Normothermic Kidney Perfusion Phase 1

A single centre pilot study of prolonged normothermic perfusion of deceased donor kidneys prior to transplantation.

ISRCTN:	*To be registered prior to study commencement*
Ethics Ref:	*TBC*
Protocol Version:	1.0 (23rd October 2020)
Chief Investigator:	Professor Peter J. Friend Professor of Transplantation Director of Oxford Transplant Centre Nuffield Department of Surgical Sciences University of Oxford UK
Investigators:	Mr Simon Knight (University of Oxford) Prof Constantin Coussios (University of Oxford) Mr James Hunter (University of Oxford) Prof Rutger Ploeg (University of Oxford) Dr Annemarie Weissenbacher (University of Innsbruck) Mr Richard Dumbill (University of Oxford)
SME partner:	Mr Craig Marshall (OrganOx Ltd)
Patient representatives:	Mr Steve Rogers (Patient Representative)
Sponsor:	University of Oxford
Funder:	NIHR i4i
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
Conflicts of Interest:	Peter Friend is a co-founder, Chief Medical Officer and Consultant to OrganOx Limited and also holds shares in the company. Constantin Coussios is a co-founder, Chief Technical Officer and Consultant to OrganOx Limited and also holds shares in the company. Simon Knight has received consultancy fees from OrganOx Limited for assisting in the design of previous clinical trials. Craig Marshall is Chief Executive Officer for OrganOx Ltd.

STUDY SYNOPSIS

Trial Title	Normothermic Kidney Perfusion Phase 1	
Short Title	NKP1	
Clinical Phase	Phase I (IDEAL 2a)	
Trial Design	Single centre prospective 3-stage cohort study	
Trial Participants	Adult recipients of deceased donor kidney transplants	
Planned sample size	36 patients (12 per stage)	
Intervention	Normothermic machine perfusion (NMP) with oxygenated perfusate containing red blood cells, for increasing time durations pre-transplant: • Stage 1: Between 2 and 6 hours NMP • Stage 2: Between 2 and 12 hours NMP • Stage 3: Between 2 and 24 hours NMP	
Control	Matched historical controls (2:1) who have undergone deceased donor kidney transplantation on or after 01/10/2016 at the Oxford Transplant Centre	
Follow-up duration	12 months (primary outcome reporting at day 30)	
Planned trial period	36 months	
	Objectives	**Outcome measures/endpoints**
Primary	To assess the safety and feasibility of a normothermic perfusion device for the prolonged ex-vivo perfusion of deceased donor kidneys prior to transplantation, and to establish suitability of this technique for progression to a future efficacy or utilisation trial.	30-day graft survival (primary) Organ discards Adverse events Adverse device events/device errors
Secondary	To study the clinical outcomes and function of kidneys preserved by prolonged ex-vivo normothermic perfusion	3 and 12-month graft survival 30-day, 3- and 12-month patient survival Incidence of Delayed Graft Function (DGF) Incidence of Primary Non-Function (PNF) Early measures of graft function including functional DGF (fDGF), and day 2 creatinine reduction ratio 30-day, 3- and 12-month measures of graft function including estimated GFR, and serum creatinine trajectory 30-day, 3-month and 12-month proteinuria Acute rejection rate

Exploratory	To study the effects of prolonged ex-vivo normothermic preservation on post-transplant reperfusion injury	Pre-perfusion, and post-reperfusion histology Serial measure of biochemical markers of function and injury in the recipient post-operatively
	To identify biomarkers and machine perfusion parameters during NMP that are predictive of clinical outcome following transplantation	Machine perfusion parameters (including perfusate flow, gas flows, perfusate pressure, and urine output) Urine and perfusate biochemistry and acid-base balance Serial measurement of perfusate injury biomarkers
	To characterise the performance of the machine perfusion system during perfusion of human kidneys for transplantation	Biochemistry in urine and perfusate during NMP Insulin and glucose concentration during NMP Anti-factor Xa and free haemoglobin levels during NMP Perfusate microbiological culture
Device Name	OrganOx *metra K* normothermic kidney device	
Device Manufacturer	OrganOx Ltd, Oxford, UK	
Device Classification	Class IIa medical device	
Length of time device has been in use	First in man study (prior experience in discarded human and animal kidneys perfusion). The device does not currently possess a CE mark.	

PROTOCOL SIGN-OFF

Chief investigator:	Signature…… Name…… Date……
Investigators:	Signature…… Name…… Date…… Signature…… Name…… Date…… Signature…… Name…… Date…… Signature…… Name…… Date…… Signature…… Name…… Date…… Signature…… Name…… Date…… Signature…… Name…… Date……
Sponsor's representative:	Signature…… Name…… Date……
Senior statistician:	Signature…… Name…… Date……

INVESTIGATOR SIGNATURE PAGE

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

I understand that I may terminate or suspend enrolment of the study at any time if it becomes necessary to protect the best interests of the study subjects as advised by the DSMC. 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 the principles of Good Clinical Practice, the Declaration of Helsinki, and the moral, ethical and scientific principles that justify medical research. The study will be conducted in accordance with all relevant laws and regulations relating to clinical studies and the protection of patients.

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

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

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

Signature…… Name…… Date…….

ABBREVIATIONS

ADE	Adverse Device Effect
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CA	Competent Authority
cfDNA	Cell-free Deoxyribonucleic Acid
CIT	Cold Ischaemia Time
CMV	Cytomegalovirus
CRF	Case Report Form
DBD	Donation after brain death
DCD	Donation after circulatory death
DGF	Delayed Graft Function
DSMC	Data safety and monitoring committee
DPMP	Donors per Million Population Per Year
DRI	Donor Risk Index
ECD	Extended Criteria Donor
eCRF	Electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
EGL	Early Graft Loss
ESOT	European Society for Organ Transplantation
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GST	Glutathione S-Transferase
HD	Haemodialysis
HDF	Haemodiafiltration
HDU	High Dependency Unit
HF	Haemofiltration
HLA	Human Leucocyte Antigen
HMP	Hypothermic Machine Perfusion
ICU	Intensive Care Unit
IFU	Instructions for Use

IL-18	Interleukin 18
INR	International Normalised Ratio
ITU	Intensive Therapy Unit
IUD	Intrauterine Device
IVC	Inferior Vena Cava
KIM-1	Kidney Injury Molecule-1
L-FABP	Liver-type Fatty Acid Binding Protein
LDH	Lactate Dehydrogenase
MAP	Mean Arterial Pressure
MHRA	Medicines and Healthcare Products Regulatory Authority
NHSBT	National Health Service Blood and Transplant
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NMP	Normothermic Machine Perfusion
OCTRU	Oxford Clinical Trials Research Unit
OTC	Oxford Transplantation Centre
PIL	Patient Information Leaflet
PNF	Primary Non-Function
QUOD	Quality in Organ Donation
R&D	Research and Development
REC	Research Ethics Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SITU	Surgical Intervention Trials Unit
TMC	Trial Management Committee
USADE	Unanticipated Serious Adverse Device Event
UW	University of Wisconsin

DEFINITIONS

We will use the following definitions throughout this trial:

- Delayed Graft Function, DGF – we will use the following functional definition of DGF (fDGF): 'serum creatinine increases or remains unchanged or decreases <10% / day for the first 3 days post-transplant'. Additionally, we will record all post-operative use of dialysis.
- Primary Non Function, PNF – persistent dialysis dependence at 3 months post-transplant.
- Early Graft Loss, EGL – failure of perfusion resulting in a proven (radiologically +/- histologically) non-viable graft, or graft nephrectomy for any cause, within 30 days of transplantation.
- 30-day graft survival – a functioning graft in a patient who does not require chronic dialysis.

1. ADMINISTRATIVE INFORMATION

1.1 TITLE

"Normothermic Kidney Perfusion Phase 1"

1.2 TRIAL REGISTRATION

This trial protocol will be registered with the ISRCTN prior to study commencement.

1.3 PROTOCOL VERSION

1.3.1 CURRENT VERSION

Version number: 1.0

Issue date: 23rd October 2021

1.3.2 PREVIOUS VERSIONS

Details of previous versions and amendments to this protocol are detailed in appendix A4.

1.4 STUDY FUNDING

National Institute for Health Research (NIHR) Invention for Innovation (i4i).

The funding agency will not take part in nor has the ultimate authority over the study design; collection, management, analysis or interpretation of data; writing of the report; and the decision to submit the report for publication.

1.5 SPONSOR

The trial is sponsored by the University of Oxford. Contact details:

Joint Research Office
Boundary Brook House,
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ctrg@admin.ox.ac.uk
01865 616480

The sponsor will approve this protocol prior to study commencement.

1.6 TRIAL PERSONNEL

1.6.1 INVESTIGATORS

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1.6.2 SME PARTNER

SME Partner

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1.6.4 TRIAL STATISTICIAN

Trial Statistician

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Oxford Clinical Trials Research Unit (OCTRU)
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Nuffield Orthopaedic Centre
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Oxford, OX3 9DU

1.6.5 CLINICAL TRIALS UNIT

Oxford Clinical Trials Research Unit (OCTRU) incorporating the Surgical Interventional Trials Unit (SITU) group
The Botnar Research Centre

Nuffield Orthopaedic Centre
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Oxford, OX3 9DU

1.7 ROLES AND RESPONSIBILITIES

Chief and co-investigators

The chief and co-investigators will be responsible for:

- Design and conduct of the study
- Preparation of protocol and revisions
- Preparation of trial-specific instructions
- Advising the REC, CA and DSMC chairman of serious adverse events (SAE)
- Organisation and management of device training
- Organisation and management of logistics
- Publication of study reports
- Identification and recruitment of patients to the study
- Conducting clinical procedures in accordance with the protocol and standard operating procedures
- Data collection and completion of electronic CRFs
- Follow-up of study participants

Co-investigators will also be members of the trial management committee.

Clinical Trials Unit

The Oxford Clinical Trials Research Unit (OCTRU) incorporating the Surgical Interventional Trials Unit (SITU) is the designated clinical trials unit for this trial and will be responsible for:

- Database design
- Oversight of the management of data collection
- Statistical analysis of trial data
- Providing data for regular DSMC meetings
- Oversight of monitoring the trial
- Oversight of site initiation and close-out

Database design

OCTRU will develop and maintain the trial database and electronic clinical reporting forms. The database will have reporting functionality in order to capture data entry and verification, which the trial manager (research fellow) will utilise.

Trial Manager

The trial manager is a Research Fellow based outside of OCTRU and SITU and ensure that regulatory standards are maintained and that the trial is conducted according to the principles of GCP. The Trial Manager will also be responsible for:

- Organisation of trial management committee meetings
- Maintaining and applying for the study's regulatory approvals
- Ensure local investigator's compliance with the trial protocol
- Clinical monitoring
- Regular reporting to the sponsor and funder on the progress of the clinical investigation.
- Data cleaning

Trial statistician

The trial statistician will be responsible for approving the format of the data collection, preparing the data for analysis, performing the analysis and presenting the trial results as outlined in this protocol.

1.8 COMMITTEES

1.8.1 TRIAL MANAGEMENT COMMITTEE

The trial management committee (TMC) will be responsible for:

- Agreement of the final protocol
- Reviewing progress of the study and, if necessary, agreeing to changes to the study protocol and/or standard operating procedures to facilitate the success of the study
- Reviewing new studies that may be of relevance to the current protocol
- Receiving and reviewing details of any concerns raised by the DSMC

All co-investigators, SME partners and patient representatives will be trial management committee members. These individuals are:

- Professor Peter Friend
- Mr Simon Knight
- Professor Constantin Coussios
- Mr James Hunter
- Dr Annemarie Weissenbacher
- Professor Rutger Ploeg
- Mr Richard Dumbill
- Mr Steve Rogers
- Mr Craig Marshall

The committee membership will also include a senior representative from the Clinical Trials Unit.

1.8.2 DATA SAFETY AND MONITORING COMMITTEE

The data safety and monitoring committee (DSMC) is responsible for:

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

As a result of the reviews the DSMC may make recommendations to the TMC, including premature termination of the trial, should they feel it is indicated.

Members of the data safety and monitoring committee are:

- Professor Nizam Mamode, Professor of Transplant Surgery, Guy's and St. Thomas' NHS Trust
- Professor Michael Nicholson, Professor of Transplant Surgery, University of Cambridge
- Virginia Chiocchia, Medical statistician, University of Bern

2. INTRODUCTION

2.1 KIDNEY TRANSPLANTATION – CURRENT TRENDS

Kidney transplantation is the best available treatment option for many patients with end stage renal failure. Kidney transplantation is associated with improved life expectancy, with reduced medium and long-term mortality accounting for an overall doubling of life-span from 10 years (dialysis) to 20 years (transplant). Quality of life studies have shown the benefit of transplantation over dialysis, including in older patients(1,2). The cost effectiveness of kidney transplantation is well-recognised: for example, a recently published Scandinavian study demonstrated that, compared to dialysis, a kidney transplant saves €380,000 over 10 years, representing 66-79% of the expected healthcare costs of a patient requiring renal replacement therapy(3).

60,000 patients in the UK currently receive renal replacement therapy (RRT), in the form of dialysis or transplantation: of these 53% have functioning kidney transplants(4). Transplantation is more prevalent amongst younger patients (66% of those younger vs. 31% of those older than 65 years). The demand for kidney transplantation is increasing, due to a 4% annual increase in the population of patients requiring RRT. Also, progressively improving results have broadened the applicability of transplantation, particularly in older patients and those with comorbidities. This has increased demand on the limited organ resource: meanwhile the proportion of 'ideal' donors (younger brain-dead donors with a non-cardiovascular cause of death) has declined due to improvements in road safety and medical services. The median waiting time for a kidney transplant in the UK is 829 days, with around 250 patients dying on the waiting list each year(5).

2380 deceased donor kidney transplants were carried out in the United Kingdom in 2017-18. However, at the end of this year, 4819 patients were registered actively for a kidney on the national waiting list(5). This shortfall is typical for kidney transplant services around the globe. The factor that prevents the NHS (and healthcare providers in other countries) from providing a transplant for every patient who would benefit is that of donor organ supply.

Much progress has been made in the UK to increase the number of organ donors. The annual number of deceased donors has increased from 809 (2007-8) to 1575 (2017-8), an increase of 95%. The rate of increase in transplant numbers, although substantial, does not reflect the same degree of change: this is because many of the additional donors are less suited to the needs of patients. Nonetheless, to maximise the number of transplants, the transplant community has increasingly turned to the use of older and higher risk donor organs, those which would not have been considered acceptable for transplant previously.

Higher risk kidneys include those from donors declared dead by cardiovascular criteria (donation after circulatory death, DCD) rather than neurological criteria (donation after brain death, DBD), as well as donors with additional comorbidities, including older age, cardiovascular disease and diabetes (extended criteria donors, ECD). High risk donor organs of this sort are much more likely to be declined for clinical transplantation, sometimes because of clear evidence of non-viability (e.g. obvious vascular and/or parenchymal disease), but much more commonly due to uncertainty as to whether the organ will provide adequate function after the transplant.

Many such kidneys are either not accepted for transplantation at the time of donor offering (and not retrieved) or retrieved and then declined after inspection. In the UK in 2016-17, 2822 kidneys were offered, of which only 2390 (85%) were transplanted. If even half of these discarded kidneys were transplanted, these would represent an additional 216 transplants per year. Finding ways to transplant a higher proportion of available organs without compromising the outcome is one of the major challenges facing organ transplant clinicians. Even a modest increase in organ utilisation would bring vital benefit to patients on transplant waiting lists.

The reasons for this low level of organ utilisation are two-fold: (i) organs suffer damage during the transplant process of brain death, organ retrieval, preservation and reperfusion; (ii) the criteria whereby organ viability is assessed are inadequate: there is a lack of objective and reliably predictive metrics. It is very likely that many of

the organs currently discarded could be transplanted successfully if: (i) improved methods were available to reduce the damage caused by the transplant process, and (ii) more reliable methods were available to assess organ viability.

2.2 Methods of Organ Preservation

For many years, standard practice has been to cool kidneys after removal from the donor for storage and transport on ice (static cold storage; SCS). This involves flushing the organ with a specialist solution designed primarily to prevent cell swelling as cell membrane functions cease with decreasing temperature. More recently, there has been a resurgence of interest in hypothermic machine perfusion (HMP), pumping cold preservation solution through the circulation of the organ throughout preservation. There is evidence that this form of preservation is superior to SCS, especially in the context of extended criteria donor organs(6,7).

However, neither SCS nor HMP achieve physiological conditions which would allow: (i) the organ to remain in a functioning state; (ii) injury sustained before/during retrieval (e.g. hypoxia) to be reversed; (iii) direct measurement of the function of the organ to assess its potential to function after transplantation. In particular, cooling and hypoxia are known to be most damaging to ECD and DCD organs, the categories most affected by poor utilisation.

2.3 Normothermic Machine Perfusion

We are proposing to test a novel approach to the practice of kidney transplantation, normothermic machine perfusion, which maintains the organ in a physiological, functioning state during preservation. This potentially fulfils the three criteria defined in the previous paragraph and would, if successful, unlock the potential of many donors/donor organs that are currently not used. Our group has previously developed comparable technology for the liver(8) and recently published a Phase 3 trial showing clear evidence that NMP greatly reduces preservation-related damage, reduces the rate of discard of retrieved organs (by 50%) and allows (54%) longer preservation times. NMP may, therefore, benefit not only transplant outcome and organ utilisation, but also allocation of organs, operating theatre logistics and cost(9).

A previous systematic review of machine preservation techniques in kidney transplantation identified just one human clinical study of normothermic preservation from the group of Hosgood/Nicholson(10). The remaining evidence came from experimental animal studies. The review found that, in these experimental studies, NMP was associated with lower peak creatinine values than HMP or cold storage, as well as improved survival compared to HMP. Given the limitations of this previous review, we have conducted an up-to-date systematic literature search to identify more recent evidence for the experimental and clinical application of NMP.

The evidence from transplanted organs that kidney preservation by NMP is superior to SCS is primarily based upon clinical and experimental studies from Hosgood/Nicholson and Selzner(11-14). Pre-clinical studies by the former group demonstrated the technical feasibility of delivering therapy to the organ during normothermic perfusion(15-17). This group was also the first to demonstrate that a short (one to two-hour) period of NMP prior to transplantation following SCS yielded significant improvements in post-transplant metabolic function and reduced tubular injury compared to SCS alone(17,18). These findings were translated into humans in a series of seminal studies which provided evidence suggesting that a 60-minute period of normothermic perfusion following SCS makes it possible to transplant marginal kidneys more reliably and with improved immediate graft function compared to SCS alone(19-22).

Since reporting the first kidney transplant in man after a 60 minutes period of NMP Nicholson et al. have developed a score for organ quality assessment during perfusion(20). This is a cumulative points score based on perfusion of a large number of discarded human kidneys, incorporating 3 main parameters: (i) macroscopic assessment (1 to 3 points); (ii) renal blood flow - more or less than 50 ml/min/100g (0 or 1 point); (iii) total urine output – more or less than 43 ml (0 or 1 point). The total score therefore ranges from 0 to 5 points, 1 the best and 5 the worst quality.

Nicholson and Hosgood proposed that kidneys with a score 1 to 3 can be transplanted successfully, kidneys with a score of 4 may be suitable for transplantation, and that those with a score of 5 should not be considered for transplantation(20,21) . As well as no improvement in perfusion parameters during the 60 minutes of NMP, kidneys scoring 5 showed a low level of tubular function compared to the kidneys with a score 1 to 4(20,21) . This is the only score to date that attempts to quantify the quality of a human kidney during NMP to determine its suitability for transplantation.

The Cambridge group has now applied this technique of NMP and quality scoring in a clinical trial, using organs declined by other transplant centres. They first reported the transplantation of a pair of DCD kidneys declined for transplantation by all UK transplant centres because of inadequate in situ perfusion. During 60 minutes of NMP the patchy areas cleared in both kidneys prompting the decision to transplant these organs; both recipients had immediate graft function(22) . With this evidence, combined with that from the discarded kidney study, Nicholson and Hosgood are conducting a Phase 3 randomised controlled trial in DCD kidney transplants. This UK-based multicentre trial compares the efficacy of a 60-minute period of pre-transplant NMP, as previously used, with conventional SCS. The primary outcome is the rate of delayed graft function (DGF); results are expected by 2020(23).

2.4 Prolonged perfusion periods and Urine recirculation

All clinical studies to date have targeted short-term durations of perfusion (1 to 2 hours), with the intention of recovering cellular energetics prior to reperfusion in the recipient(11,18,22). However, recent work by Selzner *et al*. in a porcine model of kidney transplantation, demonstrated that longer, up to 16-hour periods of NMP following SCS are superior to both SCS alone and short-duration NMP; this is in terms of both tubular injury and post-transplant organ function(24,25).

There are no published studies of clinical kidney transplantation after NMP perfusion for longer than 2 hours. However, our own research group, supported by a previous i4i grant, has recently published the experience with 24-hour NMP of discarded human kidneys(26). Based on experience gained in developing the NMP liver device, we used a circuit based on a single unit of red blood cells, a combination of air and CO_2, and automated regulation of gas flows, temperature and arterial pressure.

This is also the first report of NMP with urine recirculation. In order to manage the problems of perfusate composition that would occur in a 24-hour perfusion of a diuresing kidney, we tested a closed perfusion circuit with urine recirculation. We first calculated the metabolic effects of urine recirculation regarding the accumulation of metabolic waste products. Allowing for the relative mass of the kidney alone (versus the body mass) and relative volume of the circuit (versus the human circulation), there need be no theoretical concern about waste product accumulation (i.e. the need for 'dialysis' of the perfusate) for periods of up to several months.

Eleven kidneys, retrieved with the intention of clinical transplantation but then declined, were perfused, six from DBD donors and five from DCD donors. Of these, three kidneys were perfused using Ringer's lactate solution to replace excreted urine volume, and eight using urine recirculation to maintain perfusate volume without fluid replenishment. In all cases NMP was associated with stable or slightly improved histological appearances of the renal tubules. There was urine production in both donor organ groups. All kidneys with urine recirculation were readily perfused for 24 hours (n=8) and maintained physiological perfusate sodium levels, whilst in kidneys without urine recirculation (n=3) NMP could only be achieved for a shorter time of 7.7±1.5 hours and was associated with significantly higher perfusate sodium levels. We concluded that NMP of human kidneys for 24 hours appeared to be feasible when associated with urine recirculation(26).

Regarding longer periods of NMP, our own group's recent phase I and phase III studies in human liver transplantation have provided evidence of the superiority of prolonged NMP over SCS for both conventional and extended criteria donor organs(8,9). As part of the EU-funded Consortium for Organ Preservation in Europe (COPE), a multicentre liver transplant trial showed clear advantages of this more physiological preservation

technique. The most important findings were of significantly lower levels of peak AST (hepatocellular injury marker and validated surrogate parameter for graft and patient survival, and the primary endpoint of the trial), despite a 50% reduction in the proportion of livers that were discarded after retrieval, and a 54% increase in the mean preservation time(9).

2.5 SUMMARY

The existing evidence therefore demonstrates the safety and feasibility of a relatively brief period (1-2 hours) of normothermic perfusion in the clinical setting, as well as longer periods of perfusion in the experimental setting. The proposed study will develop NMP for prolonged periods in the clinical setting, potentially transforming the clinical approach to higher-risk donor organs.

3. OBJECTIVES

3.1 HYPOTHESIS

Prolonged (2-24 hour) normothermic ex-vivo perfusion of the kidney is safe and feasible in the clinical setting.

3.2 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.2.1 PRIMARY OBJECTIVE AND OUTCOME MEASURES

Primary objective

To assess the safety and feasibility of a normothermic perfusion device for the prolonged ex-vivo perfusion of deceased donor kidneys prior to transplantation, and to establish suitability of this technique for progression to a future efficacy or utilisation trial.

Primary outcome measure/endpoint

The primary outcome is 30-day graft survival. This is defined as a functioning transplant in a patient who does not require chronic dialysis. This is primarily a safety endpoint, representing organs that have been safely and successfully preserved and transplanted.

We will also document adverse events, adverse device events and device deficiencies, as well as organ discards.

3.2.2 SECONDARY OBJECTIVES AND OUTCOME MEASURES

Objective	**Outcome Measures**
To study the clinical outcomes and function of kidneys preserved by prolonged ex-vivo normothermic perfusion.	1. 3 and 12-month graft survival (defined as a functioning graft and independence from dialysis) * 2. 30-day, 3- and 12-month patient survival * 3. Early measures of graft function: a. Incidence and frequency of the use of dialysis in the first 7 days post-transplant* b. Incidence of functional Delayed Graft Function (fDGF), defined as failure of serum creatinine to fall by at least 10% per day for the first 3 days * c. Day 2 creatinine reduction ratio ((serum creatinine day 1 – serum creatinine day 2) / serum creatinine day 1) * d. Total proteinuria each day measured as milligrams of urinary protein by 24-hour urine collection, post-operative days 1-4. e. Total urine production, post-operative days 1-4. 4. Incidence of Primary Non-Function (PNF), defined as persistent dialysis dependence at 3 months post-transplant * 5. 30-day, 3- and 12-month measures of graft function: a. Estimated GFR (CKD-EPI formula (27), appendix 5) * b. Month 3 to month 12 serum creatinine gradient, defined as month 12 serum creatinine concentration (µmol/l) – month 3 serum creatinine concentration (µmol/l)* c. Proteinuria – as a binary variable defined using published KDIGO cut-offs* (28) (appendix 5) and quantitated by protein-creatinine ratio. 6. Incidence of acute rejection, defined as biopsy-proven acute rejection diagnosed in accordance with the Banff criteria (29) * * Outcome measures marked with an asterisk will also be collected in the cohort of matched historical controls.

To study the effects of prolonged ex-vivo normothermic preservation on post-transplant reperfusion injury	1. Pre-perfusion and post-reperfusion histology a. H&E and PAS staining b. KIM-1 immunohistochemistry 2. Serial measurement of injury biomarkers such as serum NGAL and LDH post-operative days 1-4.
To identify biomarkers and machine perfusion parameters during NMP that are predictive of clinical outcome following transplantation	1. Machine perfusion parameters (including perfusate flow, perfusate pressure, gas flows, and urine output). 2. Serial measurement of markers of renal function during perfusion, including urine-perfusate sodium gradient, and on-pump proteinuria. 3. Serial measurement of injury biomarkers during perfusion, such as NGAL, KIM-1, L-FABP, GST, LDH, AST, IL-18, and cell-free DNA
To characterise the performance of the machine perfusion system during perfusion of human kidneys for transplantation	1. Urine and perfusate biochemistry and acid-base balance 2. Insulin pharmacokinetics during NMP 3. Glucose and oxygen metabolism during NMP 4. Anti-factor Xa levels and free Hb during NMP 5. Perfusate microbiological culture (end-perfusion)

4. TRIAL DESIGN

This is a prospective, 3-stage cohort study investigating gradually increasing durations of NMP prior to transplantation of up to 24 hours. Upon offer of an eligible deceased donor kidney, recipient eligibility will be confirmed and consent will be taken. The kidney will be transported to the Oxford Transplant Centre (OTC) under static cold storage, as per standard practice.

Upon arrival at the OTC, the kidney will be prepared on the back-table and placed on the OrganOx metra K normothermic kidney device. The duration of preservation will be dictated by study stage and logistical considerations (as determined by the implanting consultant surgeon):

- **Stage 1:** Minimum 2 hours, maximum 6 hours NMP (12 patients)
- **Stage 2:** Minimum 2 hours, maximum 12 hours NMP (12 patients)
- **Stage 3:** Minimum 2 hours, maximum 24 hours NMP (12 patients)

Note: For reasons of safety, interim reports will be drawn up after recruitment of patients 10 and 22. These reports should be prepared and finalised, if possible, by the time of recruitment of patients 12 and 24, respectively. The trial will not proceed to recruitment of patients 13 and 25 respectively (i.e. will not move from stage 1 to stage 2, and from stage 2 to stage 3) without consideration of the interim reports by the DSMC and the TMC, and approval to continue recruitment.

The trial will be conducted as Phase I/ IDEAL 2a development study; as such we will identify and report on any modifications to the device or indications for use that evolve during the study(30).

Enrolled patients will participate in the study for 12 months, with outcomes assessed during the initial inpatient stay and at study visits at day 30 post-transplant and at month 3 post-transplant. Additional biochemical and survival data will be collected at 12 months through linkage to the NHSBT registry and/or correspondence with the referring renal unit.

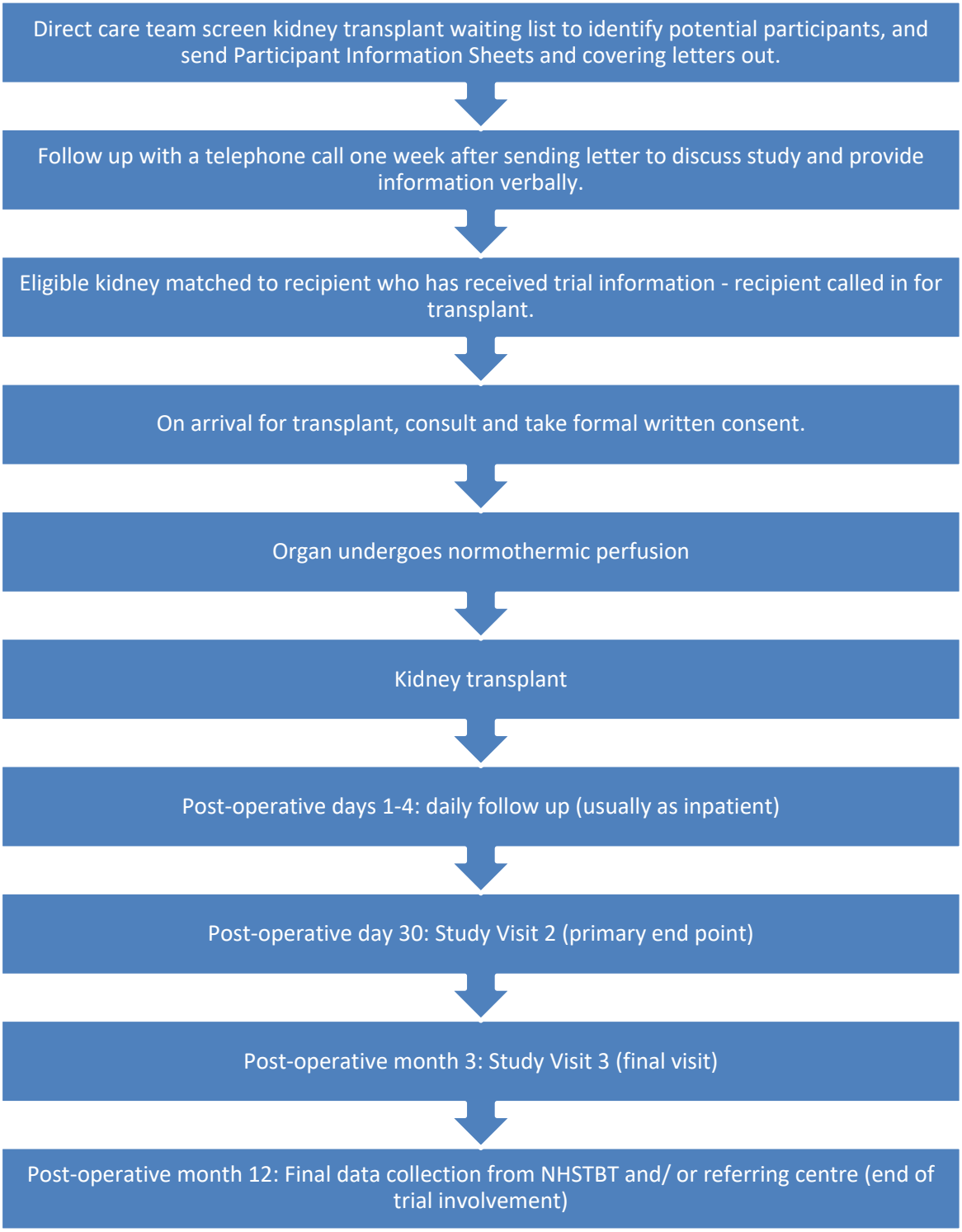
Data will be collected into a secure central online electronic database using electronic case report forms.

A historical control cohort will comprise deceased donor kidney transplant recipients from the Oxford Transplant Centre transplanted since October 2016. Control patients will be matched 2:1 to study participants based upon donor type, preservation time, and donor risk index.

The study will close after the final patient has completed 12 months follow-up. Final analysis will take place after all endpoints have been collected.

Anticipated flow of patients through the trial is depicted in *figure 1*.

Figure 1: Participant flow through the trial



5. PARTICIPANT IDENTIFICATION

5.1 STUDY SETTING

Recruitment will take place at the Oxford Transplant Centre (OTC). The centre performs around 150 deceased donor kidney-alone transplants per annum.

5.2 TRIAL PARTICIPANTS

Participants will be adult patients active on the waiting list for kidney transplantation at the Oxford Transplant Centre (OTC).

5.3 ELIGIBILITY CRITERIA

All eligibility criteria must be met at the time of enrolment.

5.3.1 DONOR CRITERIA

Inclusion

- Kidneys from deceased donors aged above 16 years
- DCD or DBD
- Accepted for transplantation according to local criteria
- Cold ischaemia time (CIT) prior to NMP no greater than 10 hours

Exclusion

- Donor kidneys that would not be accepted according to local criteria
- Donor kidneys accepted as a pair for dual transplant
- CIT greater than 10 hours prior to initiation of NMP

5.3.2 RECIPIENT CRITERIA

Inclusion

- Male or female, aged 18 years or older
- On waiting list for kidney transplantation at Oxford Transplant Centre, Oxford
- Provided informed consent for participation in the study
- Able and willing to comply with all study requirements (in opinion of investigator or deputy)
- Fit to proceed with kidney transplantation

Exclusion

- Not willing or unable to provide informed consent
- Recipients aged less than 18 years
- Participation in an investigational study likely to affect interpretation of the trial data
- Undergoing living donor kidney transplantation
- Undergoing dual kidney transplantation
- Undergoing transplantation of other organ(s) in addition to the kidney
- Substantial risk of transplant not proceeding – e.g. risk of positive cross-match (in opinion of Investigator and/ or implanting surgeon)

- Other significant disease or disorder which, in the opinion of the Investigator, may: (i) put the participant at risk by participating in the study; (ii) influence the result of the study; (iii) affect the participant's ability to participate in the study

5.4 MATCHED CONTROLS

Matched controls will be selected from an anonymised database of transplant operations done locally over the preceding three years. Matching will be performed algorithmically as described in section 6.5.2 and appendix 4.

Matched controls inclusion criteria

- Kidneys from donors aged 16 years or over.
- DBD or DCD.
- Recipients underwent transplantation of a deceased-donor kidney on or after 01/10/2016

Matched controls exclusion criteria

- Underwent transplantation of other organ(s) in addition to the kidney
- Underwent dual kidney transplantation
- Underwent living donor kidney transplantation
- 12-month eGFR not available

6. TRIAL PROCEDURES

The participant timeline is illustrated in appendix A2. The following section provides details of this timeline and all study procedures.

6.1 RECRUITMENT

The emergency nature of kidney transplantation means that once a potential recruit is called in for a transplant there will only be a 3-6 hour window for the consent and screening process to occur. This does not allow sufficient time for the potential participant to consider the implications of participating in the study. For this reason, all patients who fulfil the entry criteria and who are on the waiting list for kidney transplantation at the Oxford Transplant Centre will be approached in advance of the study by post or by electronic means, followed by provision of information verbally at either a routine clinic appointment, during inpatient admission, or by telephone. This telephone call or face-to-face consultation will be at least 7 days after sending an initial letter and Participant Information Sheet out. Detailed information will be given both verbally and in the form of a Participant Information Sheet. The Trial Manager and/or a researcher listed on the study delegation log will give the information. A list of patients on the waiting list who have received the written trial information, and who have had a verbal discussion about the trial, will be maintained at the Oxford Transplant Centre by the members of the trial team. This list will be accessible to the kidney transplant recipient coordinators, and the members of the trial team.

The database of matched controls will be drawn from follow-up data held locally for research and audit purposes at the Oxford Transplant Centre. The data on matched controls used for the trial will include no patient identifiable data and will contain data required for matching as detailed in section 6.5.2 alongside the specified endpoints.

6.2 INFORMED CONSENT

6.2.1 RECIPIENT CONSENT (STUDY VISIT 1)

Formal written consent will be taken when patients are called in for transplant. Patients will only be eligible if they have had at least 24 hours to consider the written trial information sent to them. Two copies of the consent form will be made with the original to be kept in the medical notes, one copy placed in the site file, and one copy given to the participant.

When a suitable donor organ becomes available and is allocated to a recipient who (i) has been provided in advance with the trial information, (ii) has been deemed to be fit to proceed with kidney transplantation, and (iii) is low-risk for a positive cross-match result, the recipient will be approached by a member of the Trial team and the study will again be discussed. The patient will be asked if they would like to take part in the study; if so they will be asked to sign and date the informed consent document. If the patient is not willing to proceed in the study at this stage, then organ preservation will be carried out using conventional procedures and transplanted in accordance with local protocol. In addition to trial consent, a consenting recipient will also be required to complete a surgical consent form for the transplant procedure in accordance with standard local policy.

No study specific procedures will be performed until the patient has signed and dated the latest approved version of the informed consent form. Written and verbal versions of the Participant Information Sheet and informed consent form will be presented to potential participants on their arrival for transplant, detailing the exact nature of the study, the implications and constraints of the protocol, the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. The participant will be allowed as much time as possible to consider the information, as well as the opportunity to question the investigator or other members of the clinical or research team, to decide whether to participate in the study.

Written informed consent will be taken and documented by means of dated signatures from both the participant and the member of the research/clinical team 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 suitably qualified individual;
3. authorised to do so by the Chief Investigator.

A copy of 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. All participants who receive a normothermically perfused kidney will be followed up for safety end-points as far as possible; however, no new study-specific samples would be taken following withdrawal of consent. Grounds for recruitment of additional participants in the event of withdrawal are described in section 6.8.3.

Each subject must also give permission for the sponsor and regulator's representatives to review their hospital records if required.

The subject's general practitioner/family doctor will be informed of their participation in the study. A letter to the participant's GP will be produced and sent out after they have consented to join the study, and undergone transplantation.

There is a small risk that participants in this study may lose capacity to consent to continued involvement in this study. Rarely, it is necessary for patients to be cared for on an intensive care unit (ICU) or a high dependency unit (HDU) for a short period of time following a kidney transplant. They would occasionally remain sedated following their surgery as part of this higher- level care. We would continue to take urine and blood samples in accordance with the protocol whilst their capacity is impaired in this manner, in the immediate post-operative period. In the event of a prolonged loss of capacity to consent to continued

involvement in the trial, we would provide their designated next of kin with information about the study (as the Patient Information Sheet) and seek agreement from them as about consultee about continuing to collect data from the participant whilst their capacity is impaired.

6.2.2 DONOR CONSENT

Explicit consent from the donor family is not required for participation in the study as the donation process is unaltered by participation in the trial (no intervention occurs prior to donation), and the intervention does not affect the transplantability of the donor organ.

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

6.2.3 TRIAL SAMPLES

Informed consent to join the trial includes consent for the following trial samples:

- Regular samples of perfusate and urine produced by the kidney during the process of normothermic perfusion, as detailed in appendix A3.
- Two kidney biopsy specimens (both intra-operatively at the time of transplant; one pre-perfusion and one post-reperfusion) as described in appendix A1. These biopsy specimens will be divided into two; one half will be examined and scored using the Modified Remuzzi scoring system as detailed under 6.5.3 as part of this trial. The other half will be stored for future analysis in a research biobank, as detailed below under 6.2.4.
- A urine sample of approximately 10ml on the day of transplant (pre-operative, taken following insertion of the urinary catheter after induction of anaesthesia); from post-operative day 5 until discharge; and at the 30-day and 3-month follow up appointments.
- A 24-hour urine collection on post-operative days 1 to 4 inclusive.
- Trial-specific blood tests up to 10ml (total) per day on the day of transplant (pre-operative); post-operatively each day until discharge; and at the 30-day and 3-month follow up appointments. Trial-specific sampling will usually only be required for NGAL measurement and biobanking; all other blood-based biochemical trial endpoints are anticipated to be met through routine blood samples which are required as part of standard patient care. A total volume of 10ml is sufficient for all required trial measurements and biobanking (see below) in the event that bloods are not taken for routine clinical care, and therefore represents a maximum amount that would be required.

Note that at our institution, insertion of a urinary catheter following induction of anaesthesia and subsequent removal on post-operative day 4 is standard protocol.

6.2.4 SAMPLES FOR BIOBANK

In addition to those samples required as part of this trial, additional biological samples will be obtained to be stored for use in future studies of the mechanism of action of normothermic perfusion, utilising the existing transplant biobank infrastructure at the OTC. Specifically, we will obtain two biopsy specimens (both intra-operatively; one pre-perfusion and one post-reperfusion) from the kidney; half of each biopsy specimen will be stored in the biobank for use in future analysis. We will obtain regular urine and perfusate samples during perfusion. We will also obtain blood (total up to 10ml, including that required for trial-specific investigations under 6.2.3) and urine samples (total up to 10ml, including that required for trial-specific investigations under 6.2.3) from the recipients daily starting following the induction of anaesthesia for transplantation (day 0), until discharge. The schedule for collection of blood from participants post-operatively, and amounts to be taken, are detailed in appendix A1. The schedule for collection of specimens during perfusion is detailed in appendix A3.

Patients are required to authorise these procedures by signing the additional consent item on the consent form. The consent form will identify them by name and/or patient study number and will be sent to the tissue bank along with their samples. Patients can withdraw permission to use their samples in these ways at any time,

without affecting their participation in the study. Banked samples will be managed in accordance with applicable host institution policies and the Human Tissue Act (HTA) requirements.

6.2.5 Procedure in the event of organ reallocation after the start of perfusion

It is possible that due to the detection of a last-minute contraindication to proceeding with transplantation such as an unexpected positive cross-match result, the kidney will have to be reallocated to a different recipient after consent has been taken and perfusion has been started. In this very uncommon eventuality, the kidney may be reallocated either locally or nationally. If the kidney is reallocated to a local recipient, it will be offered to that patient as a kidney which has already begun perfusion as part of the trial. The patient will be provided with three options: 1) to accept the kidney but not consent to joining the trial (in which case their data will not be collected for the purposes of the trial, and this perfusion will be replaced), 2) to accept the kidney and consent to join the trial, 3) to decline the kidney In the event that the kidney is re-allocated nationally, it will be offered as a kidney that has undergone a period of normothermic machine preservation. If it is accepted by an alternative transplant centre, perfusion will be terminated and the kidney will be cold-stored from the point of acceptance.

6.3 Screening and eligibility assessment

6.3.1 Recipient assessment

All patients on the transplant waiting list at the OTC will have been screened for suitability for transplantation; further screening assessment is not required as part of this trial. On offer of a suitable donor organ an approach will be made for consent as described in section 6.2.1.

On admission to hospital, the recipient will be assessed for fitness to proceed to transplant according to local procedures. If a recipient is deemed unfit for transplant at the time of admission, they will no longer remain active on the transplant waiting list and as such will be excluded from the trial. Any data collected from these individuals will not be included in the trial and will be deleted. Should a recipient who is deemed unfit for transplant subsequently become fit, be re-activated on the transplant waiting list, and be called in again for transplant, they would be eligible to join the trial as any other recipient.

6.3.2 Donor assessment

The role of the transplant recipient co-ordinator is to facilitate calls for organ offers and co-ordinate most aspects of the transplant process. On receiving an organ offer, the local recipient co-ordinator and on-call transplant surgeon will ascertain baseline demographic information from the NHSBT electronic offering system to assess eligibility of the kidney for inclusion in the trial.

6.4 Baseline assessments

6.4.1 Donor demographics

Donor demographics to be recorded will include the following:

- Age
- Sex
- Ethnicity
- Cause of death (CVA, hypoxia, trauma, other)
- Type of donor (DBD, DCD)
- Donor height
- Donor weight
- Donor history of hypertension

- Donor inotrope use
- Number of days in hospital prior to retrieval
- Donor risk index (DRI) (31)
- Last and peak serum creatinine
- Last and peak eGFR
- Donor proteinuria and/or protein:creatinine ratio
- CMV status
- Cold preservation solution (University of Wisconsin, Marshall's solution)

6.4.2 Recipient demographics

Recipient demographics to be recorded will include the following:

- Age
- Sex
- Aetiology of renal failure
- Native urine output (unimpaired/ reduced (10-500ml)/ absent)
- Dialysis status/duration
- History of hypertension
- History of diabetes
- Height and weight
- Number of previous transplants
- Calculated reaction frequency (CRF, %)
- HLA mismatches (A, B, DR)
- CMV status

6.4.3 Recipient baseline tests

Bloods taken on admission for transplant in line with departmental protocol/ routine clinical care will be recorded, including:

- Serum creatinine
- eGFR (if pre-dialysis)
- Haemoglobin concentration
- White cell count
- Lymphocyte count
- Neutrophil count
- Platelet count

In addition, a blood sample (5ml) and urine sample (5-10ml) will be taken following the induction of anaesthesia and insertion of a urinary catheter. The blood sample will be biobanked. The urine sample will be tested for protein: creatinine ratio, and the remainder of the sample biobanked.

6.5 Trial interventions

6.5.1 Normothermic perfusion

Following the routine retrieval procedure at the donor hospital the kidney will be placed in ice-cold perfusion solution and transported to the OTC under static cold storage as per standard NHSBT practice. Meanwhile the on-call surgical team will ensure that one unit of red blood cells of donor blood type is available for perfusion.

On arrival at the OTC, the kidney will be removed from storage and prepared for transplantation as per standard unit practice. Kidneys are routinely biopsied during the back-table procedure at our institution for biobanking; in the event that a trial kidney is not biopsied for the established biobank it will be biopsied at this point (pre-perfusion) for the purposes of the trial. The procedure for preparing the device for use and placing the organ on

the device is described in detail in the instructions for use (IFU) document (version xxx). Preservation will be monitored at all times by a research fellow or member of the surgical team. If perfusion problems are encountered which cannot be resolved rapidly, the kidney will be cold flushed and placed back on ice.

The procedure for removing the kidney from the device is also described in the IFU. Implantation and reperfusion of the kidney will proceed as per the usual practice at the Oxford Transplant Centre.

The duration of machine perfusion will be dictated by the stage of the study and by operating theatre logistics, with the aim of progressively extending the maximum allowable period of preservation during the study. The stages of the study are as follows:

- **Stage 1:** Minimum 2 hours, maximum 6 hours NMP (12 patients)
- **Stage 2:** Minimum 2 hours, maximum 12 hours NMP (12 patients)
- **Stage 3:** Minimum 2 hours, maximum 24 hours NMP (12 patients)

For kidneys with multiple arteries, the approach to perfusion will be determined by the anatomical configuration and implantation plan. The options include back table reconstruction to create a single inflow, use of a Y-connector, and suture of the aortic patch to a vascular graft. Arterial cannulation is an alternative to suturing the aortic patch to a vascular graft. If perfusion is not technically possible, the kidney will be preserved using standard static cold storage and transplanted as soon as is logistically possible. In this circumstance the reasons for failure to perfuse will be recorded and reported in the trial report. For the purposes of the trial, the perfusion will be replaced.

6.5.2 Matched controls

The heterogeneous nature of the donor population means that a contemporary randomised control cohort would be poorly matched. For this reason, we will identify two matched historical control transplants for each study patient, from patients who have undergone deceased donor kidney transplantation since 01/10/2016 at the Oxford Transplant Centre. The matching of cases to controls will be based on (i) donor type (DBD/DCD), (ii) induction immunosuppression, (iii) Cold Ischaemic Time, and (iv) Donor Risk Score (appendix A5 and (31)). Further details including the matching algorithm are given in Appendix 4.

6.5.3 Recording of operative and perfusion parameters

The following data will be collected from the NHSBT HOT B form and the implanting surgical team:

Donor timings

The times to be recorded for DBD donors are as follows:

- Cessation of donor circulation (cross clamp)
- Start of cold perfusion (should be the same unless technical problem)
- Kidney removal and placement on ice
- Arrival time at OTC
- Time kidney removed from storage to bench
- Initiation of normothermic machine preservation
- Cessation of normothermic machine preservation (cold flush)
- Time of removal from ice for implant
- Time of reperfusion in recipient

The times to be recorded for DCD donors are as follows:

- Withdrawal of support
- Onset of functional warm ischaemia (SBP < 50 mmHg)
- Cessation of donor circulation

- Start of cold perfusion
- Kidney removal and placement on ice
- Arrival time at OTC
- Time kidney removed from storage to bench
- Initiation of normothermic machine preservation
- Cessation of normothermic machine preservation (cold flush)
- Time of removal from ice for implant
- Time of reperfusion in recipient

Preservation parameters

In addition to timings, a number of other preservation parameters will be recorded. These will include:

- Quality of *in-situ* perfusion (graded poor, moderate, good by donor surgeon)
- Quality of bench perfusion (graded poor, moderate, good by recipient surgeon)
- Weight of the kidney at start and end of preservation
- Perfusion parameters (logged automatically by the device):
    - Arterial pressure (in mmHg)
    - Arterial flow rate (in ml/min)
    - Perfusate pO_2, pCO_2 and pH
    - Perfusate temperature (^oC), and urine production (ml/h)
- Perfusion solution used for aortic perfusion
- Perfusion solution used for organ transport
- Number of donor arteries, veins and ureters
- Method of arterial connection
- Hosgood/Nicholson quality assessment score (QAS) (32):
    - Score of 1-5 points, composed of:
        - Macroscopic assessment of the kidney: excellent perfusion (1 point), moderate perfusion (2 points) poor perfusion (3 points)
        - Renal blood flow (ml/min/100g): ≥ 50 (0 points), < 50 (1 point)
        - Total urine output (ml): ≥ 43 (0 points), < 43 (1 point)

Perfusate and urine samples will be taken periodically during NMP as detailed in appendices A1 and A3 to assess:

- Biochemical parameters including pH, sodium concentration, protein concentration, lactate, and glucose
- Injury markers including LDH and AST.
- Novel biomarkers of oxidative stress and renal injury, such as NGAL, KIM-1, L-FABP, IL-18, GST, and cell-free DNA.
- Pharmacokinetic properties of substances used during perfusion including insulin concentration, and anti-Xa activity.

In addition to these pre-specified outcomes, additional biological samples will be taken for the Oxford biobank at prespecified timepoints as detailed in appendices A1 and A3. These will be stored for future exploratory analyses.

At the end of preservation, a sample of perfusate/storage solution will be taken for microbiological culture.

Operative parameters

These will include:

- Total operative time: defined as time from knife-to-skin to skin closure.

- Anastomotic time (secondary warm ischaemia): defined as time between removal of organ from ice to organ reperfusion.
- Use of vasopressors prior to and after reperfusion
- Intraoperative transfusion of blood products measured in units.
- Number of arterial, venous and ureteric anastomoses
- Details of any vascular reconstruction performed

Histological evidence of ischaemia-reperfusion injury

Graft biopsies will be taken before connection to the normothermic perfusion device, and immediately prior to abdominal closure and examined for evidence of injury. Modified Remuzzi score, the acute tubular injury sub-score (0 = absent; 1 = loss of brush borders or vacuolation of tubular epithelial cells; 2 = cell detachment or cellular casts; 3 = coagulation necrosis), and KIM-1 positivity will be reported.

6.6 CONCOMITANT CARE

All other aspects of the retrieval procedure will be carried out according to local policies and national guidelines.

Recipient management including the implantation procedure, postoperative care, immunosuppression and other medications, and post-transplant monitoring will follow local protocols.

6.7 STUDY VISITS

6.7.1 INPATIENT STAY

Patients will be assessed daily by the clinical team and managed according to normal local protocols.

Outcome assessment

The following post-operative biochemical outcomes will be recorded:

- Daily serum samples during the inpatient stay, up to day 7, to include:
    - Serum creatinine
    - eGFR (using the CKD-EPI formula)
    - Full blood count
    - Serum C-reactive protein
    - Serum NGAL (post-operative days 1-4 only)
    - Serum LDH (post-operative days 1-4 only)
    - Samples for storage in the Oxford Biobank for use in future research (see appendix A1)

- Daily urine samples during the inpatient stay, up to day 7, to include:
    - Proteinuria (by 24 hour urine collection post-operative days 1-4)
    - Sample for storage in the Oxford Biobank for use in future research (see appendix A1)

The first measurements should be taken at 12 to 24 hours post-transplant. For subsequent measurements, in the event that more than one measurement is taken in a 24-hour period, the measurement taken closest to the specified time-point should be used. Approximately 10ml of blood in addition to that required for routine clinical care will be taken on each day. It is anticipated that most patients will be discharged before day 7 – in this case data will be collected daily for the duration of their inpatient stay. It is common practice for patients to be seen in clinic a few days after discharge following transplantation; if a routine post-transplant clinic visit falls in the first 7 days then this visit may be used as an opportunity to collect follow up data including blood and urine samples for research as if the patient were still an inpatient.

Other outcomes to be recorded include:

- Daily urine output (up to post-operative day 4)

- Total length of hospital stay (days)
- Requirement for renal replacement therapy (haemodialysis (HD), haemodiafiltration (HDF), haemofiltration (HF))

Safety outcomes

- Recipient infection (defined as a clinically diagnosed and treated infection)
- Biopsy-proven acute rejection episodes
- Ureteric complications (ureteric strictures - anastomotic and non-anastomotic, urine leaks)
- Vascular complications (bleeding, renal artery stenosis, renal artery thrombosis, renal vein thrombosis)
- Reoperation rate
- Any other adverse event

Severity will be graded according to the Clavien-Dindo classification(33) as described in Appendix A6.

Immunosuppression

Details of induction immunosuppression and maintenance immunosuppression (including doses) at discharge from hospital will be recorded.

6.7.2 STUDY VISIT 2 – DAY 30

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate. Follow up will be as close to 30-days post-operative as possible; dates 30 days ± 1 week from the date of operation will be acceptable.

Outcome assessment

The following biochemical outcomes will be recorded at day 30 post-transplant:

- Serum creatinine
- eGFR (using the CKD-EPI formula)
- Full blood count
- Serum C-reactive protein
- Urine protein: creatinine ratio

Other outcomes to be recorded include:

- Graft and patient survival at day 30 post-transplant
- Requirement for renal replacement therapy (HD, HF, HDF) at any time

Safety outcomes

- Recipient infection (defined as a clinically diagnosed and treated infection)
- Biopsy-proven acute rejection episodes
- Ureteric complications (ureteric strictures - anastomotic and non-anastomotic, urine leaks)
- Vascular complications (bleeding, renal artery stenosis, renal artery thrombosis, renal vein thrombosis)
- Reoperation and readmission rates
- Any other adverse event

Severity will be graded according to the Clavien-Dindo classification(33) as described in Appendix A6.

Immunosuppression

Details of maintenance immunosuppression (including doses) at day 7 and day 30 post-transplant will be recorded.

6.7.3 STUDY VISIT 3 – MONTH 3

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate. Follow up will be as close to 3 months post-operative as possible; dates 3 months ± 2 weeks from the date of operation will be acceptable.

Outcome assessment

The following biochemical outcomes will be recorded at day 30 post-transplant:

- Serum creatinine
- eGFR (using the CKD-EPI formula)
- Full blood count
- Serum C-reactive protein
- Urine protein: creatinine ratio

Other outcomes to be recorded include:

- Graft and patient survival at day 30 post-transplant
- Requirement for renal replacement therapy (HD, HF, HDF) at any time
- Primary non-function (PNF)

Safety outcomes

- Recipient infection (defined as a clinically diagnosed and treated infection)
- Biopsy-proven acute rejection episodes
- Ureteric complications (ureteric strictures - anastomotic and non-anastomotic, urine leaks)
- Vascular complications (bleeding, renal artery stenosis, renal artery thrombosis, renal vein thrombosis)
- Reoperation and readmission rates
- Any other adverse event

Severity will be graded according to the Clavien-Dindo classification (33) as described in Appendix A4.

Immunosuppression

Immunosuppression will follow the local protocol; details of maintenance immunosuppression (including doses) at 6 months post-transplant will be recorded.

6.7.4 LATER OUTCOMES

Whilst the final study visit will be at 3 months, we also intend to collect graft and patient survival data, adverse event information and serum biochemistry values from consenting participants at 12 months post-transplant. Data collection time points are outlined in detail in Appendix 2. Patients transplanted at the Oxford Transplant Centre are referred back to their original renal unit at 3 months post-transplant; therefore these data will be collected from referring centres and will be measured as part of routine clinical care, not requiring additional visits or interventions on the part of the trial participant. Where data are unable to be obtained, they will be requested from the NHSBT transplant database. Data will be collected from as close to 12 months post-operative as possible; dates 12 months ± 1 month from the date of operation will be acceptable.

The parameters to be recorded are:

- Patient and graft survival
- Serum creatinine
- eGFR (using the CKD-EPI formula)
- Acute rejection episodes

Where measurement has been performed as part of clinical care, spot urinary protein: creatinine ratio at 12 months will also be recorded.

6.8 PARTICIPANT RETENTION

All enrolled patients completing the 30-day follow-up assessment will be regarded as having completed the primary objective of the study. All patients 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 (e.g. travelling costs) as a result of study participation.

6.8.1 PATIENT-LED WITHDRAWAL

It is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. In the event of a patient choosing to withdraw from the trial, the reason for withdrawal must be documented on the eCRF. Such patients will be asked whether they wish to withdraw from future trial procedures only, or trial procedures and remote data collection. The options available to participants wishing to withdraw consent will be:

a) In the event that perfusion has begun but transplantation is yet to take place – participant withdraws consent for the kidney to continue with normothermic preservation and wishes to undergo transplant as soon as feasible, but agrees to continue with follow up as per protocol;
b) Post-transplant – participant has requested to withdraw from active participation in the trial including future trial-specific tests, but agrees to continued remote data collection as per protocol. In this instance we would continue to collect safety data (instances of adverse events) and data on graft function as recorded during routine clinic reviews, from routine laboratory tests, and as recorded by NHSBT and the referring renal centre up to 12 months post-transplant; samples collected to the point of withdrawal will be retained for the study and may be used in analysis. This may include samples and data taken for use in future research.

c) Pre- or post-transplant – participant withdraws consent for both further tests/ trial specific procedures and the future collection of data. In this instance we would continue to collect safety data (instances of adverse events) and data regarding kidney function as recorded during routine clinic reviews and by NHSBT up to 12 months post-transplant. The scope of the data collected would be agreed on a case-by-case basis with the Data Monitoring and Safety Committee.

6.8.2 INVESTIGATOR-LED WITHDRAWAL

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. We will keep all data accrued to the point of withdrawal, as is stipulated in the trial consent form. In the event that a patient loses capacity to consent to continue in the trial we would continue with collection of safety data. The scope of the data collected would be agreed on a case-by-case basis with the Data Monitoring and Safety Committee.

Possible reasons for investigator-led withdrawal of a participant from the trial include:

a) Ineligibility overlooked at screening or arising before transplantation (for example, positive cross-match)
b) Significant perfusion protocol deviation precluding transplantation

The investigators will not seek to withdraw any participant from study follow up who has received a kidney which has undergone normothermic perfusion; as far as is possible, all protocol-stipulated follow-up information will be collected. In the event of loss to follow-up, all available information will be used in the study analysis.

6.8.3 RECRUITMENT OF ADDITIONAL PARTICIPANTS

In this early phase study, analysis of sufficient numbers of participants who adhered to all important aspects of the protocol is vital. Therefore, additional patients will be recruited to replace participants in the primary analysis in the following circumstances:

- Withdrawal under 6.8.1 (a) where the kidney has been perfused for less than two hours;

- Ineligibility overlooked at screening, or detected prior to transplantation;
- Significant perfusion protocol deviation deemed likely to affect the outcome by the Data Safety and Management Committee;
- Significant non-compliance with trial requirements, for example non-compliance with routine immunosuppression, that may prejudice the outcome occurring within 30 days of transplantation;
- Unavailability of the primary outcome data;
- Additional circumstances agreed on a case-by-case basis with the Data Safety and Management Committee.

Protocol stipulated data collection will continue for these participants wherever possible. Participants whose withdrawal led to the recruitment of additional participants will be clearly marked on the database.

Samples collected to the point of withdrawal will be retained for the study and may be used in analysis. This includes samples and data taken for use in future research.

6.9 DEFINITION OF THE END OF THE TRIAL

The end of trial is the point at which all the data has been entered and queries resolved.

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7. THE ORGANOX METRA K KIDNEY PERFUSION DEVICE

7.1 DEVICE DESCRIPTION

7.1.1 ORGANOX LIMITED

OrganOx Limited is a late-stage medical device development company that was founded in April 2008 as a spin-out from the University of Oxford.

7.1.2 THE ORGANOX KIDNEY PERFUSION DEVICE

The OrganOx *Metra-k* is a class IIa normothermic preservation device for use in human kidney transplantation. It perfuses the donor kidney with oxygenated perfusate, nutrients, and a number of medications at normal body temperature to replicate ideal physiological conditions and preserve the organ for at least 24 hours. The device provides information as to the haemodynamic, synthetic and metabolic function of the kidney whilst being perfused which may assist the clinician in assessing the organ's suitability for transplantation.

7.1.3 THE ORGANOX KIDNEY PERFUSION DEVICE BASE UNIT

The OrganOx normothermic perfusion device incorporates a centrifugal pump, an oxygenator and heat exchanger, reservoir, flow probes, pressure sensor, infusions and blood gas analyser together with tubing and connector components. The device is comprised of three main components:

- a reusable base unit which contains software and hardware
- a disposable plastic circuit
- a set of perfusion solutions suitable for 24 hours perfusion

7.1.4 DISPOSABLE SET

The disposable set used with the core base unit of the OrganOx device contains all the disposables used with each organ recovery on the device and comprises:

1. A disposable tubing set, including a blood reservoir, perfusion lines, a blood oxygenator and centrifugal pump-head together with flow and pressure sensors.
2. An organ storage bowl which is pre-connected to the tubing set to contain the organ while on the device.
3. Blood gas sensors for monitoring pO_2, pCO_2 and pH by means of in-line blood gas analysis.

The disposable set will be supplied with a range of organ connection components for use as determined by the anatomy of the kidney, the operative plan, and the implanting surgeon. This includes a venous cannula, a ureteric cannula, and arterial connection options including vascular graft (to which an aortic patch can be sutured), and machine perfusion clamps.

7.1.5 PERFUSION SOLUTIONS

The primary fluid for perfusing the organ is packed red cells supplied from the Oxford blood transfusion centre and supplemented by 20% human albumin solution (CSL Behring or Grifols) and saline to normalise the haematocrit and osmolarity. Further additions are made to the perfusate to support the kidney (in a similar mode to current preservation solutions such as Viaspan® or those used in machine preservation devices such as the Waters RM3® or the Organ Recovery Systems LifePort®). All primary solutions required will be attached to the circuit during set-up and before the kidney is attached, and are prepared immediately before the organ is attached to the device.

The perfusate comprises:

- Up to 1 unit of packed red cells of donor blood group
- 100 ml of 20% human albumin solution

- Saline to make up the total priming volume (as described in the IFU; priming volume 480ml).

The haematocrit of a unit of packed red cells is variable, as is the volume. Therefore, the final haematocrit of the perfusate will be variable. The target haematocrit will be consistent with the normal range (that seen physiologically and during cardiopulmonary bypass).

In the event that Human Albumin Solution is unavailable, gelofusin is an acceptable alternative solution for dilution.

Before connection of the kidney the blood-based perfusate is supplemented with:

- Meropenem and micafungin as antimicrobials.
- Low molecular weight heparin (enoxaparin 8000 units) or fondaparinux (2.5mg) as an anticoagulant
- Sodium bicarbonate (8.4%, 5-15ml) as a buffer for adjusting the pH of the perfusate before the kidney is placed on the device or after the kidney has warmed to 37°C (at the discretion of the research and operating teams).
- Calcium gluconate or calcium chloride (10%) to correct the binding of citrate to calcium (starting iCa^{2+} will be titrated to the physiological range 1.1-1.3mmol/L).
- Insulin to achieve physiological concentration.
- Glucose if required, to achieve physiological concentration (≥5mmol/L)
- Verapamil, 2.5mg

During the perfusion the following may be infused at a constant rate, or given as bolus doses, as required:

- Insulin – provided as a continuous infusion or bolus doses to maintain physiological concentration
- Glucose – provided as a constant infusion or bolus doses of glucose or parenteral nutrition solution to maintain physiological concentration

Epoprostanol sodium (Flolan 0.5mg, at a rate of 2-10µg/min), glyceryl trinitrate (GTN, at a rate of 0.1-1mg/min), and further doses of verapamil (2.5mg) may be used during perfusion, to optimise the microcirculation.

The aim in supplementing the perfusate as above is to replicate normal physiology whilst optimising perfusion parameters and organ oxygenation. Optimum dosing strategy for these perfusate supplements is unknown. Therefore, a range of possible doses is permitted and will be administered at the discretion of the investigators, with changes within the ranges expected as clinical experience with the device develops. This iterative approach to optimisation is in line with this study's characterisation as an IDEAL 2a phase investigation. All iterative changes to the perfusion procedure will be recorded in detail.

7.2 Device Safety

In designing the perfusion device, OrganOx has made every attempt to maintain the current practices of organ retrieval and transplant teams, in order to minimise the risk of complications or errors that would prevent a successful retrieval. From a regulatory standpoint, it is important to note that the device is an organ preservation system and its use does not involve direct connection to either the donor or recipient at any time.

The device has been designed according to ISO 13485, the standard that stipulates the requirements for a comprehensive management system for the design and manufacture of medical devices. In addition, ISO 14971 specifies a process for a manufacturer to identify the hazards associated with medical devices to estimate and evaluate the associated risks, to control these risks, and to monitor the effectiveness of the controls. As part of the development of the device an extensive risk analysis has been undertaken and the risks identified and minimised in accordance with this standard. As a result, any remaining risk can only be investigated by a clinical transplant study.

The OrganOx perfusion system is based on the principle that it is indirect patient contact and all of the perfusion solutions, additives and packed red cells must be removed from the organ prior to transplant. Therefore, following the completion of the perfusion, the perfusion solution is flushed out of the organ with a cold preservation solution (University of Wisconsin or Marshalls). OrganOx has deliberately designed the operation of the device such that it will require minimal changes to current transplant clinical practice. Device safety has been evaluated in accordance IEC 60601-1:2005+AMD1:2012+AMD2:2020, IEC 60601-1-2:2014+AMD1:2020 for electrical safety and ISO 11135:2014 for sterilisation of the disposable set.

7.3 Device Labelling

All components of the OrganOx system (reusable base unit and disposable set) will be labelled by OrganOx as "Exclusively for Clinical Investigation". Labelling will also include the Sponsor name, contact details and a unique trial identifier.

The disposable set is sterile, custom-made, and intended for use in this clinical investigation. As such it will be labelled with:

- An indication permitting the sterile packaging to be recognised as such,
- A declaration that the device is in a sterile condition,
- The method of sterilisation
- The name and address of the manufacturer
- A description of the device
- The words 'exclusively for clinical investigations'
- If the device is custom-made, the words 'custom-made device'
- The month and year of manufacture,
- An unambiguous indication of the time limit for using or implanting the device safely expressed at least in terms of year and month
- An instruction to check the instructions for use for what to do if the sterile packaging is damaged or unintentionally opened before use

7.4 Device Accountability

Device accountability will be undertaken at the Oxford Transplant Centre throughout the study for the reusable unit(s) and disposable sets (sterilisation/assembly batch number and disposable set number). The manufacturer and lot number for each perfusion solution will also be recorded on the case report forms (CRFs). The Oxford Transplant Centre will maintain a log of usage of both the retained unit, disposable set and perfusion solutions used throughout the study recording the lot number used against each subject (on the CRF).

7.5 Device Maintenance

Device cleaning and routine maintenance will be the responsibility of the investigator storing the device. Full details for cleaning and routine maintenance required will be provided in the instructions for use (IFU), and appropriate training will be provided as part of the device training described in section 11.3.

7.6 Device logistics

7.6.1 Logistical considerations

Two OrganOx metra-k machines will always be available to the study team. They will be stored securely at the Churchill Hospital. Perfusions will take place in the operating theatre suite at the Churchill Hospital, with back-table preparation of the graft and connection to the machine performed under sterile conditions in the emergency operating theatre. Once a perfusion is running the device is designed to be enclosed and transportable; therefore, to minimise occupancy of the emergency theatre, once a perfusion is running and

stable the device will be moved outside the operating theatre. It will remain in the theatre suite and supervised by the Clinical Research Fellow at all times.

7.6.2 Procedure for return of unused, expired or malfunctioning device

Base unit

In the event that a base unit malfunctions and needs to be returned to OrganOx for investigation and/or repair, OrganOx will be contacted at the earliest opportunity. Engineering expertise is available at all times. Response time is rapid as the engineering laboratory where the device has been developed and has undergone pre-clinical testing is located at the Churchill Hospital site and the manufacturer is based in Oxford.

Disposable sets

Disposable sets which are unused at the end of the trial, or expire during the trial, will be disposed of. In the event that a component of the disposable set malfunctions the set will be returned to OrganOx for further investigation as appropriate.

7.7 Certification

The OrganOx Metra-K is not a CE marked device. This trial is not for the purpose of obtaining Regulatory Approval
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8. SAFETY MONITORING AND REPORTING

8.1 SAFETY REPORTING

8.1.2

8.1.1 DEFINITIONS

Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) whether or not related to the investigational medical device.

Serious Adverse Event (SAE)

'Serious adverse event' means any adverse event that led to any of the following:

- Death
- Serious deterioration in the health of the subject, that resulted in any of the following:
    - life-threatening illness or injury,
    - permanent impairment of a body structure or a body function,
    - hospitalisation or prolongation of patient hospitalisation
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
    - chronic disease,
- Fetal distress, fetal death or a congenital physical or mental impairment or birth defect

Note: A planned hospitalisation for pre-existing condition, or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be a SAE for example transplant ureteric stent removal, peritoneal dialysis catheter removal, and haemodialysis catheter removal.

These are handled under the AE reporting system.

Adverse Device Effect (ADE)

An adverse event related to the use of an investigational medical device.

Note: This definition includes any events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational device. This definition also includes any event resulting from user error or form intentional misuse of the investigational device.

Serious Adverse Device Effect (SADE)

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

Unanticipated Serious Adverse Device Effects (USADE)

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

Note: Anticipated: an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report.

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.

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.

Severity definitions

The following definitions will be used to determine the intensity of a specific event. This is not to be confused with serious. Severity does not determine if the event meets the definition of seriousness.

Mild: awareness of signs or symptoms, that does not interfere with the subject's usual activity or is transient that resolved without treatment and with no sequelae.

Moderate: a sign or symptom, which interferes with the subject's usual activity.

Severe: incapacity with inability to do work or perform usual activities.

Severity will additionally be graded according to the Clavien-Dindo classification (appendix A6)

8.1.2 Anticipated Adverse Events

General

- Infection (chest, urine, blood, bile, wound, abdominal)
- Fluid collection (abdominal, pleural)
- Renal dysfunction
- Cardiac failure
- Respiratory failure

Events related to the disease / condition /surgery

- Rejection
- Delayed graft function
- Admission for suspected rejection
- Occurrence and treatment of abdominal or wound infection
- Occurrence and treatment of lymphocele/seroma
- Respiratory failure requiring mechanical ventilation
- Hospitalisation for pre-existing condition that has not deteriorated.
- Clinically significant abnormal laboratory finding or other abnormal assessments that is associated with the condition being studied (unless judged by the investigator as more severe than expected for the patient's condition).

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

8.1.3 Procedures for 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 using the electronic Safety (Adverse Event and Device Deficiency) reporting form. 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. Should an adverse event be recorded as any of the following:

- Serious
- Arising from a device deficiency
- Arising from a user error
- Categorised as 'probable' or 'causal relationship' with respect to causality and use of the perfusion device

An Adverse Event and Device Deficiency reporting form will be triggered which must be completed by an appropriately qualified investigator.
Device deficiencies and user errors not falling into the categories of ADEs or SADEs should be reported via the online data collection tool and will be collected by the study investigators for investigation by the manufacturer.

8.1.4 Assessment of causality

The following definitions will be used in the assessment of causality for each Adverse Event:

Not related: relationship to the device or procedures can be excluded when:
- The event is not a known side effect of the product category the device belongs to or of similar devices and procedures;
- The event has no temporal relationship with the use of the investigational device or the procedures;
- The serious event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible;
- The discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious event;
- The event involves a body-site or an organ not expected to be affected by the device or procedure;
- The serious event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
- The event does not depend on a false result given by the investigational device used for diagnosis 17, when applicable;
- Harms to the subject are not clearly due to use error;

In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious event.

Unlikely: the relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.

Possible: the relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition

or/and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.

Probable: the relationship with the use of the investigational device seems relevant and/or the event cannot reasonably explained by another cause, but additional information may be obtained.

Causal relationship: the serious event is associated with the investigational device or with procedures beyond reasonable doubt when:

- The event is a known side effect of the product category the device belongs to or of similar devices and procedures;
- The event has a temporal relationship with investigational device use/application or procedures;
- The event involves a body-site or organ that
    - the investigational device or procedures are applied to;
    - The investigational device or procedures have an effect on;
- The serious event follows a known response pattern to the medical device (if the response pattern is previously known);
- The discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious event (when clinically feasible);
- Other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
- Harm to the subject is due to error in use;
- The event depends on a false result given by the investigational device used for diagnosis, when applicable;

In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious event.

The above definitions will be applied to each adverse event by the appropriately qualified investigator. For any adverse events categorised as 'Not Related', 'Unlikely', or 'Possible' (and which are not serious), further investigation/ review will not be required. Adverse events categorised as 'Probable' or higher, or any adverse events categorised as serious, will be recorded using the electronic Adverse Event and Device Deficiency reporting form and forwarded to the Trial Management Committee for review and investigation/ further reporting as appropriate.

8.1.5 Reporting Procedures for All serious Adverse Events and device deficiencies

Reporting of all Serious Adverse Events and device deficiencies will be done in accordance with the requirements of the Competent Authority and REC and as per the CTU SOP on Safety Oversight in Regulated Medical Device Trials (OCTRU SOP GEN-053).

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 CI/trial team as soon as possible after becoming aware of the event but no later than 24 hours from becoming aware of the event.

Adverse event and serious adverse event reporting will be via the electronic data collection tool using the trial safety form, with SAEs and AEs which are identified as arising from a device deficiency; arising from a user error; or categorised as 'Probable' or 'Causal relationship' with respect to causality and use of the perfusion being forwarded to the Trials Co-ordinator and clinical reviewers by the reporting tool. The clinical reviewers are the Chief Investigator and co-investigators. Reporting by email (situ@nds.ox.ac.uk) will provide a backup system in the event that the online data collection tool is unavailable.

The local investigator may be required to provide additional information on the SAE or device deficiency in the form of a written narrative. This should include a copy of the completed Safety Reporting 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 chief investigator and clinical reviewers using the same electronic Safety Reporting form.

On submission of an electronic Safety Reporting form, the trial manager, chief investigator 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 DSMC immediately and to the device manufacturer, competent authority and the REC.

All other reported SAEs will be reported to the DSMC, REC, and competent authority within the timeframe specified by the competent authority. This will include any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate. This will not include SAEs that may be expected as part of the risks of kidney transplant surgery. Adverse device events (regardless of seriousness) and device deficiencies will also be reported to the device manufacturer, again within the timeframe specified by the competent authority. All SAEs will be followed up to resolution or until the event is considered stable. The DSMC will review the accumulating data at regular intervals as described under section 8.1.2. Interim safety reporting to the MHRA will be supplied by the trial team as required.

Electronic Safety Reporting Forms will be supplied to the site for use in the event that the electronic reporting system fails and that an email submission is required.

SAE reporting will begin at the point of enrolment and continue until the last patient recruited has completed 12 months of follow-up. Patients transferred back for on-going care to referring centres will have their adverse event data sent to the patient's specialist where relevant to their ongoing care. Patient cards will be provided to all participants of the study, with a contact telephone number (research nurse / researcher) to inform regarding the occurrence of SAEs.

8.2 STUDY SUSPENSION OR EARLY TERMINATION

The DSMC or sponsor may recommend suspension or termination of the study for significant and documented reasons. An investigator, ethics committee or regulatory authority may suspend or prematurely terminate participation in the study. 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. Specific, pre-specified grounds for early suspension or termination of the study are outlined in section 9.1, below.

The sponsor shall consider terminating or suspending the participation of a particular 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 chief investigator and sponsor shall keep each other informed of any communication received from either the ethics committee or the regulatory authority.

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

If suspension or premature termination occurs,

a) the sponsor shall remain responsible for providing resources to fulfil the obligations from the study protocol and existing agreements for following up the subjects enrolled in the study, and
b) the chief investigator or authorized designee shall promptly inform the enrolled subjects

9. STATISTICS

9.1 INTERIM ANALYSES

Reports for the DSMC will be drawn up after recruitment of patients 10 and 22. These reports should be prepared and finalised by the time of recruitment of patients 12 and 24, respectively. The trial will not proceed to recruitment of patients 13 and 25 respectively (i.e. will not move from stage 1 to stage 2, and from stage 2 to stage 3) without consideration of the interim reports by the DSMC and the TMC, and approval to continue recruitment.

In addition, to minimise risk associated with the introduction of this new technology there will be an element of continuous monitoring throughout the trial. Should any two out of six consecutive recipients experience Early Graft Loss in the first 30 days, defined as transplant nephrectomy or proven non-viability, recruitment will be paused to allow for investigation. Reports will be drawn up to include detailed information specific to the cases that experienced EGL, and summary data for all cases recruited up to the point where the trial was paused. The TMC and DSMC will consider these reports and either permit recruitment to continue with the rolling total reset to 0 (e.g. if the reasons for failure were clearly unrelated to the trial); permit recruitment to continue with the rolling total reset to 1 or other conditions as necessary; pause recruitment until 30-day follow up data is available for all patients recruited up to that point and reconsider; or stop the trial. Recruitment will only be restarted with approval of the TMC and DSMC.

9.2 DESCRIPTION OF STATISTICAL METHODS

9.1.1 OUTCOMES

Primary outcome reporting will occur after collection and analysis of data at 30 days following the enrolment of the last patient to the trial.

The intervention (NMP) will be compared against historical controls (SCS) for selected primary and secondary outcomes. These will include:

Clinical outcomes:

- 30 day (primary outcome), 3- and 12- month graft survival
- 30-day, 3- and 12- month patient survival
- Incidence of Primary Non-Function (PNF)
- Incidence and duration of use of post-operative dialysis
- Length of hospital stay
- Re-operation and readmission rates
- Incidence of acute rejection
- Incidence of positive perfusion culture (subdivided into saprophytic and pathogenic organisms)(34)

Post-operative measures of graft function:

- Incidence of functional Delayed Graft Function (fDGF)
- Day two creatinine reduction ratio

Graft function up to one year post-transplant:

- 30-day, 3- and 12- month eGFR (CKD-EPI formula)

- Serum creatinine trajectory (month 12 minus month 3)
- 30-day, 3- and 12- month proteinuria (as a binary variable, defined as using the KDIGO cut-offs as in appendix A5 (28))

We will report the adverse event rate subdivided by type, all organ discards, and all adverse device events/ device errors.

12-month eGFR is pre-specified as the most foremost secondary end-point of interest.

Descriptive statistics will be provided for all primary and secondary outcomes. Mean and 95% confidence intervals, medians, interquartile range and range will be provided for continuous data, frequency and percentage for categorical data. The main analysis will focus on the participants with no important protocol deviations that led to the recruitment of additional participants.

Sensitivity analysis will also consider the participant with such protocol deviations. An additional sensitivity analysis will repeat summaries for key outcome measures including participants who were "replaced" during the trial.

Reports to the DSMC committee will be separate from the final analysis described here, and items included will be agreed with the committee prior to the first meeting.

9.1.2 Additional analyses

Proteinuria

As an exploratory analysis, we will assess proteinuria by means of 24-hour urine collections on post-operative days 1-4. We will examine whether on-pump proteinuria as reflected by perfusate-urine albumin gradient predicts post-operative proteinuria, as well as day 30, month 3, and month 12 protein: creatinine ratio. We will also assess whether the last measured on-pump perfusate-urine albumin gradient predicts DGF. We will describe how the perfusate: urine albumin gradient changes during NMP, and how total urine protein excretion changes from post-operative day 1 to day 4. We will perform a pre-specified sub-group analysis considering separately patients with a native urine output and an abnormal day 0 (pre-transplant) urine protein: creatinine ratio (>200mg/g; appendix 5), vs patients with no native urine output or a normal pre-transplant protein: creatinine ratio (≤200mg/g; appendix 5).

Perfusion parameters

Secondary analyses will also investigate the ability of perfusion parameters (including renal blood flow, urine production rate, and Quality Assessment Score), to predict clinical outcome (i.e. viability assessment). These measured predictors will be examined for their ability to predict DGF, and biochemical measures of graft function (30-day, 3-month, and 12-month graft function).

The variability of biochemical parameters including perfusate glucose concentration, perfusate sodium concentration, urine-perfusate sodium decrement, urinary pH, serum pH, lactate concentration, and perfusate osmolality during perfusion will be described and reported.

Other markers indicating the stability of the perfusate, including insulin concentration, anti-Xa activity, and free haemoglobin concentration will be recorded after 1 hour of perfusion, at the predicted mid-point, and at the end of perfusion. Change during perfusion will be reported.

Biomarkers

Biomarkers such as AST, LDH, NGAL, KIM-1, L-FABP, IL-18, GST, and cell-free DNA will be measured in perfusate taken whilst the kidney is perfused on the device. Samples for these analyses will be taken at the following timepoints after the start of perfusion: 2 hours, 6 hours, 12 hours, 18 hours, and the end of perfusion (variable). The accepted tolerance for timing of these samples is ±15 minutes. We will perform exploratory analyses to assess the performance biomarkers at 2 hours, end-perfusion, and the change in concentration from 2 hours to end-perfusion for prediction of a) DGF (composite of fDGF and clinical DGF – any use of dialysis); and b) 12-month eGFR. The number of kidneys perfused to 6 hours, 12 hours, and 18 hours will be variable. Should we have a sufficient number of cases we will consider the utility of the biomarker panel at these later time points we will assess predictive performance; else we will use this data to provide a description of the change in biomarker concentration over time during NMP. The final selection of biomarkers for reporting will be based on sample availability and the best available evidence at the time.

9.1.3 Missing data

Withdrawals from the trial after implantation will be documented as per section 6.8, and timing of the withdrawals, extent of the withdrawals and reasons, where available, will be reported.

Summaries will be based on available data only, assuming that the participants with available outcome data are representative of all participants recruited.

The sensitivity of these outcomes to different assumptions about missing data will be investigated in a sensitivity analysis. Specifically, grafts will assume to have not survived in those participants with missing outcome data, and the participants will be assumed to have died prior to the relevant time points.

The results from this sensitivity analysis will be compared to the results from the main analysis.

9.2 Sample size and recruitment

This is a feasibility trial and not statistically powered. The sample size is estimated on a 12 month study plan.

The basic group size of 12 is based on a compromise between feasibility, precision and regulatory considerations(35).

9.3 Deviation from the statistical analysis plan

Any deviation from the original statistical analysis plan will require justification in the final study report.

10. Data collection and management

10.1 Data collection methods

10.1.1 Source data

Source documents are where data are first recorded, and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the eCRF), clinical charts, laboratory reports, pharmacy records, radiographs, correspondence, device accountability records, recorded data from automated instruments, and records from medico-technical departments involved in the clinical investigation.

eCRF entries will be considered source data if the eCRF is the site of original recording (e.g. there is no other written or electronic record of the data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

10.1.2 Data recording

Data collection will be achieved using secure internet accessible forms. Data will be input by study investigators/co-ordinators trained in the use of the system prior to receiving log-in details. Data will be uploaded to a central trial specific database.

Demographic data, laboratory results and survival data will be recorded on the data collection system.

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

Biopsies will be analysed by the Oxford Centre for Histopathology Research (OCHRe) and the biopsy findings recorded in the main database.

10.2 DATA MANAGEMENT

10.2.1 DATA FORMS AND DATA ENTRY

As described in section 10.1.2, data will be directly entered onto online forms, which is stored in a database maintained on a virtual server at the University of Oxford. Validation rules will ensure that data are entered in the correct format, within valid ranges and minimise the chance of missing data. Data already entered will be retrievable for viewing through the data entry system. The extent of an individual user's activity in the data entry system will be limited by privileges associated with his/her login and password.

All electronic research data will be stored in a secure fashion, with data identified only by the unique participant study ID. A separate existing Clinical Audit Database will be utilised to allow identification of study participants, if required, from their unique ID – this is maintained by the Oxford Transplant Centre and is stored on an Oxford University Hospitals NHS Foundation Trust Server. This Clinical Audit database will record whether participants wish to be contacted in the future about the results of this research or participation in similar studies. This personal data will be kept by the Oxford Transplant Centre for a period of at least 10 years after the clinical investigation with the device in question has ended, or, in the event that the device is subsequently placed on the market, at least 10 years after the last device has been placed on the market.

10.2.2 DISCREPANCIES AND MISSING DATA

The central database will be monitored for discrepancies and missing data. OCTRU is responsible for maintaining and managing the database; data entry is the responsibility of sites and the trial manager. The trial manager will be responsible for the production of monthly reports to containing information and details of missing data or missed visits requiring completion.

10.2.3 SECURITY AND BACKUP OF DATA

The database will be accessible to authorised users. Access to databases will be controlled by username and password. When the user logs on to the first time, he/she will have to change the password. The password allowed has to adhere to the following rules –

- Passwords contain at least nine 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.

The OCTRU IT team is responsible for assigning username and passwords to individuals requiring access to the database. It will be responsibility of the Trial Manager to request access and request the revocation of access for the users who are no longer required to use the database via the CTU system, and they should regularly monitor the list of users granted access.

The REDCap source code and data reside on the server on which REDCap is installed. The application code and the database for the trial 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 backed up to the University managed central back up service.

The university managed backup will also be copied to an external hard drive at regular intervals at least once every month. The external drive will be stored at a different site within the University of Oxford and will always be locked.

At any time complete backups of the data are available in three different places - the data backup drive on the REDCap server (on a separate drive to where the DBMS runs), the University managed central back up service and the external site to which these backups are copied to.

10.2.4 DATA ACCESS

Direct access will be granted to authorised representatives from the Sponsor, host institutions and the regulatory authorities to permit trial-related activities including monitoring, audits and inspections.

10.2.5 DATA RETENTION

The trial coordinating centre (SITU) will archive all of the trial master file data and the trial database on restricted University of Oxford drives for a period of at least 10 years after the clinical investigation with the device in question has ended, or, in the event that the device is subsequently placed on the market, at least 10 years after the last device has been placed on the market.

10.2.6 DESCRIPTION OF HARDWARE AND SOFTWARE

The database used will be the REDCap EDC application installed on the following hardware and web stack.

- *Linux OS - CentOS Linux release 7.8.2003*
- *Apache/2.4.46*
- *MySQL Ver 14.14 Distrib 5.7.31*
- *PHP 7.2.34*

Only the OCTRU IT and the vDC hosts (Cloud Support at IT Services) have direct access to the server.

Access to the REDCap application will be maintained by the trial management team and OCTRU IT.

11. QUALITY ASSURANCE, AUDIT AND TRAINING

11.1 RISK ASSESSMENT

A risk assessment will be conducted according to OCTRU's process and a monitoring plan will be drafted to include all central monitoring activities. The trial will be conducted in accordance with the current approved protocol, Principles of GCP, relevant regulations and OCTRU standard operating procedures.

11.2 MONITORING

Due to the nature of this study and timelines and in accordance with the risk assessment in section 11.1 monitoring will be limited to central monitoring activities – there will be no site monitoring, missing data will be queried with sites where mandatory. Monitoring of the data will occur as the data is being entered into the database.

11.3 QUALITY ASSURANCE AUDITING AND INSPECTION

The Sponsor or its designated representative will assess each study site to verify the qualifications of each Investigator and the site staff and to ensure that the site has all of the required equipment. A study Initiation meeting (which may be virtual) will occur where among other things the Investigator will be informed of their responsibilities and procedures for ensuring adequate and correct study documentation.

11.4 DATA SAFETY MONITORING COMMITTEE (DSMC)

The DSMC is a group of independent experts external to the trial who assess the progress, conduct, participant safety and critical endpoints of the study. The study DSMC will adopt a DAMOCLES charter which defines its terms of reference and operation in relation to the oversight of the trial. They will review the interim analysis l and make recommendations to the CI and Investigators as to the continuation or otherwise of the trial. The group will consist of at least three members, including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source.

This Group will provide advice and recommendations to the CI and Co-investigators and may correspond directly with the Sponsor if potential safety concerns are raised.

11.5 LOCAL INVESTIGATOR AND SITE PERSONNEL TRAINING

All key site personnel must undergo relevant training in advance of the site initiation in accordance with Good Clinical Practice (GCP) guidelines. Such training will be documented.

In addition, training on the investigational device will be provided in advance of recruitment of the first patient by the central co-ordinating centre. 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 members of the clinical trials unit, and records of such training will be maintained.

11.6 STUDY DOCUMENTATION

It is the responsibility of the chief investigator to maintain complete, accurate and current study records. Each site will hold a site file, and online access to the case reporting system and other associated study specific documentation will be provided to all trial personnel. Such records will be maintained during the course of the study and for a period of at least 10 years after the clinical investigation with the device in question has ended,

or, in the event that the device is subsequently placed on the market, at least 10 years after the last device has been placed on the market.

12. PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial”.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI and the DSMC, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the relevant NHS host organisation within seven calendar days.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1 RESEARCH ETHICS AND REGULATORY APPROVAL

13.1.1 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.

13.1.2 PRINCIPLES OF GOOD CLINICAL PRACTICE

The Investigator will ensure that this trial is conducted in full conformity with the principles of Good Clinical Practice.

13.1.3 MEDICAL DEVICE REGULATIONS AND STANDARDS

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

- Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC Clinical investigation of medical devices for human subjects — Good clinical practice

13.1.4 OTHER COMPLIANCE

The trial will be conducted also in compliance with the UK Data Protection Act (2018) and all other applicable regulatory and governance frameworks including the UK policy framework for health and social care research.

13.1.5 OTHER ETHICAL CONSIDERATIONS

Participation in this trial will not affect a patient's position on the kidney transplant waiting list or their likelihood or receiving a kidney transplant. Similarly, withdrawal of a participant from the trial at any point and for any reason will not affect their position on the kidney transplant waiting list or their likelihood or receiving a kidney transplant.

The timing of transplantation is usually determined by a range of factors including waiting for pre-operative investigations, the timing of the arrival of the organ and recipient, the availability of theatre and staff, and the time of day. The process of normothermic perfusion will take place whilst patients wait for the results of routine pre-transplant investigations to return, and for the usual logistical considerations. It will not in itself unduly delay the patient proceeding to the operating theatre. This routine pre-operative preparatory process necessitates a delay of several hours, in some cases up to a day, between the recipient arriving at the transplant centre after being called in for transplant, and the recipient proceeding to the operating theatre. The process of normothermic preservation will fit around these usual timeframes and logistical considerations, and the maximum permitted preservation time will be determined by study stage.

Explicit donor consent is not required for this study, as all interventions take place remote from the donor and the study does not affect the intent to transplant any retrieved organ. There is a strong precedent for this approach, including from similar trials of normothermic perfusion of the liver run locally(8,9), hypothermic machine perfusion trials run by the COPE consortium(36) , and an ongoing trial of one-hour ex-vivo 'resuscitative' normothermic perfusion of the kidney being conducted in Cambridge currently(23).
It is possible, but unlikely, that participants will go intensive care or a high-dependency unit following transplantation and remain sedated. This would represent a temporary loss in capacity to consent to continued involvement in the trial. Appropriate consent will always be obtained prior to surgery, and the clinical consent process for surgery encompasses this possibility. In this instance we would take samples as mandated by the protocol (e.g. day 1 blood and urine) under the terms of the trial consent. In the event that there was an enduring

loss in capacity during the follow-up period, we would not seek to take further trial-specific samples but we would seek to continue to collect safety data including that regarding complications and kidney function. This is felt to be a very remote possibility.

13.1.5 Local regulatory approval

This protocol, site-specific informed consent forms and participant education and recruitment materials will be submitted for approval by the appropriate research ethics committee (REC), host institutions and regulatory authorities in each participating region. Before the study can begin, the chief investigator must have written evidence of favourable ethical opinion from an appropriate REC and approval from an appropriate regulatory authority.

Once approval has been granted, the chief investigator is responsible for ensuring that he/she complies with the terms of the approval, namely with adverse event reporting, notification of amendments, interim, annual and final reports on the progress of the study.

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.

Administrative changes to the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by the trial management committee and notified to the relevant RECs and regulatory authorities at the discretion of the trial management committee.

All other amendments to the protocol will be notified to the local regulatory authorities and research ethics committees for approval. Approved amendments will be circulated promptly to all investigators by the co-ordinating centre. Amendments will be tracked by version number and date in appendix A5 of this document.

13.3 Reporting

The chief investigator shall submit once a year throughout the clinical trial, or on request, an annual progress report to the REC, host organisation and sponsor. In addition, the end of trial notification and final report will be submitted to the MHRA, the REC, host organisation and sponsor. Timelines for submission of these reports will conform with the Medical Devices Regulations. Specifically, in the event of a temporary halt the MHRA will be informed within 15 days (or 24 hours if the sponsor brings the trial to a halt or terminates for safety reasons). The end of trial notification will be submitted with 15 days of the trial ending. The Clinical Investigation Report will be submitted within one year of the end of the trial, or within 3 months if the trial is terminated early.

13.4 Donor and recipient confidentiality

All study-related information will be stored securely both at the study sites and the co-ordinating centre. Written information will be stored in locked filing cabinets in areas with limited access. All documentation and specimens will be identified by a unique study ID number to maintain participant confidentiality. Where this is not possible (e.g. informed consent forms), these will be stored separately from any study records identified by the unique study ID.

Participant's information will not be released outside of the study without the written consent of the participant, except as necessary by regulatory authorities or to their local hospital care team.

13.5 Development of a new product/process or the generation of intellectual property

Ownership of any intellectual property generated by this trial will be covered by a separated Collaboration Agreement.

13.6 Declaration of conflicting interests

Professor Peter Friend is a co-founder, Chief Medical Officer and Consultant to OrganOx Limited and also holds shares in the company.

Professor Constantin Coussios is a co-founder, Chief Technical Officer and Consultant to OrganOx Limited and also holds shares in the company.

Mr Craig Marshall is Chief Executive Officer for OrganOx Limited.

Mr Simon Knight has received consultancy payments from OrganOx Limited for assistance in the design of previous clinical trials.

13.7 Expenses and benefits

Where possible, study visits and investigations will be conducted during routine hospital attendances. Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, in accordance with the requirements of the Declaration of Helsinki 2008.

13.8 Indemnity insurance

The University 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). NHS indemnity operates in respect of the clinical treatment that is provided.

13.9 CTU Involvement

This study will be coordinated by the UKCRC registered Oxford Clinical Trials Research Unit (OCTRU) at the University of Oxford

14. DISSEMINATION POLICY

14.1 DATA ANALYSIS AND RELEASE OF RESULTS

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

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

14.2 PRIMARY OUTCOME PUBLICATIONS

Reports relating to primary outcomes will be published in peer-reviewed journals of appropriate relevance. A final report on the primary outcomes of the study will be compiled by the chief investigator and co-investigators, and approved and signed off by each member of the trial management committee.

14.3 OTHER STUDY PAPERS, ABSTRACTS AND PRESENTATIONS

All publications, conference abstracts and presentations will require authorisation from the chief investigator and trial management committee.

APPENDICES

APPENDIX A1: BIOLOGICAL SPECIMENS

A1.1 ROUTINE CLINICAL SPECIMENS

All specimens collected as part of the recipient's routine clinical care, such as pre- and postoperative blood samples for routine laboratory analysis, will be analysed and stored locally as per normal local procedure. Results required by the trial protocol will be uploaded to the online data collection system.

A1.2 TRIAL SPECIMENS – PRE-PERFUSION

Blood and urine samples

A single blood sample will be taken from the recipient for biobanking, of up to 10ml, following induction of anaesthesia.

A single urine sample of up to 10ml will be taken from the recipient following insertion of a urinary catheter, after induction of anaesthesia. This will be analysed for protein: creatinine ratio, with the remainder of the sample used for biobanking.

Biopsies

The standard biopsy technique will be a 4mm punch biopsy, with the site over-sewn using 6-0 prolene stitch. However, in the event that on arrival at the Oxford Transplant Centre the kidney has already undergone a QUOD research biopsy (routinely 2mm punch biopsies), we will not re-biopsy pre-perfusion, and will instead use the existing sample to fulfil the trial requirements.

Biopsy segments will be divided into two and stored in pre-prepared formalin or RNAlater tubes. Samples stored in RNAlater will be frozen in liquid nitrogen and stored at -80^{o}C for long-term storage.

A1.3 TRIAL SPECIMENS – DURING PERFUSION

Perfusate and urine samples will be collected during normothermic machine perfusion. Perfusate samples will be analysed hourly for the first two hours, and 2-hourly thereafter, using a point-of-care blood gas analyser. Additional samples for point-of-care testing, laboratory measurements, and biobanking will be taken at time points as described under appendix A3.

A1.4 TRIAL SPECIMENS – POST-PERFUSION

Biopsies

A single 4mm punch biopsy will be taken after reperfusion in the recipient. Again, the standard technique is to over-sew the site with a 6-0 prolene stitch.

Biopsy segments will be divided into two and stored in pre-prepared formalin or RNAlater tubes. Samples stored in RNAlater will be frozen in liquid nitrogen and stored at -80^{o}C for long-term storage.

Blood and urine samples

Study specific samples will be processed in the Oxford Transplant Centre, Oxford Centre for Histological Research, or Oxford University Hospitals Biochemistry Laboratory for analysis, or storage in the Oxford Transplant Biobank. These samples include:

- Plasma, serum and urine samples as specified, other than those required for routine clinical care
- Pre- and post-perfusion kidney biopsies

Provision will be made on the study consent form for storage and later use of these samples for ancillary studies. Shipping of all samples will be tracked with shipping and receipt logs maintained in each site investigators file.

Timing of samples

Once daily samples of urine and blood will be taken for analysis and/or biobanking on post-operative days 1-7 whilst an inpatient or attending routine clinic follow-up appointments (as detailed in section 6.7.1), post-operative day 30, and at 3 months post-transplant. These samples will usually be taken each day during the morning, where possible to coincide with routine phlebotomy required for post-operative care. The first samples will be taken 12-24 hours post-transplant, with subsequent samples at as close to 24-hour intervals as feasible.

Regulatory aspects

All samples will be collected in accordance with national regulations and requirements including standard operating procedures for logistics and infrastructure. 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 bioresource. The stored tissues will be held under an extension of the University of Oxford's HTA license (12217).

Blood samples

At each time-point where blood is collected for the purposes of biobanking,

- 1x EDTA 4 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 should be kept at room temperature prior to separation of plasma from cellular parts. Separation of cells from plasma and serum should, where required, be achieved by centrifugation at 1500g for 10 min at room temperature as close as possible to blood collection. After centrifugation plasma and serum samples should kept at 4^{o}C, or frozen at -80 ^{o}C as required for analysis.

APPENDIX A2: TIMELINE FOR INTERVENTIONS AND ASSESSMENTS DURING THE STUDY.

For definitions of data to be collected see text and data matrix. Key: X = planned data collection.

PROCEDURES	Visits												
	1	2	3								4	5	(6)
	PIS – mailed out	Telephone call	Called in for transplant – study visit 1*								30-day ± 1 week follow up	3-month ± 2 weeks follow up	12-month ± 1 month follow up
TIME	Pre-Op	Pre-Op	D0	D1	D2	D3	D4	D5	D6	D7	Discharge			
Written trial information sent to all patients on waiting list at commencement of trial	X												
Followed up with phone-call to provide verbal explanation of trial		X											
Confirmation of eligibility and taking of informed consent			X										
Data collection – recipient demographics			X										
Data collection – concomitant medications			X										
Data collection – donor data			X										
Organ perfusion (trial procedure and data collection)			X										
Data collection – operative parameters			X										
Data collection – results of histology											X		
Blood and urine sampling (standard care + trial samples + biobank samples) **			X	X	X	X	X	X	X	X		X	X	
Data collection – clinical and biochemical follow-up			X	X	X	X	X	X	X	X	X	X	X	X
Clinical follow-up appointment												X	X	
Remote data collection														X

*note that in the event that patients are discharged before day 7, daily follow-up will cease at the point of discharge. Patients are commonly brought back to clinic within the first week after discharge – if a standard care clinic appointment falls within 7 days of transplant this may be used as an opportunity to acquire clinical and biochemical data as if the patient were still an inpatient.

** where possible samples will be collected at the time of routine sample collection.

APPENDIX A3: TIMELINE FOR MEASUREMENTS DURING NORMOTHERMIC PERFUSION

Sample type	Time points	Volume	Collection method	Total volume
Perfusate biochemistry	Hourly for the first two hours; two-hourly thereafter	1ml	Gas syringe (point of care blood gas analyser)	Up to 13ml
Urine biochemistry	Hours 1, 2, 6, 12, 18, end-perfusion	1ml	Gas syringe (point of care blood gas analyser)	Up to 6ml
Biochemistry lab panel, perfusate (LDH, AST, albumin)	Hours 2, 6, 12, 18, end-perfusion	2ml	Lab collection tube	Up to 10ml
ELISA panel (e.g. NGAL, KIM-1, L-FABP, IL-18, GST, cfDNA)	Hours 2, 6, 12, 18, end-perfusion	2ml	Lab collection tube	Up to 10ml
Biochemistry lab panel, urine (albumin)	Hours 2, 6, 12, 18, end-perfusion	1ml	Lab collection tube	Up to 5ml
Haematology lab panel (free Hb, anti-Xa)	Hours 1, mid-perfusion, end-perfusion	1ml	Lab collection tube	3ml
Biochemistry lab, perfusate (insulin)	Hours 1, mid-perfusion, end-perfusion	1ml	Lab collection tube	3ml
Biobank perfusate sample	Hours 1, 2, 4, 6, 12, 18, end-perfusion	2.5ml	Lab collection tube	Up to 17.5ml
Biobank perfusate additional	1 hour, end perfusion	2ml	Lab collection tube	4ml
Biobank urine sample	Hours 1, 2, 4, 6, 12, 18, end-perfusion	1ml	Lab collection tube	Up to 7ml

Notes

- These time-points assume the maximum perfusion duration of 24 hours. Most trial perfusions will be less than this, and as such only the time points that each kidney actually reaches during perfusion will apply.
- Total volume sampled during perfusion: up to 78.5ml.
- Free haemoglobin, anti-Xa activity, and perfusate insulin concentration will be assayed at 1 hour, the predicted mid-point of the perfusion (based on expected duration at perfusion start), and the end of perfusion.

APPENDIX A4 – HISTORICAL CONTROLS MATCHING ALGORITHM

Historical matched controls will be drawn from a pool of all single kidney-only transplants performed at the Oxford Transplant Centre, conducted from 01/10/2016 to the start of the trial, not including those transplants where outcome (12-month eGFR unless patient or graft survival is less than 12 months, in which case date of death or graft failure) is not available.

A matching algorithm has been developed, and validated by simulation, that matches each case to two controls drawn from this pool. Cases are matched to controls strictly on donor type (DBD/ DCD) and induction immunosuppression (Basiliximab/ Alemtuzumab), and then by minimising the summed percentage difference ('match score') in cold ischaemia time (CIT) and donor risk index (DRI):

$$Match\ score = \frac{CIT_{case} - CIT_{control}}{CIT_{case}} + \frac{DRI_{case} - DRI_{control}}{DRI_{case}}$$

Match scores are computed for all possible case-control pairs. The final case-control allocation is then defined as that which minimises the total match score summed across all cases. This is computed by the Hungarian method (37) implemented in R, as below.

Matching algorithm – R code

```
nkp1matchfunc = function(db=db, m_dtype, m_cit, m_dri, m_induction, nmatch=2) {

  require(dplyr)
  require(clue)
  as_tibble(db) -> pool
  pool[0,] -> controls
  tibble(nmp_dtype = m_dtype, nmp_cit = m_cit, nmp_dri = m_dri, nmp_induction = m_induction) -> cases
  cases[rep(seq_len(nrow(cases)), each = nmatch), ] -> cases

    matrix = matrix(nrow=nrow(cases), ncol=nrow(pool))
    for (i in 1:nrow(cases)){
      for (j in 1:nrow(pool)){
        abs((cases[i,]$nmp_cit - pool[j,]$cit_mins)/cases[i,]$nmp_cit) +
        abs((cases[i,]$nmp_dri - pool[j,]$dri)/cases[i,]$nmp_dri) +
        (if(cases[i,]$nmp_induction==pool[j,]$induction) 0 else 1000) +
        (if(cases[i,]$nmp_dtype==pool[j,]$dtype) 0 else 1000) -> matrix[i,j]
      }
    }

    solve_LSAP(matrix) -> solution
    for (i in 1:length(solution)){
      rbind(controls, pool[solution[i],]) -> controls
    }

  return(cbind(cases, controls))
}
```

APPENDIX A5: DEFINITIONS FOR EGFR, PROTEINURIA, AND DONOR RISK INDEX

CKD-EPI FORMULA FOR ESTIMATION OF GFR

Throughout this trial, wherever eGFR is referred to it will be computed by the CKD-EPI formula (27) :

$$eGFR = 141 \times \min(\frac{SCr}{\kappa}, 1)^{\alpha} \times \max(\frac{SCr}{\kappa}, 1)^{-1.209} \times 0.993^{Age} \times 1.018[if\ female] \times 1.159[if\ black]$$

PROTEINURIA

When used clinically, proteinuria may variously be reported at binary variable (above/ below a pathological cut-off point), or as a continuous measure. Additionally, there are a number of possible ways of measuring proteinuria (dipstick; spot-test for protein creatinine ratio; spot-test for albumin-creatinine ratio; 24-hour total urinary protein excretion). In this trial, where binary classification is required – particularly for drawing comparisons between the historical matched control cohort and the trial cohort – proteinuria will be reported as a binary variable using the following cut-offs. In the trial cohort it is specified that protein-creatinine ratio will be used at 30 days, 3 months, and 12 months. For the historical matched controls measures of proteinuria will be used preferentially; in the event that only historic measures based on albumin are available the cut-offs below will be used (NB micro-albuminuria in a historic control will not be compared to proteinuria in a trial case).

Urine collection method	**Normal (non-pathological)**	**Abnormal (pathological)**
Dipstick	Negative; trace	+ (one plus) or greater
Spot protein-creatinine ratio	<200mg/g	≥200mg/g
24-hour protein excretion	<300mg/day	≥300mg/day
(Spot albumin-creatinine ratio)	≤250mg/g (men) ≤355mg/g (women)	>250mg/g (men) >355mg/g (women)
(24-hour albumin excretion)	≤300mg/day	>300mg/day

(28)

DONOR RISK INDEX

The Donor Risk Index used by NHSBT for the kidney allocation scheme will be used for matching to historical controls (31) – not the previously published UK Kidney Donor Risk Index (UKKDRI). The NHSBT DRI for use in this trial is computed as following:

$$\begin{aligned} DRI = \exp\{\ & 0.023 \times (donor\ age - 50) - 0.152 \times \frac{(donor\ height) - 170}{10} \\ & + 0.149 \times (history\ of\ hypertension) - 0.184 \times (female\ donor) \\ & + 0.190 \times (CMV\ positive\ donor) - 0.023 \times \frac{(offer\ eGFR - 90)}{10} \\ & + 0.015 \times (days\ in\ hospital)\ \} \end{aligned}$$

APPENDIX A6: CLAVIEN-DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS

Grade	Definition
I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.
II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
III	Requiring surgical, endoscopic or radiological intervention.
IIIa	Intervention not under general anaesthesia.
IIIb	Intervention under general anaesthesia.
IV	Life-threatening complications (including CNS complications) requiring HDU/ITU management.
IVa	Single organ dysfunction (including dialysis).
IVb	Multi-organ dysfunction.
V	Death of a 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.

APPENDIX A7: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issues	Author(s) of changes	Details of changes made

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(

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