor and donor kidney

*Inclusion:* All potential consecutive Maastricht category III DCD donors[8] (awaiting circulatory death – controlled), aged 50 years or older from the collaborating donor regions reported to Eurotransplant (ET) / National Health Service Blood and Transplant (NHSBT) are eligible for randomisation.

Potential donors will be managed by the local transplantation coordinator according to loco-regional and ET/NHSBT guidelines.

*Exclusion:*

- Kidney deemed untransplantable by the retrieval surgeon. The contralateral kidney will also not be randomised as the trial is designed as a paired trial..

### 5.1.2. Eligibility criteria for the recipient

*Inclusion:*

- At least 18 years of age
- Listed for renal transplantation due to end stage renal disease on the ET or NHSBT renal waiting lists within one of the participating centres
- Willingness to comply with the protocol procedures for the duration of the study, including scheduled follow-up visits and examinations

*Exclusion:*

- Scheduled to undergo multi-organ transplantation
- Planned dual kidney transplantation

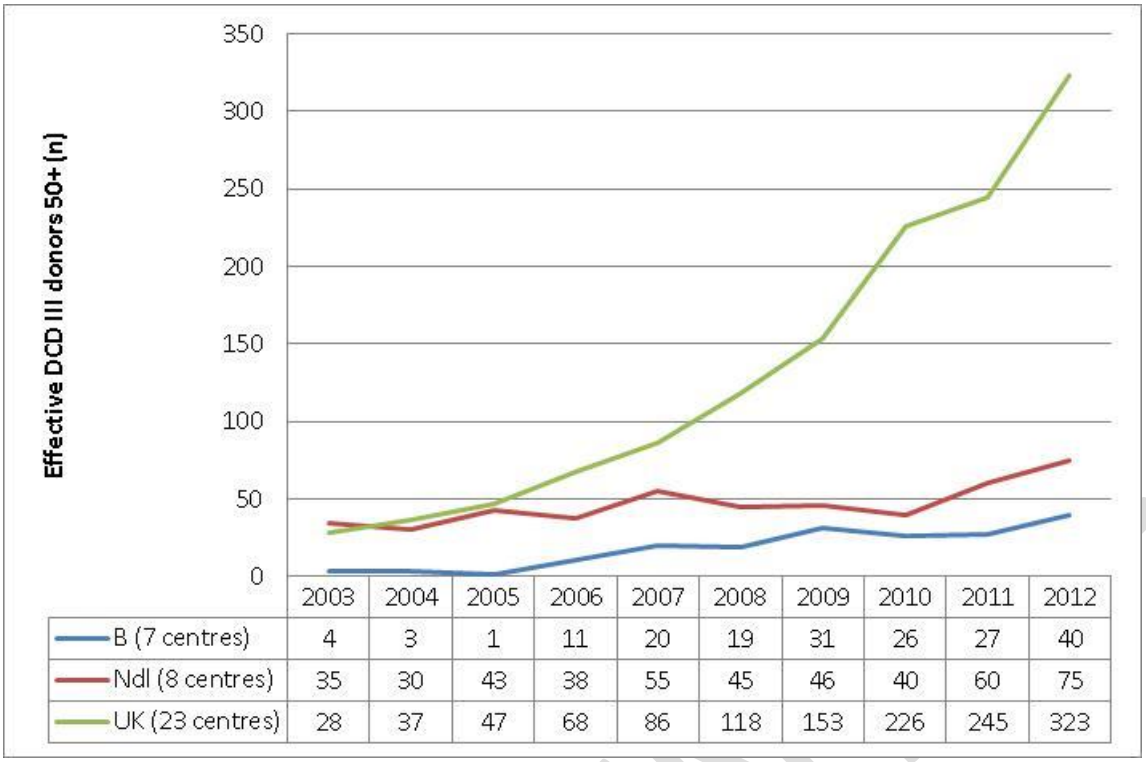
# 6. Recruitment

Based on expected numbers to be enrolled in the participating centres per country (see Fig. 2) we estimate an inclusion period of 12 to 18 months.

A conservative estimate for the number of inclusions per country per year is:

- Belgium: 40 donors per year
- The Netherlands: 75 donors per year
- United Kingdom: 60 donors per year

Strategies to promote recruitment will consist of the necessary logistical help for the donor and recipient centres to limit the time investment needed for the trial. Since the trial includes all kidney transplant centres in Belgium and the Netherlands, and it is endorsed by ET and the NHSBT, recruitment will be facilitated.




| | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 |
|---|---|---|---|---|---|---|---|---|---|---|
| B (7 centres) | 4 | 3 | 1 | 11 | 20 | 19 | 31 | 26 | 27 | 40 |
| Ndl (8 centres) | 35 | 30 | 43 | 38 | 55 | 45 | 46 | 40 | 60 | 75 |
| UK (23 centres) | 28 | 37 | 47 | 68 | 86 | 118 | 153 | 226 | 245 | 323 |

**Fig. 2** Number of effective DCD III donors ≥ 50 y in Belgium (B), the Netherlands (Ndl), United Kingdom (UK).[4, 5] UK: effective kidney donors, B / Ndl DCDs not specified whether kidneys were donated; however liver only in DCD is uncommon. Data from NHSBT and ET.

## 6.1.Consent

### 6.1.1. Informed consent of donors

When national law requires, informed consent for participation in the trial will be requested from the donor's relatives. Each donor/donor family will have been given the opportunity to consent previously for their tissue to be used for research using routine site-specific consent forms. These consent forms will be checked to ensure the eligibility of the donor for this study prior to continuation of this kidney into the study if national law requires this.

During the course of the study, the donor's details will be kept anonymous (specific study identification codes will be used for each study donor). Donor data will only be made available to authorised staff of the study sponsor, its authorised representatives and regulatory authorities.

### 6.1.2. Informed consent of recipients

In order to maximise the potential benefit of pre-transplant machine perfusion, the donor kidney needs to be placed on the machine as soon as possible after retrieval. This will inevitably be before the potential recipient arrives in the hospital, and usually some time before that recipient has been identified (see Fig. 3). It would be desirable to have fully informed consent from the recipient prior to any intervention being undertaken on the kidney. However the necessity to maximise the length of time on the machine means that it will usually not be possible to consent the recipient ahead of time. Hypothermic machine perfusion preservation is a standard technique. Subjects will therefore be asked to consent to prospective collection of their data for study purposes and to obtain additional blood samples during the transplantation at the time of their admission to the transplant centre (see

Appendix 3: Samples). Furthermore, an informed consent will be requested at time of admission to obtain a tissue sample during transplantation if this is not routine in the recipient's transplant centre. All information sheets and consent forms have been translated into English, Dutch, French and German.

One option that has been considered is to consent all the potential recipients on the waiting lists at the participating centres. This would amount to consenting over two thousand patients. However, it is unlikely that a particular patient on the waiting list is called in for a transplant during the period of the study as waiting times are long. Furthermore, 216 (108 in each arm ) patients will be transplanted with a randomised kidney, meaning a less than 0.1% chance of a waitlisted patient to receive a randomised kidney.

The proposed protocol with deferred consent is a pragmatic solution to the problem of consent timing. It might be argued that it is unethical not to perform such a study to optimise an established treatment. Equally does the recipient have any rights over the kidney until the transplant has been performed and it is then considered to be theirs? Indeed, it may happen that the intended recipient is found unsuitable, either medically or immunologically (a positive cross-match), and the kidney will be re-allocated to another patient.

There are a number of precedents that follow a similar approach (Machine Preservation Trial, ISRCTN83876362; Machine Perfusion in asystolic donor kidney transplantation, ISRCTN95022818; Pre-transplant machine perfusion of heart-beating donor kidneys prior to renal transplantation, ISRCTN35082773); Cardiac Death kidney Machine Perfusion trial, ISRCTN 50082383. We hope that our suggested solution is acceptable.

In case the recipient does not wish to consent, he / she will still receive the kidney that was allocated to him/her by ET or NHBST and will receive standard of care. In such an event, his / her data will not be collected and study samples will not be taken.

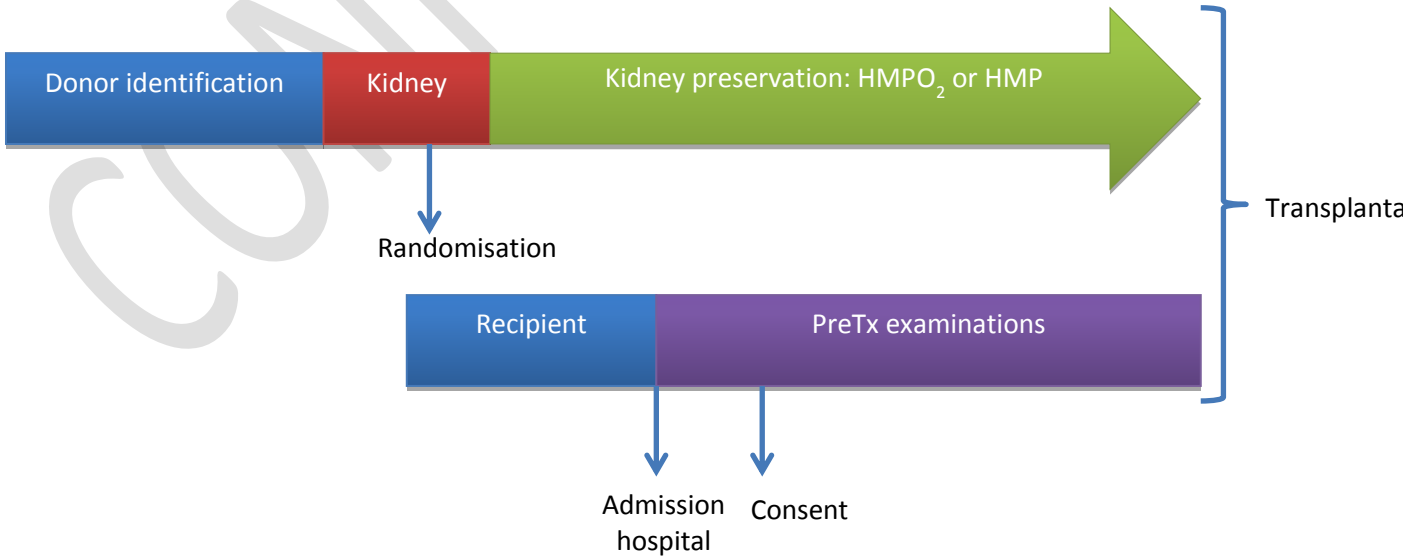



**Fig. 3 Timing of consent**

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

Written informed consent will be documented by means of a dated signature from the participant and dated signature from the person who presented and obtained the informed consent. The person who obtains consent must be:

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

A copy of the signed patient information leaflet and 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.

In case the recipient gives consent, is included in the study but then loses capacity during the running of the trial, the participant will be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

# 7. The HMP Device

**The Kidney Assist**

The Kidney Assist machine being used in the trial is a CE marked medical device designed for isolated hypothermic oxygenated perfusion of a donor kidney during storage. The machine will be produced and delivered by Organ Assist (Groningen, The Netherlands). Organ Assist will also be responsible for training Transplant Technicians and other relevant individuals on how to operate the device as well as for maintenance and repairs to the machine as required. Organ Assist will deliver a replacement device within 24 hours in the event of a defect being reported to them. Med Assist will be responsible for logistics in Belgium and The Netherlands. A local logistics organisation will be used in the UK. The role of the logistics teams will include maintaining contact with the transplant registers (e.g. ET and NHSBT), donor coordinators, supervising the Transplant Technicians and ensuring that a Transplant Technician is present at both donor and recipient procedures. Training on the use of the Kidney Assist machine will be provided in advance of recruitment of the first patient by Organ Assist/Med Assist for Belgium and The Netherlands and their equivalent in the UK. A record of all device training will be maintained. All personnel involved in randomisation and data entry will also

be trained in the use of the online randomisation and data collection tool by the National Logistics Coordinator or Local Investigator, and records of such training will be maintained.

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

The renal artery is connected to a kidney holder and a cold preservation solution (perfusate) is pumped through the kidney while (optionally) adding oxygen to the perfusate. 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. In the COMPARE Trial, the pressure will be set at 25 mmHg and will not 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. Usually the 'flow alarm' and decreased pressure signifies a connection problem of the artery to the machine (e.g. perfusate leak) and this will be noticed minutes after the kidney is placed on the machine. If this occurs, the connection of the kidney with the Kidney Assist will be checked by the retrieval surgeon with the help of the Transplant Technician 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 CRF by the Transplant Technician.

Perfusate and kidney are cooled to 1-4°C using crushed ice outside the sterile container. The Kidney Assist will be used as a stand-alone preservation method (up to 24 hours). If the Kidney Assist stops functioning for whatever reason, the kidney always remains submerged in preservation solution and remains cooled by the ice (i.e. cold storage on ice). The kidney does not need to be disconnected from the machine to be properly cold stored. In this case, the kidney will still be included in the intention-to-treat analysis. The kidney with the Kidney Assist will be transported from the donor to the recipient hospital according to current practice in ET/NHSBT.

The Kidney Assist has been tested to pressure changes outside of the device (in case of air transport); no disturbances in the perfusion occurred.

The Kidney Assist continuously registers renal resistance and flow measures, however these will be concealed in order to avoid the decision to accept / discard a kidney based on these parameters.

The Kidney Assist does not have an alarm to indicate that the oxygen cylinder is empty. It uses an 0.6 L oxygen cylinder with a pressure of 300 bar. At the fixed flow of 100 mL/min this allows for enough oxygen to pump the kidney for 30 consecutive hours. As a precaution to avoid the risk of an empty cylinder in a kidney allocated to oxygenated HMP, only completely full cylinders will be used in the oxygenated HMP arm. When the kidney has been stored for 28 hours, the Logistics Team is contacted to change the cylinder. This way the allocation of the kidney will remain concealed (see Section 8.2). If necessary, the ice in the reservoir (which is enough to guarantee 24 hours of cold storage) will be refilled by the Transplant Technician or personnel of the recipient transplant centre.

**The machine perfusion solution**

Kidneys will be perfused with 1000 mL Belzers Machine Perfusion Solution.[30] This solution is intended for the in vitro flushing and temporary continuous machine perfusion preservation of explanted kidneys. Belzers Machine Perfusion Solution is a sterile, isotonic non-pyrogenic solution with the following approximate concentrations: osmolarity of 300 mOsm, sodium concentration of 100 mEq/L, potassium concentration of 25 mEq/L, and pH of 7.4 at room temperature. A commercially available form of Belzers Machine Perfusion Solution will be used, the same form in all kidneys included in the study.

# 8. Methods: Assignment of interventions

## 8.1.Allocation

### 8.1.1. Sequence generation

Kidneys from the same donor will be randomly assigned to HMP with or without oxygen in pairs and hence 1:1 allocation with stratification by country (Belgium, the Netherlands and UK) will be performed by a computer generated randomisation schedule.

### 8.1.2. Timing of randomisation

Kidney pairs will be randomised after retrieval when they are inspected on the back table by the retrieval surgeon. After he / she has declared that both kidneys are transplantable, the pair will be randomised as described in Section 4.1.

### 8.1.3. Allocation concealment mechanism

Allocation concealment will be ensured by the use of a web-based trial database with telephone back-up available. Allocation will not be revealed to anyone except the Transplant Technician and the Logistics Coordinator.

### 8.1.4. Implementation

Once all criteria for inclusion have been checked and both the kidneys have been deemed transplantable by the retrieval surgeon (this is at the stage of the back-table inspection of the kidneys during the retrieval), the Transplant Technician will log in to an online data collection and randomisation tool and will randomise the transplantable kidneys from this donor. This will require compliance with inclusion and exclusion criteria. Baseline donor characteristics will be required prior to release of the randomisation groups. This will ensure that the donor is enrolled prior to allocation.

### 8.1.5. What if the kidney cannot be connected to the Kidney Assist?

When, after randomisation, it is apparent that the kidney cannot be connected to the Kidney Assist, the kidney will be cold stored and transplanted as usual. It will be registered as a 'failure to connect the kidney to the Kidney Assist' in the eCRF. A reason for this failure will be recorded. This 'failure' can be expected to happen when:

- An aortic patch is too small for a reliable connection to the Kidney Assist. 2 sizes of cannula holders are available: 7mm x 20mm, 9mm x 30mm. At the start of the trial a solution for multiple arteries on different patches will be available.
- Too many renal arteries prevent a safe connection of the kidney to the Kidney Assist.

As one can only truly ascertain whether a safe connection of the kidney with the Kidney Assist can be made upon actually attempting a connection, we chose to randomise kidneys as a pair before this attempt has been made. This will allow us to document the 'real-life' incidence of a failed cannulation, i.e. where the technology would not be usable in clinical practice.

## 8.2.Blinding

- All members of the retrieval team, the international allocation offices, the transplant team, the team responsible for patient follow-up, and the recipients themselves will be blinded. The only people aware of the randomisation will be the Logistics Team that operates independent of the procurement/transplant teams and will be trained not to reveal the allocation.
- The intervention concerns the addition of oxygen to the perfusate. In order to accomplish this a full oxygen cylinder needs to be connected to the Kidney Assist and the cylinder needs to be opened by turning the regulator so that oxygen flows at a prespecified rate (see the separate document: Instruction manual Kidney Assist). The regulator will be concealed thus effectively blinding anyone besides the Logistics Team that turns the regulator (ON/OFF) and conceals it by placing a non-transparent cap over the regulator and fixing this in place with tape. If later during the trial it is discovered that the tape has been broken and as a consequence blinding might be broken, this will be reported to the National Investigator and mentioned in the eCRF.
- Furthermore, the decision to accept/discard a kidney will not be influenced by perfusion characteristics (e.g. flow, vascular renal resistance) as these parameters will remain concealed to everyone involved in the trial by placing a non-transparent sticker on the display after priming of the machine. This will be done by a member of the Logistics Team. Only after the kidney has been removed from the Kidney Assist can a member of the Logistics Team download and store flow and resistance data for later analysis.
- To maintain the overall quality and legitimacy of the clinical trial, unblinding should occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the patient. Investigators are encouraged to discuss the case with the Trial Office if he/she believes unblinding is necessary.
    - The investigator is encouraged to maintain the blind as far as possible. The actual allocation must not be disclosed to the patient and other study personnel including other site personnel, monitors, sponsors or project office staff; nor should there be any written or verbal disclosure of the code in any of the corresponding patient documents.
    - The investigator must report all cases of unblinding (with reason) as they occur on the corresponding eCRF page.
    - Unblinding is not necessarily a reason for study discontinuation.
- Emergency unblinding procedure

  No formal emergency unblinding procedure will be established. In case unblinding is needed, please refer to the previous section. After extensive discussion within the Trial Management Committee it was decided that none of all possible complications potentially related to the preservation method (e.g. discarding the kidney, PNF, …) require the absolute need to know randomisation (so whether oxygen was administered during HMP) in order to treat the recipient in the best way. All adverse events will be collected and reported as described in Section 6.2. Additionally the DMC will review unblinded data as specified in Section 1.8.3 and in the DMC charter.

# 9. Methods: Data collection, management, and analysis

## 9.1. Data collection methods

### 9.1.1. Data recording

- The data collection protocol and data collection form (eCRF) will be provided as an application on an electronic handheld device that can be either used online (with direct backup of the data) or offline if no internet connection is available (with backup of the data at a later time). Alternatively, the eCFR will also be able to be accessed online through a secured website. This will improve protocol adherence, data accuracy, user acceptability, and timeliness of receiving data.
- All internet-based forms will be properly secured. Data will be uploaded to a central database maintained at the SITU in Oxford, UK.
- A tutorial will be provided with the eCRF for all users. The user must complete the tutorial before access to the eCFR is granted and will be displayed when the user first logs in to the eCFR. The tutorial will be designed by the Trial Database Programmer.
- Donor information will be collected by the Transplant Technician and entered into the eCRF at time of donation. Donor data will be checked by the Local Investigator (or his/her delegate) at the time of transplantation. The Local Investigator is ultimately responsible for data entry and data completeness at his/her centre.
- Recipient information will be collected by the Local Investigator. He/she can also delegate this task but remains responsible for data entry and data completeness.
- The primary endpoint – 24-hour creatinine clearance at 1 year after transplantation – requires a correct 24-hour urine collection. The recipient will need to bring a 24-hour urine collection (collected in the 24 hours preceding the 1 year follow-up consultation) to the out-patient clinic of the transplant centre. Patients will be instructed in the correct methods of a 24-hour urine collection during their initial hospitalisation after transplantation. They will receive an instruction manual and the necessary materials to perform a correct collection.
- All laboratory analyses required for the primary and secondary endpoints (i.e. creatinine concentrations on urine and serum/plasma) will be performed at the clinical laboratory of the transplant centre where the recipient was transplanted. Creatinine concentration measurement will be performed by an isotope dilution mass spectrometry (IDMS) traceable method. IDMS traceability has been introduced by the National Kidney Disease Education Program (United States Department of Health and Human Services) to reduce interlaboratory variation in creatinine assay.[31] Creatinine clearance will be calculated by using the raw data and the following formula: $C_{Cr} = (U_{Cr} \times V) / P_{Cr}$
    - where $C_{Cr}$: creatinine clearance (mL/min); $U_{Cr}$: creatinine concentration in urine; V: volume of urine collection in mL; $P_{Cr}$: creatinine concentration in plasma / serum. $U_{Cr}$ and $P_{Cr}$ determined by the same clinical laboratory and expressed in the same units (e.g. mg/dL; mmol/dL).

      Creatinine clearance calculation will be an automated procedure in the eCRF to avoid calculation mistakes and account for possible variations in the formula used to calculate creatinine clearance at the clinical laboratory.

### 9.1.2. Retention

All randomised recipients completing the 1-year follow-up assessment will be regarded as having completed the study. All recipients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments, and that patients do not incur extra financial costs as a result of study participation.

However, it is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. The investigators may also withdraw a recipient from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures.

In the event of early study termination, the reason for withdrawal must be documented.

Recipients of a kidney transplant require frequent follow-up visits within the first year. Willingness to comply with these frequent follow-up visits is a prerequisite to be listed as a potential kidney transplant recipient. Furthermore, the COMPARE Trial does not require additional effort of the participant outside of normal follow-up procedure besides – in a few transplant centres – a 24-hour urine collection at a prespecified time point that is a routine and harmless procedure. To assure standardised acquisition of those 24-hour urine collections, participant will be guided through the procedure step by step and be referred to a participant's brochure. This will also make it easier for participants to adhere to the study protocol. As such we do not foresee many participants to be lost to follow-up. However, in case a participant discontinues the trial or is lost to follow-up, last follow-up data will be used to attain secondary endpoints and missing data will be appropriately handled (see Section 9.2)

## 9.2.Data management

### 9.2.1. Data forms and data entry

As described above, 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 is entered in the correct format, within valid ranges and minimise the chance of missing data. Data already entered will be retrievable for viewing through the data entry system. The database and forms will be password protected. Each individual responsible for data entry will receive a personal password and will only be allowed to complete and see those data sections related to his/her level of responsibility and permissions within the COMPARE Trial.

Data will be entered at the level of individual participating centres by Investigators or their delegates and Transplant Technicians (for donor data and perioperative recipient data). At a second level, these data will be checked for completeness and correctness and amended where necessary by the National Investigator or his/her delegate. Furthermore, a third level of data checking will be performed the Trial Coordinator who will oversee data entry.

### 9.2.2. Discrepancies and missing data

The central database will be monitored for discrepancies and missing data. The Trial Coordinator will be responsible for the database, and if such discrepancies are identified the Trial Coordinator will be responsible for identifying the problem and contacting the local centre to ensure resolution. The Trial

coordinator will be responsible for the production of monthly reports to each participating centre containing information and details of missing data requiring completion. Every possible effort will be made to try to attain any missing data. When data is missing because of loss to follow-up, withdrawal of consent, … data will be imputed for patients with missing information if data loss is more than a minor amount, as specified in section 5.3.

### 9.2.3. Security and backup of data

The database 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 to the database server. The port used by the server will be blocked. Access to the database 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 password allowed has to adhere to the following rules

- Passwords contain at least eight characters.
- Passwords contain both alphabets and numbers.
- Passwords contain a mixture of both upper case and lower case characters.
- Passwords contain at least one numeric character.
- Passwords contain a minimum of five different characters.
- The maximum sequential repetition of a character allowed is two.
- Passwords are changed at least once a year.

Each centre involved in the COMPARE Trial will have one Super User who will be responsible for assigning user name and passwords to individuals requiring access to the database. It will be responsibility of the Super User to revoke access for the users who are no longer required to use the database. The username and password for the Super User will be assigned by the Trial Coordinator. The Trial coordinator and the Super Users will regularly monitor the list of users granted access.

Each centre will have its own website and access to a website will only be allowed through a fixed set of IP addresses belonging to the institution. This will ensure that only a fixed set of users belonging to a specific institution can access the database. The websites will be created by the Trial Database Programmer.

The database will only be accessible through a fixed list of websites. Where an individual requires access to the complete database, it will be possible to provide such individuals links to data dumps within the website. Before completion of the trial, only the statistician of the DMC is allowed full access to the data.

The source data on the server resides in the database server which is installed on the drive where the operating system is installed. The data in the database will be backed up on a separate drive on the server. This will be done automatically once every day. The backed up data will also be encrypted and copied to another drive, the complete contents of which will be backed up to the University of Oxford managed central back up service.

The backed up data will also be copied to an encrypted external hard drive at regular intervals at least once every month. The external drive will be stored at a different site and will always be locked.

Every time changes are made to a web application, the web applications will be backed up. The backed up web applications along with a copy of the active application will also be copied to an encrypted external hard drive at regular intervals along with the databases once every month.

At any time complete backups of the data are available at least in three different places: MySQL server where the source data is stored, data backup folders (on a separate drive to where the DBMS runs) and the encrypted external hard drive.

A secure password system will be used to limit access to the online data management tool. All data will be transmitted over a secure connection and stored in a password-protected central database, the password for which will be regularly changed.

All data reports will be prepared using a unique study ID, preventing the identification of individual subjects from the report data.

Regular backups of the main database will be made to an off-site location.

#### 9.2.4. Data retention

All data in the central database will be stored by the study sponsor for at least 15 years following study close-out. Informed consent forms will be stored by the recipient transplant centre.

#### 9.2.5. Description of hardware and software

The database will be developed using ASP.NET (.NET Framework 4.0/4.5) and the database server used will be MySQL (see Section 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 Data programmer and the only other person who has access to the server is the departmental (Nuffield Department of Surgical Sciences, Oxford, United Kingdom) IT officer.

# 10. Statistical Analysis

## 10.1. Statistical methods

The primary analysis of the COMPARE trial will be an intention-to-treat analysis of the paired kidneys comparing intervention (oxygenated HMP) against control (non-oxygenated HMP) for all primary and secondary outcomes. The primary endpoint, GFR, will be analysed using paired t-test if normally distributed or Wilcoxon signed rank test otherwise, with imputation of GFR if this is unavailable. Outcomes will be reported overall and by country. Binary outcomes will be assessed using McNemar's test and logistic regression to adjust for prognostic factors. Continuous outcomes will be compared using paired T-test if normally distributed, otherwise using the Wilcoxon signed rank test. 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 or alternative validated methods if the proportional hazards assumption is not met. 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 secondary analysis will also assess treatment effect for the unpaired kidneys, which will be combined with the results from the paired kidneys' treatment effect in a weighted meta-analysis.

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

## 10.2. Sample size

The study is powered for a paired analysis of kidneys from the same donor that are randomised to each of the two study arms. A correlation coefficient of 0.4 has been used for the function of 2 kidneys from the same donor based upon analyses of kidney transplants in the United Kingdom, Belgium and the Netherlands. The study is powered to detect a mean difference of 8 ml/min (the smallest difference in GFR thought to be of clinical significance by an expert team of transplant physicians and nephrologistst) in measured GFR between the 2 kidneys, considering a standard deviation of 20ml/min and an expected mean measured GFR in the control group of 40 ml/min at one year after transplantation. For a power of 90% (β=0.10) and 5% significance level (2-sided α=0.05), 83 pairs of kidneys will need to be analysed. Assuming that 5% of the recipients will be lost to follow up and 3% will suffer PNF and 15% of kidneys will be declined by the recipient centre (sometimes the contralateral kidney will be transplanted), 108 kidney pairs will be randomised to each of the study arms. Assuming that 30% of the kidneys will be deemed not transplantable before randomisation by the retrieving surgeon and 20% of donors will be lost in the early stages (e.g. donor procedure cancelled or donor hospital not reached in time), 194 donors are required to be screened in order to achieve 83 paired kidneys transplanted. This will ensure an intention-to-treat (ITT) analysis powered to a 90% level. This will be the primary outcome analysis. Assuming that an extra 15% of kidneys will eventually end up in static cold storage (SCS) due to inability to cannulate or to technical failure of the machine, the above sample size will ensure an 85% power for the per-protocol (PP) analysis i.e. not including these SCS kidneys. Kidneys from donors that result in 1 transplanted kidney will be kept in the study. Results from these "unpaired" kidneys will be used to assess the treatment effect for unpaired kidneys. The treatment effect from both the 'paired' kidneys and 'unpaired' kidneys can then be weighted and used in combination in a type of meta-analysis. This will be a secondary analysis.

### 10.2.1. Health Economic 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 (pre-transplant at time of consent) and at each 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), radiological investigations, biopsies and other procedures, outpatient visits and visits to the family doctor. Resource use will be identified from case report forms, hospital episode statistics/insurer claims, where available in the country of residence, and from patient self-reporting using a simple log/questionnaire (to assess out-of hospital resource use).

The EQ-5D-5L questionnaire can be found in the Appendix 4: EQ-5D-5L quality of life questionnaire~~EQ-5D-5L quality of life questionnaire~~

The resource log can be found in Appendix 5: Resource log

#### 10.2.2. 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 as described in the DMC charter.

# 11. Safety

## 11.1. Adverse Events Definitions

*Adverse Event (AE)*

Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs whether or not related to the study intervention. 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.

*Serious Adverse Event (SAE)*

An adverse event that:

- Led to death
- Resulted in serious deterioration in the health of the subject that:
    - resulted in a life-threatening illness or injury
    - resulted in a permanent impairment of a body structure or a body function
    - required in-patient care or prolongation of hospitalisation
    - resulted in medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.

This includes device deficiencies that might have led to a serious adverse event if:

a) suitable action had not been taken or

b) intervention had not been made or

c) circumstances had been less fortunate.

These are handled under the SAE reporting system.

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.

*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 from intentional misuse of the investigational device.

*Serious Adverse Device Effect (SADE)*

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

*Unanticipated Serious Adverse Device Effects (USADE)*

Any serious adverse device effect which, by its nature, incidence, severity or outcome, has not been identified in section 10.4.

*Device Deficiency*

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling. Device deficiencies resulting in SADEs will be managed as detailed in section 10.5.

Device deficiencies that did not lead to an adverse event, but could have led to a medical occurrence if suitable action had not been taken, or intervention had not been made or if circumstances had been less fortunate will also be managed as detailed in section 10.5.

*Use error*

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

## 11.2. 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.

## 11.3. Grade of severity

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

## 11.4. Anticipated Adverse Events

- Infection (chest, urine, blood, wound, abdominal)
- Cardiac failure
- Cardiac arrhythmias
- DGF
- PNF
- Venous or arterial thrombosis
- Renal artery stenosis
- Fluid collection (around transplanted kidney)
- Lymphocoele
- Bleeding
- Admission for suspected rejection
- Rejection
- Graft loss
- Ureteral necrosis
- Ureter leak
- Ureteral stricture
- New Onset Diabetes Mellitus
- Respiratory failure requiring appropriate treatment
- Hospitalisation for pre-existing condition that has not deteriorated

The investigator will exercise his/her medical judgment in deciding whether a postoperative laboratory finding falling outside the relevant reference range 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.

## 11.5. Protocol-defined Events

All anticipated adverse events are protocol-defined events and do not require immediate reporting.

- The following anticipated adverse events need to be reported within one week after becoming aware of the event irrespective of seriousness criteria:
    - Venous or arterial thrombosis
    - Rejection
    - Graft loss

- All other anticipated adverse events will be reported at the time of study visits (day 7, 3, 6 and 12 months after transplantation) irrespective of seriousness criteria.
- The following adverse events are very common features in kidney transplant recipients and are not considered adverse events for the purpose of the trial:
    - Gastrointestinal problems (nausea, constipation and/or diarrhoea) related to the use of immunosuppressive drugs (such as mycophenolate acid derivatives)
    - Hypertension as a pre existing condition or induced by immunosuppression
    - Headaches related to immunosuppression
    - Anaemia, leukopenia or thrombocytopenia related to immunosuppression
    - Transient hyper/hypocalcemia, hyper/hyponatremia, hyper/hypokalaemia, hyper/hypophosphataemia, hypomagnesemia are expected during the peri-operative period after kidney transplantation
    - Transient abnormal liver function tests induced by medication given to a kidney transplant patient
    - Peripheral oedema and hypoalbuminemia in the peri-operative period related to filling status and peri-operative management (until first 3 months after kidney transplantation)

## 11.6. Recording adverse events and device deficiencies

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

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

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

It is the responsibility of the Local Investigator to collect all directly observed adverse events and all adverse events spontaneously reported by the subject. In addition each subject should be questioned about adverse events at each visit. Adverse events should be recorded on provided adverse event data collection forms within the eCFR.

## ~~1.1.~~11.7. Reporting procedures for all serious adverse events

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

It is the responsibility of the 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. Details to be included in the report are as Section 10.5.

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.

All Protocol-defined events will be reported as per protocol (see section 11.5)

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.

### ~~1.2.~~11.8. Surgical complication classification

If any surgical complications arise in the first 30 days after transplantation, they will be classified according to the Clavien-Dindo classification detailed below:

The Clavien-Dindo classification[32] (Table 3~~Table 3~~) 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 3 **Clavien-Dindo classification of surgical complications**[32]

| Grades | Definition |
|---|---|
| **Grade I** | Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.<br>Acceptable therapeutic regimens are: drugs as anti-emetics, antipyretics, analgesics, diuretics and electrolytes, and physiotherapy.<br>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.<br>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. |

CNS: brain haemorrhage, ischemic stroke, subarachnoid bleeding, but excluding transient ischemic attack; IC: intermediate care; ICU: intensive care unit

# ~~2.~~12. Monitoring & Quality Assurance

## ~~2.1.~~12.1. 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.

## ~~2.2.~~12.2. 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 the authorised representatives of the sponsor, REC, government regulatory authorities, and institution compliance and quality assurance groups, and provide adequate access to all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.).

## ~~2.3.~~12.3. Data monitoring

### ~~2.3.1.~~12.3.1. Data Monitoring Committee

The trial has a data monitoring committee (DMC) which consists of at least three independent members including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DMC will periodically review accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. The DMC will advise the Trial Management Committee and WP8 of the COPE project (Study design, statistical analysis, and cost-effectiveness) if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms.

A separate DMC charter will contain full details of the committee and its roles and reporting structure.

### ~~2.3.2.~~12.3.2. Data quality assurance

2 levels of data checking are built into data retrieval (see Section 8.2). Furthermore, 10% of the data will be externally audited every 12 months that the study is recruiting. If data accuracy is poor (i.e. more than 25 mistakes per 10000 data fields are made[33]) additional audits will be done and the necessary steps to assure increased data accuracy and completeness will be implemented. Data auditing is coordinated by the Trial Coordinator.

### ~~2.3.3.~~12.3.3. Study documentation

It is the responsibility of the Local Investigator to maintain complete, accurate and current study records. Each investigator will be provided with access to online case reporting system and other associated study specific documentation by the National Investigator. 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.

### ~~2.3.4.~~12.3.4. 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 5, 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 Ethics Committee. Failure to comply with and/or inability to meet these regulations may jeopardize further participation of the investigator or investigative site in this and future clinical studies.

## ~~2.4.~~12.4. Study Close Out and Early Termination

Study close out will occur 1 year following recruitment of the last recipient to the study, after the last follow-up visit of this patient.

The DMC may advise the Principle Investigator to suspend or prematurely terminate the study either at an individual investigation site or the entire study for significant and documented reasons. The Principle 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 Principle Investigator and Sponsor shall keep each other informed of any communication received from either the local Ethics Committee or the Competent Authority.

National Investigators will ensure compliance with national requirements regarding study closing. In the UK, local Ethics Committees need to adhere to an early termination procedure in case of such an event. Local Ethics Committees need to be notified by completion of a report within 15 days of the early termination or 90 days at normal end of the study.

If, for any reason, the Sponsor suspends or prematurely terminates the study at an individual investigation site, the Sponsor shall inform the responsible Competent Authority as appropriate and ensure that all local Ethics Committees are 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 Local Investigators and local Ethics Committees.

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 Principle Investigator or authorized designee shall promptly inform the enrolled subjects at his/her study site, if appropriate.

# ~~3.~~13. Ethics and dissemination

## ~~3.1.~~13.1. Research ethics and approval

This protocol, site-specific informed consent forms and participant education and recruitment materials will be reviewed and approved by appropriate Ethics Committees in the participating countries and local approvals of all participating centres before the start of the trial. Approval will be obtained in writing, stating the identity of the clinical trial, the date of review, the documents reviewed and a list of the names and titles of the committee members. Where necessary, approval of the competent authorities will be obtained.

Any substantial amendment to the protocol will be submitted for local approval. The Local Investigators will be responsible for informing their host institutions of all problems involving risks to patients according to national regulations.

## ~~3.2.~~13.2. Protocol Amendments

Any change or addition to this study protocol which may impact on the conduct of the study, potential benefit to the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures or significant administrative aspects will require a formal written amendment to the study protocol and approval from National Ethics Committees and host institutions.

All other amendments to the protocol will be notified to the National Ethics Committees and host institutions – if required by national law. Approved amendments will be circulated promptly to all investigators by the co-ordinating centre. Amendments will be tracked by version number and date. All amendments will be provided in English.

## ~~3.3.~~13.3. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the current revision of the Declaration of Helsinki on Biomedical Research involving Human Subjects.

## ~~3.4.~~13.4. ICH guidelines for good medical practice

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

## ~~3.5.~~13.5. Medical Device Regulations

The Investigator will ensure that this trial is conducted in full conformity with:

- European Commission Medical Device Guidelines relating to the application of the EU Directives on Medical Devices
- Guide to European Medical Device Trials and BS EN ISO 14155

## ~~3.6.~~13.6. Confidentiality

All study-related information will be stored securely at the study site. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded identification number only to maintain participant confidentiality. All databases will be secured with password protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant identification numbers to other identifying information will be stored in a separate, locked file.

## ~~3.7.~~13.7. Declaration of a conflict interest

None

## ~~3.8.~~13.8. Access to data

The Principle Investigator and Trial Management Committee members will have access to the cleaned data sets. The Central Investigator will have access to the full cleaned data sets. Access to the cleaned data set for their centre can be obtained by Local Investigators not part of the Trial Management Committee after a formal request describing their plans is approved by the Trial Management Committee.

## ~~3.9.~~13.9. Indemnity Insurance and Compensation

The University of Oxford has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd., at Lloyd's of London).
Host institution indemnity operates in respect of the clinical treatment that is provided. In the UK centres, this indemnity is provided by the NHS.
Participants will not incur any extra costs related to taking part in the COMPARE Trial.

## ~~3.10.~~13.10. Dissemination policy

### ~~3.10.1.~~13.10.1. Data analysis and release of results

By conducting the study, the Local Investigators agree that all information provided by the sponsor and co-ordinating centre will be maintained by the Local Investigators and the site personnel in strict confidence. It is understood that the confidential information provided to Local Investigators will not be disclosed to others without authorization from the sponsor and/or PI.

The scientific integrity of the study requires that all data must be analysed study-wide and reported as such.

### ~~3.10.2.~~13.10.2. Primary outcome publications

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 UZ Leuven (i.e. Central Investigator and PI), 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 UZ Leuven 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 UZ Leuven. 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

### ~~3.10.3.~~13.10.3. Other study papers, abstracts and presentations

Study investigators wishing to publish secondary data analyses are encouraged to submit a proposal to the COPE publication committee for approval, this must first pass by the National Investigator and PI of the COMPARE Trial. The proposal author, if accepted, may decide on the lead author in each publication resulting from such a proposal.

# Appendices

## 1. Data collection parameters

### Donor

**Donor identification**

Donor number
Name of donor centre: _____
Date of admission (dd-mm-yyyy): __-__-____
Date of donor operation (dd-mm-yyyy): __-__-____
Donor date of birth (dd-mm-yyyy): __-__-____
Donor sex: [ ] male [ ] female
Donor weight: ____ kg
Donor height: ____ cm
Donor BMI: ___ kg/$m^2$
Donor blood group: [ ] O [ ] A [ ] B [ ] AB
HLA-typing: A ____ B ____ DR ____
Kidney left donated: [ ] yes [ ] no
Kidney right donated: [ ] yes [ ] no
Liver donated: [ ] yes [ ] no
Lung donated: [ ] yes [ ] no
Heart donated: [ ] yes [ ] no
Pancreas donated: [ ] yes [ ] no
Bowel donated: [] yes [] no
Tissue donated: [] yes [] no

**Donor pre-operative clinical data**

Diagnosis:

[ ] multi trauma
[ ] trauma capitis
[ ] cerebro vascular accident
[ ] primary brain tumor
[ ] anoxia
[ ] other : specify _____

Diabetes Mellitus (IDDM): [ ] yes [ ] no
Alcohol abuse: [ ] yes [ ] no
Cardiac arrest (during ICU stay prior to retrieval procedure) [ ] yes [ ] no
Systolic blood pressure (mean): _____ mmHg
Diastolic blood pressure (mean): _____ mmHg
Hypotensive period (syst. < 100 mmHg): [ ] yes [ ] no
Mean diuresis / hr. last 24 hrs.: ____ ml
Donor anuria / oliguria (< 500 ml/24h): [ ] yes [ ] no
Vasopressors:

Dopamine: [ ] yes [ ] no | Last dose: _____ µg/kg/min

Dobutamine: [ ] yes [ ] no | Last dose: _____ µg/kg/min
(Nor)adrenaline: [ ] yes [ ] no | Last dose: _____ µg/kg/min
Others: _____ | Last dose: _____ µg/kg/min

Other relevant data: _____

**Donor laboratory values** (last available values)

Hb: _____ mmol/l or mg/dL
Ht: _____%
pH: _____
pCO2: _____ Pa
pO2: _____ Pa
Urea: _____ mmol/l or mg/dL
Last creatinine: _____ µmol/l or mg/dL
Max. creatinine: _____ µmol/l or mg/dL

### Donor operation data

Time of withdrawal of life supporting treatment(dd-mm-yyyy - hh:mm): __-__-____ __:__
Time of mean arterial pressure below 50 mmHg (dd-mm-yyyy - hh:mm): __-__-____ __:__
Time of start no touch period (dd-mm-yyyy – hh:mm): __-__-____ __:__
Time of circulatory arrest (dd-mm-yyyy - hh:mm): __-__-____ __:__ (start of no touch period)
Length of no touch period:_____ min
Time of confirmation of death (dd-mm-yyyy – hh:mm): __-__-____ __:__
Time of start in-situ cold perfusion (dd-mm-yyyy - hh:mm): __-__-____ __:__
Systemic flush solution used: [] UW [] HTK [] Marshall's
Preservation solution used for cold perfusion: [ ] UW [ ] HTK [] Marshall [] other: _____
Volume of solution used for cold perfusion:___ ml
Heparin: [ ] yes [ ] no

### Donor operation left kidney / right kidney

Preservation modality: [ ] HMP with oxygen [ ] HMP without oxygen
Randomisation act completed [] yes [] no (ie valve left open or closed and gauge concealed)
Number of renal arteries: ____
Arterial damage: [ ] yes [ ] no
Venous damage: [ ] yes [ ] no
Ureteral damage: [ ] yes [ ] no
Parenchymal damage: [ ] yes [ ] no
Washout perfusion left kidney: [ ] homogenous [ ] patchy [ ] blue
Removal time left kidney (dd-mm-yyyy hh:mm): __-__-____ __:__
Visible perfusion defects: [ ] yes [ ] no
Time of start machine perfusion (dd-mm-yyyy hh:mm): __-__-____ __:__
Recipient centre: _____

### Samples

Donor blood samples taken (2 tubes, 1 pearl, 1 gold): [] yes [] no
Donor blood samples centrifuged: [] yes [] no

Time of departure technician from donor hospital (dd-mm-yyyy hh:mm): __-__-____ __:__
Time of arrival at perfusion centre (dd-mm-yyyy hh:mm): __-__-____ __:__
End time of entire procedure for this technician (dd-mm-yyyy hh:mm): __-__-____ __:__
Remarks _____

## Machine preservation period

LEFT kidney + RIGHT kidney
Perfusate at 15 min of MP taken: [] yes [] no
Perfusate at 1 h of MP taken: [] yes [] no
Perfusate at end of MP taken: [] yes [] no
Oxygen tank changed: [] yes [] no
Date and time oxygen tank changed (dd-mm-yyyy hh:mm): __-__-____ __:__
Ice container replenished: [] yes [] no
Date and time ice container replenished (dd-mm-yyyy hh:mm): __-__-____ __:__

## Recipient

**Recipient identification**

Kidney received: [ ] left kidney [ ] right kidney
ET/NHSBT donor number: |_|_|_|_|_|_|
ET/NHSBT recipient number: |_|_|_|_|_|_|
Name recipient transplantation centre: _____
Recipient date of birth (dd-mm-yyyy): __-__-___
Recipient weight (kg)
Recipient height (cm)
Recipient gender: [ ] male [ ] female
Recipient ethnicity: black: [] yes [] no
Renal disease: _____

- Glomerular diseases
- Polycystic kidneys
- Uncertain etiology
- Tubular and interstitial diseases
- Retransplant / Graft failure
- Diabetes
- Hypertensive nephroangiosclerosis
- Congenital, rare familial, metabolic disorders
- Renovascular and other renal vascular diseases
- Neoplasms
- Other (specify)

Number of previous transplants: _____
Pre-transplant diuresis: _____ ml/24h
Recipient blood group: [ ] O [ ] A [ ] B [ ] AB
Recipient HLA-typing: A ____ B ____ DR ____
ET-urgency: [ ] 0 [ ] 1 [ ] 2 [ ] 4

Number of HLA mismatches: A |__| B |__| DR |__|

**Recipient operation – peri-operative data**

Transplantation date (dd-mm-yyyy): __-__-__
Time stop machine perfusion (dd-mm-yyyy hh:mm): __-__-____ __:__
Kidney discarded / untransplantable: [] yes [] no
If yes: reason _____
Perfusate at end of MP taken: [] yes [] no
Time on the machine: hh:mm
Start time operation (induction of anesthesia) (dd-mm-yyyy hh:mm): __-__-____ __:__
Mannitol: [ ] yes [ ] no
Diuretics: [ ] yes [ ] no
Dopamine: [ ] yes [ ] no
Hypotensive period (syst. < 100 mmHg): [ ] yes [ ] no
lowest systolic blood pressure: ____ mmHg
lowest diastolic blood pressure: ____ mmHg
duration hypotensive period: ____ minutes
CVP at reperfusion: _____ mmHg
Incision: [ ] med. laparotomy [ ] extraperitoneal (ie hockey stick incision)
Transplant side: [ ] left [ ] right
Arterial problems:
[ ] no
[ ] ligated polar artery
[ ] reconstructed polar / hilar artery
[ ] repaired intima dissection
[ ] other _____ (specify)
Venous problems: [ ] yes [ ] no
Start time anastomosis (dd-mm-yyyy hh:mm): __-__-____ __:__
Time of reperfusion (dd-mm-yyyy hh:mm): __-__-____ __:__
Total anastomosis time: ____ minutes
Cold ischaemia period: _____ hours _____ minutes
Remarks: _____

**Samples**

Perfusate at end of MP taken: [] yes [] no
Blood samples immediately after anesthesia taken (2 tubes, 1 pearl, 1 gold): [] yes [] no
Blood samples processed: [] yes [] no
If no reason: _____
Blood samples immediately 1 hour after reperfusion taken (2 tubes, 1 pearl, 1 gold): [] yes [] no
Blood samples processed: [] yes [] no
If no reason: _____
Biopsy 45 min to 1 hour post-reperfusion taken: [] yes [] no
If yes: time of biopsy (dd-mm-yyyy hh:mm): __-__-____ __:__
If no reason: _____

**Recipient operation – perfusion characteristics**

Intra-operative diuresis: [ ] yes [ ] no [ ] unknown

Remarks: _____

**Kidney graft function day 1 – 7**

Graft failure within first 14 days [] yes []no

Date of graft failure (first dialysis session) (dd-mm-yyyy): __-__-____

Primary cause of graft failure:

- [ ] immunologic
- [ ] preservation
- [ ] technical - arterial
- [ ] technical – venous
- [ ] infection – bacterial
- [ ] infection – viral
- [ ] other

Graft removal: [ ] yes [ ] no

Death: [ ] yes [ ] no

Date of death (dd-mm-yy): __-__-____

Cause of death: [ ] Tx-related [ ] non-Tx-related

Serum creatinine day 1-7

eGFR at day 7

Dialysis requirement day 1 - 7

Dialysis type: [ ] CAPD [ ] hemodialysis

If only 1 dialysis session:

Required for hyperkalemia or fluid overload: [] yes [] no

Hypotensive periods first 24 hours post-transplant

Hypotensive period I: [ ] yes [ ] no

- duration: _____ minutes
- lowest systolic blood pressure: _____ mmHg
- lowest diastolic blood pressue: _____ mmHg

Hypotensive period II: [ ] yes [ ] no

- duration: _____ minutes
- lowest systolic blood pressure: _____ mmHg
- lowest diastolic blood pressue: _____ mmHg

**Immunosuppressive therapy and rejection**

Prednisolon: [ ] yes [ ] no

Cyclosporin: [ ] yes [ ] no

Tacrolimus: [ ] yes [ ] no

Azathioprine: [ ] yes [ ] no

MMF: [ ] yes [ ] no

ATG: [ ] yes [ ] no

IL-2 receptor antagonists: [ ] yes [ ] no

Other immunosuppressive drugs: _____
Number of treatments for rejection day 1 - 14:
Prednisolon: ____
Other: ____
Rejection biopsy proven: [ ] yes [ ] no
Calcineurin inhibitor toxicity day 1 - 14 (based on levels): [ ] yes [ ] no
Remarks: _____
Dopamine and furosemide
Post-operative dopamine: [ ] yes [ ] no
Post-operative furosemide: [ ] yes [ ] no

**Discharge / readmission at 3, 6, 12 months**

Date of primary post-Tx discharge (dd-mm-yyyy): __-__-____
Readmission : [ ] yes [ ] no
Reason for readmission: _____
Nr. of in hospital days after readmission: _____
Complications interfering with graft function not mentioned above: _____
Other complications and/or adverse events: _____

**Follow-up at 3, 6, 12months post transplant**

Graft failure during follow up: [ ] yes [ ] no
Date of graft failure (dd-mm-yyyy): __-__-____
Primary cause:

- [ ] immunologic
- [ ] preservation
- [ ] technical - arterial
- [ ] technical – venous
- [ ] infection – bacterial
- [ ] infection – viral
- [ ] other

Graft removal: [ ] yes [ ] no
Death: [ ] yes [ ] no
Date of death (dd-mm-yyyy): __-__-____
Cause of death: [ ] Tx-related [ ] non-Tx-related
Serum creatinine at 1 month: _____ µmol/l or mg/dL
eGRF at 3, 6, 13 months: _____ ml/min. (cf earlier)
Creatinine clearance at 12months: _____ ml/min
Currently on dialysis: [ ] yes [ ] no
dialysis type: [ ] CAPD [ ] hemodialysis
Date of last dialysis (dd-mm-yyyy): __-__-____
Nr. of rejection periods: _____
Total nr. of dialysis treatments post-Tx: _____
Complications interfering with graft function not mentioned above: _____
Other complications and/or adverse events: _____

Serial Number of the used Kidney Assist device
Lotnumber of the used disposable(s)
Used patchholder (small, large, double artery)
artificial patch used (yes/no) If yes, what size (small, large).

## 2. Local investigators

| Participating Centre | Local Investigator |
|---|---|
| Universitaire Ziekenhuizen Leuven, Belgium | Ina Jochmans, surgeon |
| Universitair Ziekenhuis Antwerpen, Belgium | Dirk Ysebaert, surgeon |
| Universitair Ziekenhuis Brussel, Belgium | Jacques Sennesael, Lissa Pipeleers, nephrologists |
| Université Catholique de Louvain, Belgium | Tom Darius, surgeon |
| Université Libre de Bruxelles, Belgium | Dimitri Mikhalski, surgeon |
| Centre Hospitalier de Liège, Belgium | Laurent Weekers, nephrologist |
| Universitair Ziekenhuis Gent, Belgium | Caren Randon, surgeon |
| Universitair Medisch Centrum Groningen, the Netherlands | Sijbrand Hofker, surgeon |
| Maastricht Universitair Centrum, the Netherlands | Ernst van Heurn, surgeon |
| Leids Universitair Centrum, the Netherlands | Jan Ringers, surgeon |
| Universitair Medisch Centrum Utrecht, the Netherlands | Paul Berger, surgeon |
| Amsterdams Medisch Centrum, the Netherlands | Mirza Idu, surgeon |
| Universitair Medisch Centrum Nijmegen, the Netherlands | Van der Vliet, surgeon |
| Erasmus Medisch Centrum, the Netherlands | Frank Dor, surgeon |
| VU Medisch Centrum, the Netherlands | Arjan Hoksbergen, surgeon |
| Oxford University Hospitals Trust, United Kingdom | Isabel Quiroga, surgeon |
| **Addenbrooke's Hospital, Cambridge** | Kourosh Saeb-Parsy, surgeon |
| **Royal Free Hospital, London** | Neal Banga, surgeon |
| **Royal London Hospital** | Rajesh , surgeon |
| **Hammersmith Hospital, London** | Vassilios Papalois, surgeon |
| **Cardiff and Vale University Health Board, Cardiff** | Laszlo Szabo, surgeon |
| **Queen Alexandra Hospital, Portsmouth** | Keith Graetz, surgeon |
| **University Hospital Coventry and Warwickshire, Coventry** | Habib Kashi, surgeon |
| **City Hospital, Nottingham University Hospitals, Nottingham** | Keith Rigg, surgeon |
| **St George's University Hospital, London** | Ali Ahmed, surgeon |
| **Guy's Hospital, London** | Nikolaos Karydis, surgeon |

# 3. Samples

## 3.1. Rational

The purpose of the sampling of blood, perfusate in kidney biopsies is to underpin the clinical trial in kidney transplantation by assessing (immuno) histopathological criteria of DCD donor kidneys in both arms, treated with or without oxygen, 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, mechanistic research into molecular mechanisms of injury and repair during organ preservation using serum, perfusate, and tissue samples will be done in separate studies after close out of the main trial.

It is recognised that DCD kidneys are increasingly used 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.[34, 35]Other scoring system include the Maryland Aggregate Pathology Index (MAPI)[36] and the total chronic Banff score.[37] In the USA, centres that use pre-implantation histological criteria for kidney assessment end up discarding between 20-50% of 'high risk' kidneys.[38]

In addition, our understanding of the molecular mechanisms that lead to DCD kidney dysfunction and early graft lost is not fully developed, whilst the implications of warm ischaemia on cellular injury are well recognised.[39] 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 DCD kidneys have also not been universally or widely accepted.[40]

## 3.2. Aims of sample collection

- Use of the reperfusion biopsies to histologically evaluate DCD kidneys following hypothermic machine perfusion with or without oxygenation
- 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.

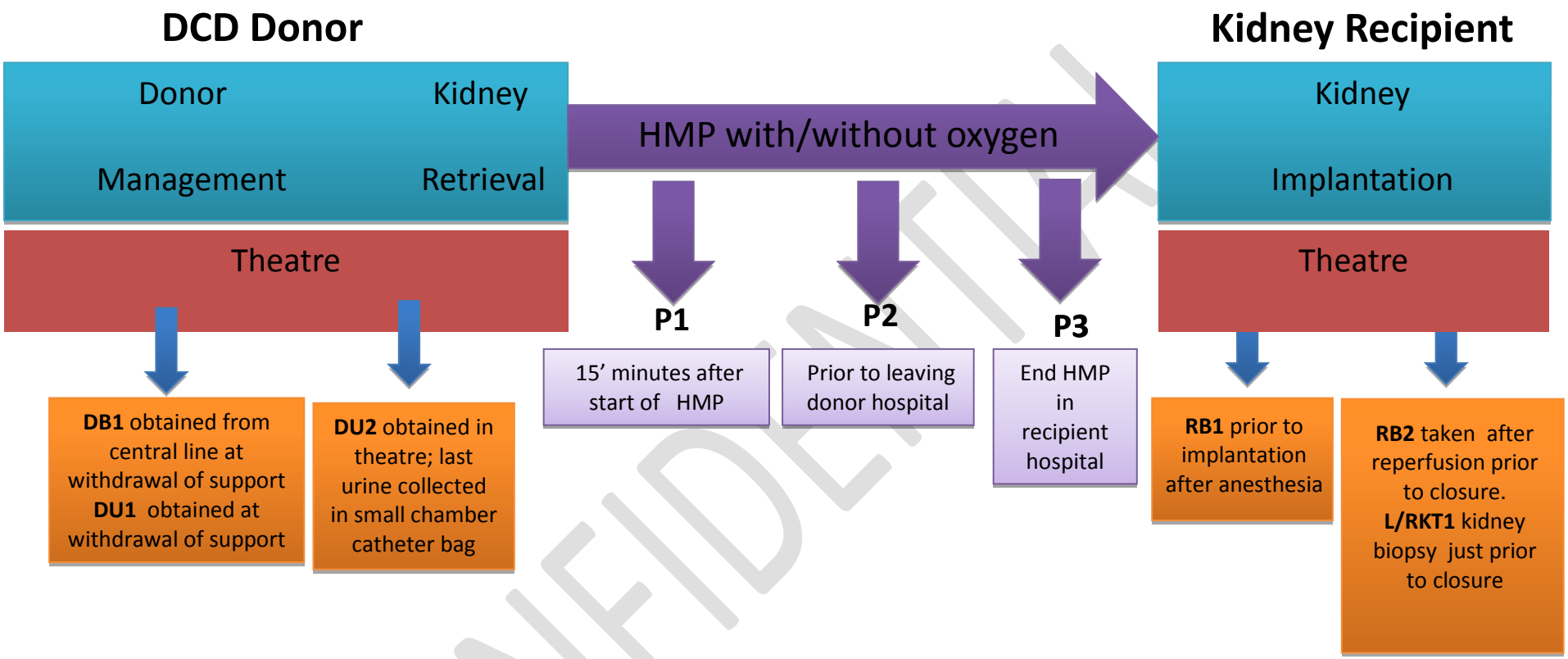
## 3.3. Timing of samples

Samples will be procured throughout the transplant process, as depicted in Table 4. A 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 RNAlater and formalin. The biopsy site is over-sewn with a Prolène stich, according to the surgeon's preference. 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.[41]

**Table 4** Sample collection in the COMPARE trial

| Sample | Type | Time points | Notes |
|---|---|---|---|
| **Donor Procedure Samples** | | | |
| DB1 | Donor Blood | Prior to kidney explant | At withdrawal of support |
| **DU1** | Donor Urine | Prior to kidney explant | At withdrawal of support |
| **DU2** | Donor Urine | Prior to kidney explant | Last urine collected |
| **Preservation Samples** | | | |
| P1 | Perfusate | 15 min after HMP start | At the donor hospital |
| P2 | Perfusate | 1 h after HMP start | Before leaving at the donor hospital |
| P3 | Perfusate | At stop of HMP | In the recipient hospital |
| **Recipient Procedure Samples** | | | |
| RB1 | Recipient Blood | Prior to transplant | Taken after induction of anaesthesia |
| RB2 | Recipient Blood | 1 hour after reperfusion | Taken during anaesthesia |
| Bx | Kidney tissue | 45 min - 1 h after reperfusion | Taken during anaesthesia, immediately before closure |




**P:** perfusate; **DB**: donor blood; **DU**: donor urine; **RB**: Recipient blood; **L/R KT** : Left/right kidney tissue biopsy

Fig. 4 **Sample collection during the clinical trial in WP4. P: Perfusate, DB: Donor blood, RB: Recipient blood, L/R KT1: Left/right kidney biopsy**

### 3.4. Blood samples

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. Centrifugation will not be achievable in some scenarios e.g. during transportation etc. SOPs reflect practical time points for the handling and processing of samples. These SOPs are available to the Medical Ethics Committee upon request.
After centrifugation plasma and serum samples will be kept at 4°C.

### 3.5. Perfusate samples

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.

### 3.6. Biopsies

Biopsies during kidney transplantation are part of the standard of care. A biopsy of a kidney graft before or shortly after reperfusion is routinely taken to document baseline histology in the donor kidney that serves as a reference for later comparison of indication biopsies, e.g. when an acute rejection is suspected. Depending on centre practice and surgeon preference, these 'reference biopsies' are either taken before the kidney is reperfused or shortly after reperfusion (during surgery). For this trial, the biopsy will be taken after reperfusion and will be divided into two parts; one part will be used for the routine clinical testing, the other part will be processed and stored for use in the COMPARE trial.

As in the standard of care, the final decision to take a biopsy lies with the surgeon. If the surgeon estimates the risks of complications of taking a biopsy (e.g. bleeding in a patient on heparin) too high, then no biopsy will be taken. This will be noted in the eCRF with a reason as to why the biopsy was not taken. Not taking a biopsy for medical reasons will not be considered a protocol violation.

### 3.7. 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 [42] (Fig. 5)
- 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 breath 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 .[43]

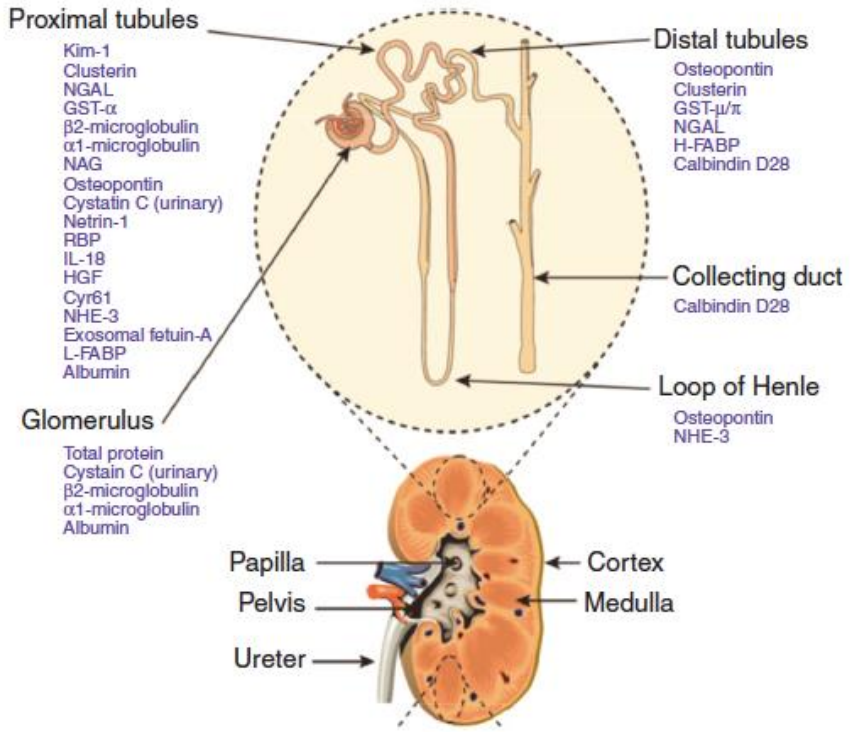



**Fig. 5** Biomarkers associated with acute kidney dysfunction (9). The biopsies will predominantly be used to assess dysfunction of the glomerulus, proximal and distal tubules.

### 3.8. Consumables, tracking and tracing, storage and analysis

All consumables needed for the sample collections and processing of samples needed for the described samples will be provided. The Transplant Technicians, responsible for collection and processing of the samples, will bring them with them to donor and recipients centres. SOPs and the tracking and tracing software/hardware and programmes are available. All samples taken outside of

clinical routine will be stored in the short term in Groningen, Maastricht and Oxford. As defined in the SOPs, samples from Groningen and Maastricht will be transported to Oxford for long-term storage and analysis as defined in Work Package 7 of the COPE project (www.cope-eu.org).

## 4. 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 ❑

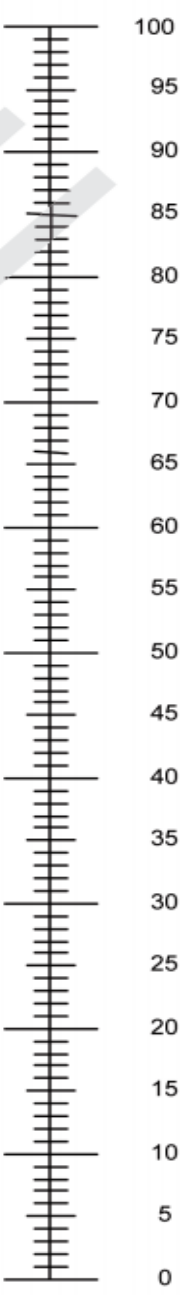
We would like to know how good or bad your health is TODAY.

- 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.

YOUR HEALTH TODAY =



## 5. Resource log

Is available as a separate file.

# 6. Ancillary studies

## 6.1. Ancillary study 1: Comparison of proteomics and metabolomics signatures in perfusate of DCD kidneys during HMP +/- O2 (COPE work plan)

### 6.1.1. Background & Rationale

The COMPARE trial aims to evaluate the therapeutic benefit of oxygen during kidney hypothermic machine perfusion on the post transplantation outcomes of kidneys obtained from extended criteria DCDs.

The hypothesis tested in this COMPARE trial is whether oxygenated is superior to non-oxygenated machine perfusion in increasing the longevity and quality of older DCD kidneys. It is suggested that the presence of O2 will replenish the energy levels in the cellular level and sustain essential cellular functions and possible facilitate the repair mechanisms and as a result improve the quality of grafts.

Analysing the protein and metabolite contents of perfusate will provide a meaningful insight in the underlying mechanisms of ischemia and repair. Similarly, proteins and metabolites that are differentially regulated among the two arms of intervention can be potential markers of injury or repair.

Research proposal:

A. To compare the proteomic and metabolomics profiles of the perfusate obtained during perfusion in the two arms of the COMPARE clinical trial.

B. To associate these profiles to the clinical outcomes post transplantation.

C. To validate published markers those have been previously identified in kidney perfusate. Among the markers published but have not been validated are GST, H-FABP, NAGL, AST.

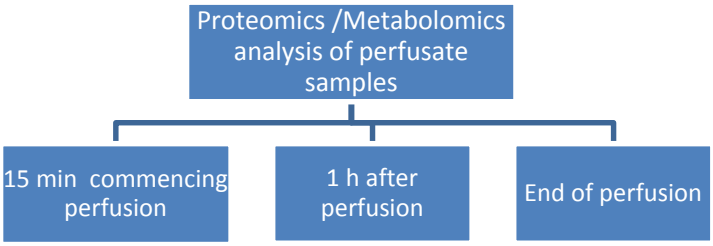
### 6.1.2. Material and methods

Phase 1: Comparison of perfusate samples by proteomics and metabolomics of the two arms of the trial: O2 vs non O2- a pilot study. N=10/group.

Phase 2 Classification of perfusate samples accord ing to the recipient kidney function at 6 months post transplantation as shown below.

We have shown that that it is possible to identify proteins in blood and urine that are related to recipients outcomes post transplantation. Initially, interrogation of recipient clinical data will classify recipients according to eGFR at 6 month post transplantation. We will analyse sequential perfusate samples to identify proteins and metabolites that are differentially regulated in the oxygenated when compared to non-oxygenated perfusate among the recipient outcome groups as listed below.

| Oxygenated HMP | vs | Non oxygenated HMP |
|---|---|---|
| 1. Immediate function | | 1. Immediate function |
| 2. Primary non function (PNF) | | 2. Primary non function (PNF) |
| 3. eGFR < 30ml/min | | 3. eGFR < 30ml/min |
| 4. 30ml/min<eGFR<52ml/min | | 4. 30ml/min<eGFR<52ml/min |
| 5. eGFR>52 ml/min | | 5. eGFR>52 ml/min |




Phase 3. Validate the shortlisted markers that are regulated among the two groups O2 vs non O2 in perfusate in parallel with known, previously published markers in perfusates.

Phase 4: Integration of all the -omics data obtained from the above analysis and the clinical data that is associated with the analysed donor samples.

#### 6.1.3. References

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### 6.2. Ancillary study 2: Transcriptomics and proteomics signatures of kidney regeneration following O2 HMP - Is the quality of kidneys obtained from older DCDs improved after oxygenated HMP? (COPE work plan)

#### 6.2.1. Background & Rationale

The COMPARE trial aims to evaluate the therapeutic benefit of oxygen during kidney hypothermic machine perfusion on the post transplantation outcomes of kidneys obtained from older DCDs.

The hypothesis tested in this COMPARE trial is whether oxygenated is superior to non-oxygenated machine perfusion in increasing the longevity and quality of older DCD kidneys.

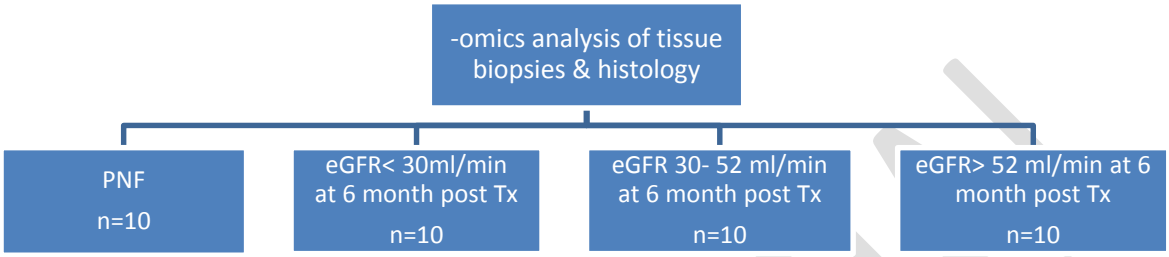
Rationale

Phase 1: Identification of differences in molecular signature of biopsies among the two groups: O2 vs non O2 - a pilot study. N=10/group.

Phase 2: Tissue biopsies obtained from paired kidneys in the two arms of the clinical trial will be grouped according to the following outcomes post transplantation: Primary non function (PNF), at 6 month eGFR < 30 ml/min, at 6 month eGFR 30 -52ml/min and at 6 month eGFR > 52ml/min . The analysis of these biopsies will result in the identification of differentially expressed genes and proteins among the analysed groups and correlate to long term function in the recipient at 6 month

post transplantation. The formalin preserved part of the same biopsies will be analysed and assessed by histology.

Combination of –omics datasets with histology and clinical outcomes will lead to the identification of molecular signatures of kidney function associated to O2 enriched perfusion. In addition this approach will lead to identification of markers or profiles of kidney quality predictive of long term kidney function.




## 6.3. Ancillary study 3: Identification of miRNAs in the oxygenated when compared to non- oxygenated tissue biopsies and perfusates as potential targets of regeneration.

### 6.3.1. Background & Rationale

The COMPARE trial aims to evaluate the therapeutic benefit of oxygen during kidney hypothermic machine perfusion on the post transplantation outcomes of kidneys obtained from extended criteria DCDs.

MicroRNAs (miRNAs) are under intense investigation as powerful regulators of various diseases with potential critical effect on disease initiation and/or progression, including kidney disease. MiRNAs represent small noncoding RNA transcripts with a length of approximately 22 nucleotides, which through post-transcriptional binding of the 39-untranslated region (UTR) of mRNA targets lead to the repression of gene/protein expression and/or translational inhibition of protein synthesis. MiRNAs regulate the expression of many genes and influence disease related pathways and signalling cascades. These short, noncoding nucleotides (~22 bases) regulate target messenger RNAs at the post-transcriptional level. Several hundred miRNAs regulate a considerable amount of the human genome and are involved in virtually all biological processes, including cellular proliferation, apoptosis.

Identification of miRNA regulation and function in renal pathology may pinpoint miRNAs as new therapeutic targets in kidney fibrosis and related diseases. MiRNA modulators have been developed to regulate downstream gene networks in vivo, thus influencing the mechanisms that underlie disease initiation or progression.

Research proposal

Comparing tissue biopsies and perfusate samples from oxygenated and non- oxygenated arms of the study will lead us to identify differentially expressed miRNAs. Initially, interrogation of recipient clinical data will classify recipients according to eGFR at 6 month post transplantation. We will analyse tissue biopsies and perfusate samples from the same recipients to identify miRNAs that are

regulated in the oxygenated when compared to non-oxygenated perfusate among the recipient outcome groups as listed below.

Phase 1:Comparison of O2 vs non O2 by microRNA in perfusate

Phase 2: MicroRNA analysis of biopsies according to recipient eGFR as shown below:

### 6.3.2. Material and methods

| Oxygenated HMP | vs | Non oxygenated HMP |
|---|---|---|
| 1. Immediate function | | 1. Immediate function |
| 2. Primary non function (PNF) | | 2. Primary non function (PNF) |
| 3. eGFR < 30ml/min | | 3. eGFR < 30ml/min |
| 4. 30ml/min<eGFR<52ml/min | | 4. 30ml/min<eGFR<52ml/min |
| 5. eGFR>52 ml/min | | 5. eGFR>52 ml/min |

Phase3: Integration of the data obtained from perfusion and biopsy analysis in addition to the results obtained from parallel studies of proteomics and transcriptomics using the same categorisation of donor biopsies for analysis.

Shortlisted microRNAs will be further analysed to explore their potential as biomarkers of organ quality or targets of potential interventions.

Number of samples: 10/group

## 6.4. Ancillary study 4: Predictive accuracy of perfusion parameters for kidney graft outcome in the COMPARE trial.

### 6.4.1. Background & Rationale

Question

1. Do perfusion parameters (i.e. renal vascular resistance [RR] and flow) during hypothermic machine perfusion (HMP) predict 1 year graft function in the recipient population of WP4 COMPARDT trial (50+ year kidneys donated after circulatory death?

2. Do perfusion parameters during HMP with oxygen predict 1 year graft function in this population?

3. Is there a difference between perfusion parameters in HMP with or without oxygen?

Rationale

We have previously shown that RR in kidneys preserved with HMP is an independent predictor for DGF and 1 year graft survival (1). However, we were not able to analyse graft function (not measured). Furthermore, the population of 50+ DCD kidneys was hardly represented in this study.

Additionally, it is not known whether the addition of oxygen has an influence of perfusion parameters. In vivo hypothermia causes vasoconstriction but hypoxia is responsible for vasodilation (likely through nitric oxide). There are no data on vasoconstriction/vasodilation during HMP, nor on the effect of adding oxygen during HMP. In that sense it is an interesting question whether the addition of oxygen during HMP changes perfusion parameters.

### 6.4.2. Material and methods

1. During the WP4 clinical trial perfusion parameters will be prospectively collected while all members of the teams involved in recipient care will be blinded to these parameters. Renal vascular resistance at 30 min, 1 hour, 4 hours, and at the end of HMP will be analysed. Univariable and multivariable regression models for 1 year graft function, DGF, PNF, and 1 year graft survival will be constructed; RR is entered as a covariate in these models. For every time point of measured RR, a new model will be constructed. Other covariates will be based on the multivariable regression performed in the COMPARE trial of WP4. Receiver operator characteristic (ROC) curves will be constructed to investigate the predictive accuracy of RR for 1 year graft function (transformed to a categorical variable with cut off based on regression analysis), DGF, PNF, and 1 year graft survival.

2. The same will be done in the HMP + O2 arm.

3. Renal vascular resistance and flow at these time points will also be compared between the HMP and HMP+O2 group. If differences are present, the underlying mechanisms should be explored, a.o. by looking at the importance of nitric oxide and nitric oxide synthethase in the tissue biopsies obtained in the WP4 COMPARE trial.

### 6.4.3. References

1 Jochmans I et al; Am J Transpl 2011, 11: 2214-2220

## 6.5. Ancillary study 5: Serum injury markers and prediction of kidney transplant outcome.

### 6.5.1. Background & Rationale

Questions

1. Can recipient plasma injury markers predict graft outcome earlier and more reliable then creatinine (changes) in kidney graft recipients from
    a. ECD kidneys that are cold stored (control arm WP3)
    b. ECD kidneys that undergo HMP+O2 after a period of CS (intervention arm WP3)
    c. 50+ DCD kidneys that are stored by HMP (control arm WP4)
    d. 50+ DCD kidneys that are stored by HMP+O2 (interventional arm WP4)
    e. 50+ DCD kidneys that are stored by HMP+O2 (interventional arm WP4)
2. How are recipient plasma injury markers influenced by the intervention.

Rationale

Ischaemia-reperfusion injury (IRI) after kidney transplantation can range from mild and shortlasting to severe and more prolonged. Markers of initial graft injury are commonly used in solid organ transplantation (transaminase in liver, troponin in heart, and amylase in pancreas transplantation). They provide early, reliable information on the severity of graft injury and its functional outcome, assisting clinicians in recipient management. Surprisingly, no markers quantifying the severity of kidney graft injury are available. Currently, initial kidney graft injury is estimated indirectly and at a late stage by assessing graft (dys)function. However, the accepted definition of DGF—need for dialysis within the first week posttransplant—is rudimentary and subjective. Delayed graft function is diagnosed several days after the initiating injury and does not identify duration or reversibility of the

dysfunction. Even standard functional markers—creatinine (clearance)—are unreliable indicators of kidney function during an episode of acute injury. Creatinine changes are not specific for parenchymal damage and occur only late after the event; creatinine clearance can remain stable in case of quite severe injury. Histopathology, the standard to detect tissue injury, is invasive, prone to sample error, and associated with interobserver variability. Moreover, there is always a delay between the actual injury and its morphological expression. We have previously shown in a porocine model of kidney autotransplantation that plasma AST, H-FABP, and NGAL reflect the severity of initial kidney graft injury and predict graft dysfunction earlier and more accurately than creatinine (clearance) and histology (1). However, there is currently very limited data assessing the value of these markers in the kidney recipient population. Furthermore, available data only looked at injury markers several days or weeks after the transplantation, as such missing the peak of IRI.

Additionally, the hypothesis of the interventions in WP3 and WP4 is that they reduce ischemic injury and consequently IRI. Therefore, we can hypothesise that end ischemic HMP+O2 (vs CS) and HMP+O2 HMP (vs HMP) will lead to reduce parenchymal injury that will be reflected by the injury markers in the recipients plasma.

#### 6.5.2. Material and methods

During the clinical trials of WP 3 and WP 4, recipient plasma samples will be collected and stored appropriately to allow determination of AST, H-FABP and NGAL at baseline and shortly after reperfusion. The evolution of these markers over time will be assessed. We will further determine their predictive accuracy for DGF, PNF, 1 year graft function, and 1 year graft survival. Univariable and multivariable regression models will be constructed. The plasma markers will be added as covariates to the model. Other covariates will be predetermined and based on the multivariate models in WP 3 and WP 4. Receiver operator characteristic (ROC) curves will be constructed to assess the predictive accuracy of the markers.

#### 6.5.3. References

1 Jochmans et al. Ann Surg 2011; 5: 784-792

### 6.6. Ancillary study 6: The autophagy-apoptosis-necrosis balance in kidney grafts after hypothermic machine perfusion with or without oxygen.

#### 6.6.1. Background & Rationale

Question

Is the autophagy-apoptosis-necrosis balance altered in kidney grafts subjected to pre-implantation oxygenated hypothermic machine perfusion (HMP) compared to HMP without oxygen and does an altered balance correlate with better graft outcome?

Rationale

Through molecular cross-talks, a delicate balance between the cell survival and cell death pathways (autophagy, apoptosis and necrosis) is maintained (1). These pathways are all affected by the ischemia-reperfusion stress and the inflammatory response following kidney transplantation, but their implications in the short- and long term graft function are still unclear. A detailed analysis of this balance of all relevant cell death/survival pathways in kidney transplantation has not been

performed. A correlation of their activities with improved graft outcome (e.g. after oxygenated HMP) will help to understand their respective roles in graft injury after kidney transplantation.

In the past decade the development of high resolution mass spectrometry techniques has advanced proteomic studies. New methodologies have been developed to study protein degradation in tissues as a physiological response to apoptosis. Looking into the patterns of protein degradation it will be possible to identify patterns of protein degradation that reveal the enzymes in action and are associated to intervention and clinical outcome.

### 6.6.2. Material and methods

Biopsies and plasma will be collected from DCD kidneys (50+) subjected to HMP with or without oxygen during the course of the FP7 COPE trial WP4. Concomitantly, plasma will be collected from the patients. During HMP, perfusate will be collected.

Biopsies will be stored in AllProtect at -80°C for protein extraction and in formalin for staining experiments. Protein lysates will be evaluated for relevant autophagy (e.g. LC3, p62/Sqstm1) or apoptosis (e.g. cleaved caspase 3, Bim) markers. Plasma and perfusate will be evaluated for necrosis and kidney function/injury markers (e.g. AST). PAS and TUNEL staining will be performed to visualize injury and cell death, resp. The analysis of these markers will determine the dynamics of these pathways after kidney transplantation. In addition, the comparison between their activities after oxygenated versus non-oxygenated HMP will allow us to correlate their extent of activation with graft outcome.

GeLC MS/MS & PROTOMAP: Reperfusion biopsy samples obtained from the two arms of intervention will analysed by 1D SDS–PAGE. Each gel lane will be sliced into ~20 evenly spaced bands. These bands will then in-gel digested with trypsin and individually analysed by reverse-phase LC-MS/MS. Finally, peptide-sequence information from each gel band is integrated into peptographs that will enable clear visualization of shifts in migration and changes in topography of proteins and their products of degradation between compared samples. These maps will reveal which proteins are cleaved, the sites of cleavage and the products of protein degradation. By looking the peptide sequence of the cleaved proteins we will be able to identify the enzymes that are in action.

Number of samples:

Number of samples per experimental group (+/- O2): 10 per group matched for age, warm and cold ischemic time.

### 6.6.3. References

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## 6.7. Ancillary study 7: The effect oxygenated kidney preservation on DNA methylation in kidney transplantation.

### 6.7.1. Background & Rationale

Question:

Does oxygenation during hypothermic machine perfusion (HMP) affect the occurrence of DNA methylation changes in the renal allograft during transplantation?

Rationale:

How short-lived ischemia during kidney transplantation can influence long-term allograft function remains unknown. We are currently investigating whether ischemia-induced epigenetic changes can provide the missing link.

Epigenetics are heritable and dynamic properties of the genome that define gene expression without alteration of the primary DNA sequence [1]. In this manner epigenetic changes are at the interface of environmental stimuli and long-lasting cellular phenotypes. One of the major epigenetic mechanisms is methylation of cytosines in gene promotor regions. The Laboratory of Translational Genetics headed by Diether Lambrechts has strong evidence that hypoxia induces DNA methylation changes. Together with Diether Lambrechts, we observed DNA hydroxymethylation differences between kidneys from living and deceased donors. We hypothesize that ischemia during kidney transplantation induces DNA (hydroxy-)methylation changes of the allograft. This could explain how ischemia and reperfusion affect long-term allograft function.

The biopsies taken as part of the COPE COMPARE study provide a solid approach to confirm our hypothesis. First, these biopsies allow for direct comparison of donor kidneys treated with or without oxygen. Second, as kidney pairs are derived from the same donor, the DNA methylome at baseline will be the same, with hypoxia the sole variable explaining DNA methylation differences. These observations will be combined with allograft function and survival.

The project aims to study and establish a novel pathway of injury in donor organs and aid in the detection of molecular markers to predict outcome of transplantation. Being potentially modifiable, epigenetic phenomena could be attractive therapeutic targets in transplantation.

### 6.7.2. Material and methods

From the post-reperfusion biopsies obtained in the COPE COMPARE trial DNA and RNA will be extracted. In a small cohort (n=2x8), the extracted DNA will be subjected to genome-wide DNA methylation and hydroxymethylation analysis by targeted bisulfite sequencing of gene promotor regions selectively isolated using capture probes. To assess the functional relevance of the observed epigenetic changes on the transcriptome, the extracted RNA will be used for RNA sequencing. This first step will investigate whether oxygenation during kidney preservation induces DNA methylation changes. If this would prove to be true, we will use the extracted DNA from a second larger cohort for whole-genome array-based methylation analysis (n=2x80), to confirm these findings and correlate the DNA methylation profiles to renal transplant function and histology. A dedicated and experienced bio-informatician will assist us with all analyses.

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