Short title/ACRONYM

Immunometabolism of Machine Perfusion Strategies (iMAPS)

PROTOCOL FULL TITLE:

MECHANISTIC EVALUATION OF MACHINE PERFUSION STRATEGIES IN DONATION AFTER CIRCULATORY DEATH LIVER TRANSPLANTATION

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CONTENTS

LIST OF ABBREVIATIONS

ADP	Adenosine Diphosphate
AE	Adverse event
AR	Adverse reaction
ATP	Adenosine Triphosphate
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body Mass Index
C	Degrees Celsius
CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials and Research Governance
CTU	Clinical Trials Unit
DAMPs	Damage Associated Molecular Patterns
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DLI	Donor Liver Index
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
EAD	Early Allograft Dysfunction
ECD	Extended Criteria Donor
GAPDH	Glyceraldehide 3-phosphate dehydrogenase
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GP	General Practitioner
GST	Glutathione S-Transferase
HOPE	Hypothermic oxygenated machine perfusion
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
INR	International Normalised Ratio
IRB	Independent Review Board
IRI	Ischaemia Reperfusion Injury
ITU	Intensive Care Unit
IVC	Inferior Vena Cava
KCH	King's College Hospital
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LDH	Lactate Dehydrogenase
L-GrAFT	Liver Graft Assessment following Transplantation scores
MEAF	Model for Early Allograft Function
MAP	Mean Arterial Pressure
MDT	Multidisciplinary Team
MELD	Model for End-stage Liver Disease
MHRA	Medicines and Healthcare products Regulatory Agency
MP	Machine Perfusion

mtDNA	Mitochondrial DNA
NADH	Nicotinamide Adenine Nucleotide
NADPH	Nicotinamide Adenine Nucleotide Phosphate
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NMP	Normothermic Machine Perfusion
NORS	National Organ Retrieval Service
NRF2	Nuclear factor erythroid 2-related factor 2
NRP	Normothermic Regional Perfusion
RES	Research Ethics Service
PGD	Primary Graft Dysfunction
PI	Principal Investigator
PIS	Participant/ Patient Information Sheet
PNF	Primary Non-Function
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RNASeq	RNA Sequencing
ROS	Reactive Oxygen Species
RRT	Renal Replacement Therapy
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SDV	Source Data Verification
SNOD	Specialist Nurse for Organ Donation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UHB	University Hospitals Birmingham
UW	University of Wisconsin preservation solution
vWF	von Willebrand Factor

STUDY SUMMARY

STUDY OVERVIEW	
Full title	Mechanistic Evaluation of Machine Perfusion Strategies in Donation after Circulatory Death Liver Transplantation
Objectives and endpoints	Primary objective: To determine the effect of different preservation strategies on the development of mitochondrial damage following reperfusion. Primary mechanistic endpoint: changes in mitochondrial complex I enzyme activity during in liver tissue samples obtained 30 minutes and 4 hours after initiating NMP. Secondary mechanistic and exploratory objectives: 1) To determine the effect of different preservation strategies on mitochondrial integrity using alternative analytical strategies. 2) To assess the overall cellular metabolic and redox state during NMP and after reperfusion in the transplant recipient. 3) To determine how the different preservation strategies impact on the levels of inflammatory mediators during NMP. 4) To determine the influence of the different preservation strategies on biomarkers of liver damage and function during NMP. 5) To compare the applicability of different preservation strategies in DCD liver transplantation. Secondary mechanistic and exploratory endpoints: 1) Quantification of oxidative damage to mtDNA, genomic DNA (gDNA) and oxidized cardiolipin in liver tissue. 2) Targeted metabolomics for polar and non-polar metabolites in liver tissue; bulk RNA-Seq experiments of liver and bile duct tissue. 3) Quantification of immune cell subsets and inflammatory cytokine levels in perfusate; single-cell RNASeq of liver tissue; release of mtDNA and cell free DNA (cfDNA) in perfusate. 4) Sequential measurements of AST, ALT, bilirubin, alkaline phosphatase, LDH, vWF, fibrinogen, D-dimer, and lactate in perfusate; bile production and bile pH, bicarbonate and glucose; liver and bile duct histological analyses; proteomic analysis of perfusate to assess predictive and exploratory biomarkers. Secondary clinical objectives: 1) To determine the impact of the 3 different MP strategies on clinical outcomes up to 12 months following transplantation.

	2) To develop objective means to assess the quality and suitability for transplantation of DCD livers. 3)To compare the applicability of different preservation strategies in DCD liver transplantation. <u>Secondary clinical endpoints</u>: 1) Surrogate post-transplant clinical endpoints of IRI damage such as reperfusion syndrome; early allograft dysfunction (as assessed by the Model for Early Allograft Function (MEAF) and Liver Graft Assessment following Transplantation (L-GrAFT) scores); clinically relevant non-anastomotic biliary complications; comprehensive surgical complication index, Clavien-Dindo complication grade; and 6-month graft/patient survival. 2) Associations between biomarkers of liver damage/function during NMP and clinical outcomes. 3) Proportion of livers retrieved and transplanted with each preservation strategy.
Type of trial	Interventional / Phase II. The patient population consists of adults listed for elective liver transplantation.
Trial design and methods	Open-label, prospective, three-arm, phase II randomized clinical trial investigating the effects of 3 different preservation techniques on pre-defined functional and mechanistic endpoints in DCD liver transplantation.
Health condition(s) or problem(s) studied	Liver transplantation
Target sample size	36 donor livers will be enrolled and approximately 30 patients will receive transplantation within this clinical trial.
Trial duration per participant:	The overall study duration will be up to 30 months. The study participant recruitment phase will be up to 18 months. Patient follow-up will be 12 months. Clinical outcome data will be collected up to 5 years post-transplant.
Inclusion/exclusion criteria:	<u>Donor Inclusion Criteria:</u> 1. DCD category III donors considered for abdominal organs-only retrieval. 2. Donor age ≥18 years. 3. Retrieval procedure allocated to the recruiting site's NORS team. 4. Donor liver accepted for a patient on the recruiting site's transplant waiting list via the standard offering process 5. Donor BMI <35kg/m^2. 6. Predicted cold ischaemic time <8 hours. 7. Donor family has given consent to use donated liver for research.

	<u>Donor Exclusion Criteria (at the time of randomisation)</u>: 1. Donor is HIV, hepatitis B (HBV HbsAg) or hepatitis C (HCV RNA) positive. 2. Any medical condition that, in the opinion of the principal investigator, would interfere with safe completion of the trial. <u>Donor Exclusion Criteria (at the time of retrieval):</u> 1. Liver weight >2.5 kg. 2. Macroscopic evidence of advanced fibrosis. 3. Functional donor warm ischaemia (defined as a period between the systolic blood pressure <50mmHg and aortic cold flush) >30 minutes. 4. Any other clinical issue that in the opinion of the surgical team constitutes a contraindication to proceed with transplantation. <u>Recipient Inclusion Criteria:</u> 1. Recipients 18 years of age or older. 2. Listed on an elective transplant waiting list. 3. Suitable to receive a DCD graft based on the liver listing MDT. 4. Willing and able to consent for the study participation. <u>Recipient Exclusion Criteria:</u> 1. High-risk surgical candidates (e.g. presence of extensive portomesenteric thrombosis, previous complex upper abdominal surgery). 2. Patients undergoing liver re-transplantation or multi-organ transplantation. 3. Patients receiving super-urgent transplantation for acute and acute-on-chronic liver failure.
Statistical methodology and analysis:	Our RCT has been specifically designed to: i) allow investigation of the early molecular events eliciting IRI in human livers under controlled experimental conditions (i.e. during ex-situ NMP); ii) eliminate the confounding effects of arbitrary donor acceptance criteria, recipient heterogeneity, and liver transplant surgery; iii) establish correlations between mechanistic data, functional endpoints and relevant post-transplant clinical endpoints. Our HYPOTHESIS is that the most effective MP strategy will be that which decreases IRI. In turn, this protection will be determined by modifying the metabolic changes induced by ischaemia that lead to ROS-induced mitochondrial damage, and/or by enhancing endogenous cellular anti-oxidant defence pathways. Utilising available literature to identify the most promising MP regimen among the 3 strategies currently employed in the clinic is problematic, given the confounding effects of patient/donor selection, lack of standardized MP protocols, and differences in surgical technique and medical care. Our goal is to ensure that if one of the 3 MP strategies is superior, then there is a high

	probability that we will select it. This requires controlling type II rather than type I error. To this end, we have chosen a three-arm phase II randomized selection clinical trial design ('pick the winner' or screening design). This design is underpowered for performing formal hypothesis testing comparing clinical efficacy endpoints across the arms, but is optimal to select the most promising intervention based on mechanistic endpoints, while reducing the unknown effects of confounders that bias most liver MP trials. Given the study design, all analyses will be based on descriptive data without testing. Continuous variables will be summarised using means and standard deviations or medians and interquartile ranges. Categorical data will be reported as numbers and frequencies. The 3 study arms will be compared for all primary and secondary outcomes. In each group we will report baseline characteristics of donor and recipients, as well as outcomes with 95 % confidence intervals. SCS corresponds to the current standard-of-care in recruiting sites and will constitute the control arm. HOPE and NRP are the 2 MP modalities currently employed in the clinic to improve DCD livers and will be considered as the experimental groups. We will report upon the proportion of eligible donors who are randomised, perfused and transplanted within each group. The primary analysis will be based on the primary mechanistic endpoint, which has been selected based on literature highlighting the key role of mitochondrial complex I in the initiation of the IRI responses (9). However, given that the mechanisms of action of MP strategies have not been adequately investigated employing human donor livers suitable for transplantation, we will use the list of secondary mechanistic endpoints to support and complement the results of the primary endpoint, and to explore if they can constitute better markers of biological activity and/or safety. In addition, we will analyse the interactions between functional endpoints and mechanistic endpoints using regression models, to identify the key biological pathways contributing to tissue damage during IRI. Finally, we will analyse the relationships between the various functional, mechanistic and biomarker endpoints assessed during NMP and between these endpoints and all post-transplant parameters independently from the allocated treatment arm to identify predictors of post-transplant outcomes (this will be restricted to livers that proceed to transplantation within 12 hours of NMP). Being a mechanistic trial, the primary analysis will be as per protocol and include all livers that are perfused under NMP conditions for at least 4 hours. In addition, a secondary analysis including recipient outcomes will include only livers actually transplanted.
STUDY TIMELINES	
Study Duration/length	The overall study duration will be up to 30 months.

	The study participant recruitment phase will be up to 18 months. Patient follow-up will be 12 months. Clinical outcome data will be collected up to 5 years post-transplant.
Expected Start Date	01.01.2024
End of Study definition and anticipated date	Last patient last visit, 31.05.2026
Key Study milestones	• Protocol submission. • Clinical trial set up • First participant recruitment • Completion of recruitment of 36 participants • Last patient last visit and completion of mechanistic studies
STORAGE of SAMPLES (if applicable)	
Human tissue samples	The samples collected as part of the study will be stored at King's College London (KCL) and/or University of Birmingham (UoB), and processed in laboratories at KCL, UoB and University College London.

1 INTRODUCTION

Liver transplant numbers do not meet the existing needs, thousands of patients remain on transplant waiting lists worldwide and many die while awaiting a life-saving organ. A key contributor to organ shortage is the discarding of viable organs coming from donors considered high-risk, for fear that they might malfunction after transplantation as a result of a phenomenon called ischaemia reperfusion injury (IRI). Most of the discarded livers are those donated after circulatory death (DCD), only 27% of which are currently utilised in the UK. The quality of DCD organs can be improved by replacing the icebox (static cold storage or SCS), which remains the main approach to preserve the livers after having been retrieved, by strategies that perfuse the livers in a machine (machine perfusion or MP). There are currently 3 MP strategies employed in the clinic: normothermic regional perfusion (NRP) is used in the donors by perfusing the liver with the donor's blood at 37 degrees Celsius, and normothermic (NMP) or hypothermic (HOPE) perfusion are used in the procured livers out of the body (using warm or cold perfusion fluids, respectively). To date, no controlled objective comparisons of these different MP strategies have been undertaken and we do not have a good understanding of their mechanisms of action. Our hypothesis is that the benefits of MP will depend on the capacity of these strategies to improve the damage to the liver cell mitochondria, which constitutes the first event that elicits IRI at the time of transplantation. To determine this, we propose to conduct a randomised clinical trial in which 36 DCD human livers will be allocated to 1 of 3 treatment arms: i) SCS; ii) NRP; and iii) HOPE. This will be followed by a period of time in NMP in order to study the IRI response and determine if the quality of the livers is good enough to proceed to transplantation. Following transplantation, patients will be followed for up to 12 months.

Our proposal will include three key objectives:

1) To investigate the role of mitochondrial damage in the IRI that takes place when DCD livers are transplanted.

2) To determine the mechanisms through which the different MP strategies influence IRI in DCD liver transplantation.

3) To develop markers to assess the quality of the livers while they are being perfused using NMP before being transplanted into patients.

Our study will allow us to decipher the mechanisms of liver IRI in humans in a much better way than what has been achieved to date. Furthermore, it will provide guidelines as to the best way of employing the MP technologies and may result in the identification of new treatments. Ultimately, our proposal will serve to improve the quality of DCD livers and increase the number of patients who can safely receive a liver transplant.

2 BACKGROUND AND RATIONALE

Organ donation inevitably includes periods of organ ischaemia, which alter cellular metabolism by compromising the delivery of oxygen and nutrients to cells. This results in: i) reduced ATP production by oxidative phosphorylation with a switch towards glycogenolysis and anaerobic glycolysis; ii) disruption of proton pumping by the respiratory chain and reversal of the FoF1- ATP synthase, which further decreases the ATP:ADP ratio; iii) intracellular acidification secondary to increased lactate production, which causes inhibition of GAPDH and contributes to the accumulation of NADH; and iv) accumulation of the citric acid cycle intermediate succinate due to reversal of the succinate dehydrogenase reaction (driven by the reduction of NADH and CoQ pools, along with the accumulation of succinate precursors, during ischaemia). Unabated, these processes cause cellular dysfunction and ultimately lead to cell death. Oxygenated reperfusion is essential to restore cell metabolism and salvage the ischaemic organ, but it causes further damage by eliciting the IRI response. IRI is initiated by a burst of superoxide production by mitochondrial complex I, which causes oxidative damage to mitochondria. As well as causing cell dysfunction, the mitochondrial disruption leads to the release of damage associated molecular patterns (DAMPs) such as mitochondrial DNA (mtDNA), which cause downstream inflammation mediated by both innate and adaptive immune responses (8).

Studies conducted in rodents indicate that the excessive generation of reactive oxygen species (ROS) upon reperfusion is mainly driven by the rapid oxidation of the succinate accumulated during ischaemia (9). Succinate oxidation drives superoxide production at mitochondrial complex I by reverse electron transport, triggering the opening of the mitochondrial permeability transition pore (mPTP), and inactivating the complex I enzyme. Rapid cooling and SCS, which remain the mainstay of organ preservation, slow succinate accumulation during ischaemia, thereby reducing tissue damage upon reperfusion (9).

However, as it takes many minutes for large human organs to cool, even SCS livers accumulate excessive succinate (10).

There are two categories of deceased donors: those where donation takes place after brain death (DBD) and those where it takes place after circulatory death (DCD). The key distinction is that DCD involves withdrawal of life supporting treatment, which results in a period of warm ischaemia at 37C lasting up until circulatory arrest and the initiation of SCS. The high metabolic activity and oxygen demand of the DCD liver at 37C amplifies the metabolic disturbances induced by ischaemia and the extent of tissue damage post-reperfusion. Clinically, this is manifested by an increased incidence of early graft dysfunction/failure and delayed complications such as progressive non-anastomotic biliary strictures, which severely limit the utilisation of DCD livers. Consequently, only 27% of DCD liver offers are currently used for transplantation in the United Kingdom (11,12).

Three different MP strategies are currently in clinical use to improve the quality of DCD livers and/or assess their viability before transplantation. Hypothermic oxygenated perfusion (HOPE) is applied after SCS by perfusing the liver ex-situ for approximately 2 hours with oxygenated preservation fluid at 10C. In a large RCT, HOPE reduced the incidence of clinically significant ischemic cholangiopathy as compared to SCS (1). Ex-situ normothermic perfusion (NMP) involves perfusing livers with a blood- based fluid at 37C (3). NMP can be initiated at any time after procurement, it can extend preservation up to 24 hours and allows functional assessment of the liver's suitability for transplantation. However, when applied after SCS, NMP does not appear to prevent complications such as ischemic cholangiopathy (4). Normothermic regional perfusion (NRP) is an in-situ MP strategy initiated immediately after confirmation of donor death. Although NRP has never been evaluated in RCTs and its mechanisms of action are poorly understood, it has been widely adopted in France and Spain based on retrospective studies showing reduced post-transplant complications (5-7).

Experiments performed in an isolated perfused rat liver model simulating DCD donation confirmed that, upon machine perfusion reoxygenation, the ischemic liver undergoes mitochondrial oxidative damage, as indicated by reduced activity of mitochondria complex I. This damage is exacerbated if exogenous succinate is added to the perfusate and improved if reperfusion is performed at 10C rather than at 37C. In this rat model, the use of HOPE, as compared to either SCS or NMP, lowers succinate levels and reduces liver damage post-transplantation (2). However, there is very limited data in humans indicating that the same mechanisms are active in clinical liver transplantation.

While much attention has been paid to the role of succinate and complex I-derived ROS upon reperfusion, mitochondrial oxidative damage is a complex process closely dependent on mitochondrial dynamics, mitophagy, and on the oxidation status of mitochondrial membrane phospholipids (in particular cardiolipin). Furthermore, in addition to mitochondria, there are other sources of ROS in the cell that can contribute to oxidative stress, such as activation of pro-oxidant enzymes (e.g. NADPH oxidases or NOX), or dysregulation of anti-oxidant defences (e.g. NRF2 pathway), all of which have been shown in animal models to regulate IRI in various tissues, including the liver (13). In addition to their potential direct impact on the initial mitochondrial ROS burst, these non-mitochondrial pathways could also affect the release of inflammatory mediators and the activation of specific immune cell subsets responsible for downstream inflammatory damage, but this remains unknown.

The extent to which the organ preservation strategies currently used in the clinic affect the mitochondrial and non-mitochondrial oxidative stress pathways outlined above has not been investigated employing viable human DCD livers suitable for transplantation. We will address these unknowns by conducting a clinical trial to determine the optimal MP preservation strategy. SCS, HOPE and NRP will be ranked based on markers of liver function, alongside measures of mitochondrial damage and other mechanistic endpoints. We will quantify the extent to which ROS causes mitochondrial injury following SCS, HOPE and NRP, and then model the IRI that would ensue within the recipient, using NMP. The use of NMP prior to transplantation as described here serves two purposes: first, it will allow us to investigate the early molecular events that elicit IRI in a controlled and reproducible manner; second, it will maximise patient safety by providing an objective assessment of the liver's suitability for transplantation.

3 OBJECTIVES

3.1 Primary Objective and Endpoint

In the current clinical trial, we intend to dissect the molecular and cellular events elicited by the IRI response under controlled experimental conditions, and to identify the mechanisms through which NRP and HOPE influence this response. This will be accomplished by comprehensively evaluating mitochondrial metabolism, oxidative stress-induced mitochondrial damage, and downstream inflammatory events that take place after reperfusing the DCD liver under NMP conditions prior to transplantation.

Primary objective: To determine the effect of different preservation strategies on the development of mitochondrial damage following reperfusion.

Primary endpoint: changes in mitochondrial complex I enzyme activity in liver tissue samples obtained 30 minutes and 4 hours after initiating NMP, as assessed by an established spectrophotometric assay quantifying the activity of the mitochondrial respiratory chain complexes I, II+III and IV (14,15).

Changes to mitochondrial metabolism play a key role in the development of the IRI response but have not been adequately studied in the setting of DCD clinical liver transplantation. The enzymatic activity of mitochondrial complex I is particularly susceptible to IRI and is considered a very sensitive marker of oxidative damage to mitochondria. The results will be expressed relative to citrate synthase activity to account for differences in mitochondrial content.

3.2 Secondary mechanistic and exploratory objectives and endpoints

Objective 1: To determine the effect of different preservation strategies on mitochondrial integrity using alternative analytical strategies.

Endpoints:

1. Quantification of oxidative damage to mitochondrial (mtDN and genomic (gDNA) DNA (liver tissue and blood), evaluated by a combination of PCR amplification of a short and a long section of mtDNA and targeted or whole exome sequencing of gDNA (16), quantification of the activity of mitochondrial respiratory complexes II, III, IV.
2. Oxidized cardiolipin (liver tissue) assessed by analysing the amount of targeted cardiolipin species that are peroxidised relative to the intact species by LC-MS/MS (17,18).

Objective 2: To assess the overall cellular metabolic and redox state during NMP and after reperfusion in the transplant recipient.

Endpoints:

1. Cellular metabolism and redox balance: quantification of succinate levels by LC-MS/MS relative to internal standard (liver tissue and perfusate) (19).
2. Targeted metabolomics for polar and non-polar metabolites using LC-MS (liver tissue), including cofactors (e.g. ATP/ADP/AMP, NADH/NAD+, NADPH/NADP, FAD, GSH/GSSG), TCA cycle and glycolysis intermediates. NADH/NAD+ and NADPH will be validated using a quantitative colorimetric assay (Biovision), and the ATP/ADP ratio further validated by bioluminescence.

3. Bulk RNASeq experiments of liver and bile duct tissue, including transcriptional analysis of inflammatory pathways, anti-oxidant pathways and mitochondrial biogenesis genes.

Objective 3: To determine how the different preservation strategies impact on the levels of inflammatory mediators during NMP.

Endpoints:

1. Quantification of myeloid and lymphoid immune cell subsets (flow cytometry) and inflammatory cytokine levels (MSD cytokine profiling) (perfusate).
2. Single-cell RNASeq of liver tissue samples to assess the contribution to the IRI response of individual cell subsets (10X platform; 3 patients per arm).
3. Release of mtDNA (perfusate).

Objective 4: To determine the influence of the different preservation strategies on biomarkers of liver damage and function during NMP.

Endpoints:

1. Sequential measurements of AST, ALT, Bilirubin, Alkaline phosphatase, LDH, vWF, Fibrinogen (NMP perfusate) and D-dimer.
2. Lactate clearance (NMP perfusate).
3. Bile production volume and composition by gas analyser.
4. Liver and bile duct histological analyses.
5. Perfusate biomarkers previously shown to predict post-transplant clinical outcomes (e.g. SMOC 1, GRFA 1 and ACYC1 by Elisa). We will collect additional perfusate samples to identify novel biomarkers through non-targeted proteomic analyses.

3.3 Secondary clinical objectives and outcome measures

Objective 1: To determine the impact of the 3 different MP strategies on clinical outcomes following transplantation.

Endpoints: Surrogate post-transplant clinical endpoints of IRI damage up to 12 months post-transplant, such as reperfusion syndrome, early allograft dysfunction (as assessed by the Model for Early Allograft Function (MEAF) and Liver Graft Assessment following Transplantation (L-GrAFT) scores), clinically relevant non-anastomotic biliary complications, comprehensive surgical complication index, Clavien-Dindo complication grade, and graft/patient survival. Clinical outcome data up to 5 years post-transplant will be collected if required.

Objective 2: To develop objective means to assess the quality and suitability for transplantation of DCD livers.

Endpoints: Associations between the biomarkers of liver damage and function during NMP with clinical outcomes post-transplantation.

Objective 3: To compare the applicability of different preservation strategies in DCD liver transplantation.

Endpoints: Proportion of livers retrieved and transplanted with each preservation strategy.

4 STUDY DESIGN

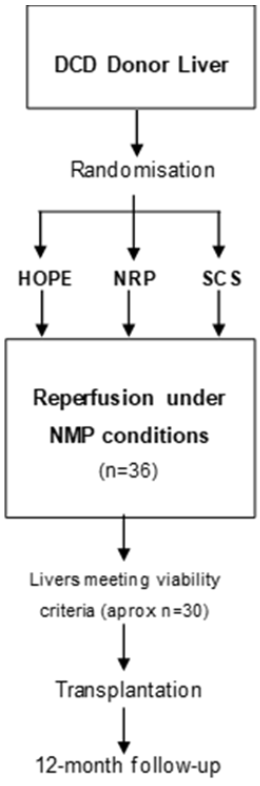


Fig. 1: Trial design

Open-label, prospective, three-arm, phase II randomized clinical trial investigating the effects of 3 different preservation techniques on pre-defined functional and mechanistic endpoints in DCD liver transplantation.

DCD donors will be randomized to one of three arms (n=12 per arm):

Arm 1 (SCS). The donor liver will be flushed in situ with 4C UW preservation solution (or HTK) through the aorta and portal vein, retrieved, and transported to the transplant centre in an icebox.

Arm 2 (NRP). The donor aorta and inferior cava vein will be cannulated, followed by descending thoracic aorta cross-clamp and initiation of perfusion (Cardiohelp device) with the donor's own blood at 37C for 2h (while monitoring pump flow, venous O2 saturation, lactate and ALT), followed by in-situ flush with 4C preservation solution as in SCS arm.

Arm 3 (HOPE). The liver will be retrieved and preserved as in SCS arm. Then, on arrival to the transplant unit, the portal vein and hepatic artery will be cannulated, and the liver perfused with hypothermic oxygenated solution (VitaSmart device) for 2h.

Regardless of the allocated arm, a period of at least 4h of end-ischemic NMP (OrganOx metra device) to evaluate their viability criteria and obtain samples for mechanistic experiments. We expect that approximately 30 livers will be considered suitable to proceed to transplantation.

5 STUDY VISITS AND INTERVENTIONS

5.1 STUDY VISITS

The trial visits will, where possible, coincide with standard-of-care routine inpatient or outpatient assessments.

Research samples (blood) post Study Visit 1 are OPTIONAL.

Screening Visit:

Patient registration and informed consent

Study Visit 1: Transplant (Day 0) and inpatient stay up to Day 7

1. Patient registration and confirmation of informed consent.
2. UKELD score.
3. Standard blood biochemistry and haematology tests, including: full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs: AST, ALT, GGT, Bilirubin, alkaline phosphatase), eGFR, International Normalised Ratio (INR). These standard routine blood tests should be done on day 0 (pre-transplantation) and repeated daily for 7 days (up to post-transplant day 7).
4. Transplantation procedure
   - Cold ischemia time, total preservation time (period from donor aortic flush to liver reperfusion in the donor body), warm ischemia time during implantation.
   - Surgical methods and technical difficulties or abnormalities (including blood loss and duration of transplant).
   - Hemodynamic status is recorded routinely and continuously. Items recorded for this study are systolic and diastolic blood pressure, heart rate and vasopressor dosage. These will be recorded 5 min before portal reperfusion, as well as 10 and 20 minutes after reperfusion to allow assessment of post-reperfusion syndrome.
5. Research samples
   - Samples will be taken at the following time points: day 0 (post-induction, 30 minutes after portal reperfusion, 2 hours after reperfusion at the time of Liver Biopsy 4), and on days 1, 3, 6 post-operation.
   - Liver Biopsy 4 – Liver Biopsy 4 will be taken 2 hours after reperfusion prior to abdominal closure (Menghini liver biopsy). Common bile duct biopsy to be performed immediately prior to biliary anastomosis.
6. Clavien-Dindo score / Adverse events

7. Anti-rejection medications including calcineurin inhibitor or mTOR inhibitor blood trough levels.

Study Visit 2: Day 30 (+/- 7 days)

1. Standard blood biochemistry and haematology tests, including: full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs: AST, ALT, GGT, Bilirubin, alkaline phosphatase), eGFR, International Normalised Ratio (INR).
2. Research samples (blood)
3. Clavien-Dindo score / Adverse events
4. Any other significant clinical events
5. Anti-rejection medications including calcineurin inhibitor or mTOR inhibitor blood trough levels.

Study Visit 3 – Day 90 (+/- 15 days)

1. Standard blood biochemistry and haematology tests, including: full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs: AST, ALT, GGT, Bilirubin, alkaline phosphatase), eGFR, International Normalised Ratio (INR).
2. Research samples (blood)
3. Clavien-Dindo score / Adverse events
4. Any other significant clinical events
5. Anti-rejection medications including calcineurin inhibitor or mTOR inhibitor blood trough levels.

Study Visit 4 – Day 180 (+/- 30 days)

1. Standard blood biochemistry and haematology tests, including: full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs: AST, ALT, GGT, Bilirubin, alkaline phosphatase), eGFR, International Normalised Ratio (INR).
2. Research samples (blood)
3. Clavien-Dindo score / Adverse events
4. Any other significant clinical events
5. Anti-rejection medications including calcineurin inhibitor or mTOR inhibitor blood trough levels.
6. An MRCP is recommended as standard-of-care (this is not part of the trial interventions).

Study Visit 5 – 1 year (+/- 30 days)

1. Standard blood biochemistry and haematology tests, including: full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs: AST, ALT, GGT, Bilirubin, alkaline phosphatase), eGFR, International Normalised Ratio (INR).
2. Research samples (blood)
3. Clavien-Dindo score / Adverse events
4. Any other significant clinical events
5. Anti-rejection medications including calcineurin inhibitor or mTOR inhibitor blood trough levels.

Long-term outcomes

All patients after liver transplantation typically remain followed up for life, with regular monitoring of blood biochemistry, haematology, and immunosuppression levels conducted by the transplant centres. Information about patient and graft survival, allograft function and biliary complications beyond 1 year may be collected by the investigators for the purpose of this study. However, the study database will not record any information beyond 1 year.

Table 1: Schedule of Assessments

ACTIVITY	Screening Visit	Visit 1								Visit 2 (D30)	Visit 3 (D90)	Visit 4 (D180)	Visit 5 (Y1)
		D0	D1	D2	D3	D4	D5	D6	D7				
Recipient meets inclusion/exclusion criteria	X	X											
Informed consent	X	X											
Donor demographics		X											
Randomisation		X											
Assessment liver suitability for transplantation		X											
Transplantation procedure data		X											
Routine blood tests		X	X	X	X	X	X	X	X	X	X	X	X
Liver biopsy		X											
Research samples*		X	X		X			X		X	X	X	X
Clinical data/Adverse events		X	X	X	X	X	X	X	X	X	X	X	X

*Research samples on D0 include samples pre-transplant, post-reperfusion, and D0 post-transplant (see Table 3) Research samples post Visit 1 are OPTIONAL.

5.2 STUDY INTERVENTIONS

All the medical devices and technologies used in this trial are CE marked and currently clinically used by majority of the UK liver transplant units. The devices are used according to the instruction of use. The staff operating the devices are provided with training and that log is maintained by the study PI.

5.2.1 Static cold storage

The static cold storage of donor organs has been the standard of care for several decades. It provides simple, cheap and relatively efficient way to preserve donor livers. During SCS at 4°C, the cellular metabolism demand decreases as much as 12-fold, but metabolic processes persist and eventually result in cellular damage, depleting adenosine triphosphate (ATP) levels (with subsequent alteration of the sodium – potassium ATP-dependent membrane cellular transport), and causing mitochondrial disturbances that eventually negatively affect cellular viability. The research focused on mitigation of the damaging consequences of cold ischaemia led to development of dedicated organ preservation fluids.

The standard preservation fluid used by the NORS teams is University of Wisconsin solution (UW; Bridge to Life Ltd, Europe), which is widely considered to be the benchmark for SCS liver preservation and will be the solution employed in this trial. The UW is an intracellular-like solution (i.e. high concentration of potassium, low concentration of sodium), contains several agents (including lactobionic acid, raffinose and hydroxyethyl starch) that result in high viscosity and prevent cells swelling during cold ischaemia, and includes glutathione and adenosine, which provide antioxidant capacity and stimulate ATP phosphate generation during reperfusion, respectively. Of note, the recruiting sites are going to use the same static cold storage preservation fluid, and this will not change throughout the study period. At the time of this protocol writing, it is uncertain which fluid (UW or HTK) is going to be used by the NORS. However, as the UW has been a well-established benchmark for SCS it would be the preferred fluid for this trial.

For standard quality donors, SCS achieves good outcomes, however, the results are often suboptimal when used for extended criteria livers, in particular from DCD donors. This has been the main reason for the rapid adoption of the novel dynamic organ preservation strategies.

The organ suitability for transplantation in SCS livers is based on the donor history, blood biochemistry, the liver visual assessment of the donor by the transplant surgeons, and in

selected cases also on a biopsy. Whilst several studies have showed that MP preservation provides superior transplant outcomes, in particular in in sub-optimal livers, due to practical and financial considerations cold storage remains the predominant preservation approach in the UK, being used in 90% of all donor livers, and over 75% of DCD livers, respectively.

The current trial design incorporates end-ischaemic NMP allowing liver viability assessment prior to proceeding with transplantation. This specific feature is included to prevent unfavourable post-transplant outcomes, which is of key importance for patients' safety, and to make clinical teams comfortable with inclusion SCS as one of the study arms.

5.2.2 Normothermic regional perfusion

Abdominal *in situ* normothermic regional perfusion is a technique to restore the circulation to the abdominal organs of the donor following circulatory arrest for the purpose of transplantation. This involves establishing a localised abdominal perfusion extracorporeal membrane oxygenation circuit and perfusing the organs with oxygenated blood at 37°C for a period of typically 2 hours. This technique, which is applicable exclusively to DCD donors, allows organs to recover from warm ischaemia, replenishes ATP reserves, and allows assessment of liver function and quality.

NRP has been shown to increase the utilisation of all abdominal organs and improve the outcomes of liver and kidneys with no adverse effects on the pancreas. When employed in liver transplant donors it is associated with better graft survival and a very low incidence of non-anastomotic biliary strictures. In kidney transplantation it has been shown to improve renal function at 12 months. For these reasons, the NRP has been promoted by the NHSBT as a preferred technique for DCD retrievals, although it is currently employed in approximately 50% of DCD liver transplantation cases in the UK.

<u>Maquet Cardiohelp device description</u>

The NRP intervention will be performed using the Maquet Cardiohelp device (Maquet Cardiopulmonary GmbH, Rastatt, Germany). This device is CE marked and currently used by all the UK NORS teams who have adopted the NRP technology and follow the national NRP protocol. It perfuses the donor abdominal compartment with warm, oxygenated blood, with several additives. NRP replicates the routinely used extracorporeal membrane oxygenation therapeutic intervention but is applied to organ donors.

The Cardiohelp device provides information about the haemodynamic and blood gas exchange parameters during perfusion, which assists the clinician in assessing the liver's suitability for transplantation.

The device incorporates a centrifugal pump, an oxygenator, oxygen blender, heat exchanger, reservoir, flow probes, pressure sensors, infusions, and blood gas analyser together with tubing and connector components. The device is comprised of four main components, and required specialised vascular cannulae to the set up and running the perfusion:

- A reusable base unit which contains software and hardware
- A Heater Unit
- A disposable perfusion set
- Vascular cannulae
- Perfusion fluid and additives

Heater Unit

The Cardiohelp Heater is a separate unit that should be topped up with water, connected to the base device, and switched on with the temperature set at 37°C. Its function is controlled via the Cardiohelp base unit.

Disposable set

A new sterile set of disposables is used for each NRP retrieval with the Cardiohelp device. It consists of a tubing set, a blood reservoir, perfusion lines, a blood oxygenator and centrifugal pump-head, pressure sensor lines, and an oxygen inflow line.

Vascular cannulae

The access to the donor large vessels to commence the NRP perfusion can be secured either in the groin using the femoral vessels, or in the abdomen with direct or indirect access to the aorta and IVC. The femoral cannulation is most appropriate in the circumstances such as younger donors with little chance of occlusive ilio-femoral arterial disease, obese donors, cases with possible delay to access the abdominal vessels (e.g. due to previous abdominal surgery), or known distal aorta and iliac vessels anomalies. The choice of cannulae for the femoral vessels varies according to the patient's size (typical sizes for femoral artery and vein are 19Fr and 25 Fr, respectively).

Aorto-iliac cannulation follows the approach used for standard DCD retrieval, using midline abdominal incision, mobilisation of the right colon and small bowel mesentery to reach the distal abdominal aorta and proximal right common iliac vessels. The choice of cannulae for the intra-abdominal vessels varies less with the patient's size (typical sizes for aorta and IVC are 24 Fr and 36 Fr, respectively).

Perfusion solution and additives

The NRP perfusion is maintained with the donor blood, that is mixed with the priming fluid added to the disposable set reservoir. This ensures that there is a sufficient volume of the circulating blood and prevents bacterial contamination. The perfusion solution is prepared immediately before the NRP procedure. Its composition is standardised and defined in the national NRP protocol and consists of:

- Hartmann's 2000 mls
- Bicarbonate 8.4%, 1ml/kg of donor weight
- Heparin 50,000 units
- Methylprednisolone: 1 gram
- Phentolamine 5mg
- Fluconazole: 400 mg
- Teicoplanin 200 mg
- Gentamicin 120mg
- Metronidazole 500 mg

During NRP the reservoir volume and the circulating blood haematocrit are monitored. If the donor is anaemic, or if there is blood loss following cannulation and initial dissection, a unit or more of donor blood group matched packed red cells may be added. Typically, if the haemoglobin is less then 6 g/dL it is corrected with 2 units, if between 6-8 g/dL with 1 unit of packed red cells. In situations when the haematocrit is acceptable, but the reservoir volume is low, Gelofusine is added as appropriate.

NRP procedure logistics and technical aspects

The SNOD should be informed early about the intention to perform the NRP and advised to order 4 units of packed red cells cross-matched to the donor to be available prior to the abdominal retrieval team arriving. Access to the operating theatre department blood gas analyser and regular (15 minutes) perfusate blood gases check is negotiated with the local team. The withdrawal of donor supportive treatment proceeds after confirmation with the operating surgeon and perfusion pump operator that the NRP circuit is ready to use. Following the cardiocirculatory arrest, 5-minute no-touch period and declaration of the donor death, the femoral or iliac vessels are cannulated, chest cavity opened, and thoracic aorta cross-clamped below the level of the left subclavian artery close to the diaphragm. A stab incision is made in the ascending thoracic aorta and a cannula is inserted, secured and left

open to atmosphere to allow monitoring of pressure and confirm absent flow in the aorta and intracranial arterial supply.

The pump must only be started once the circuit is completely connected, the thoracic aorta is cross-clamped, and the aortic arch is vented. The heater temperature should be 37°C. The air/O2 mixer should be set to deliver gas flow at 2 litres/minute with a starting FiO_2 between 21% -40%. Changes to the oxygen/air mixture may be required subsequently depending on the blood gas analysis. High oxygen concentrations may generate reactive oxygen species and can exacerbate reperfusion injury to the organs. If technically feasible the perfusion is run for two hours to recover the quality of the liver and to assess its function.

If the NORS team experiences technical difficulties (e.g. bleeding or poor flows) the NRP is stopped and the preservation converted to SCS using the standard DCD super-rapid technique. The preferred NRP duration is two hours. If this is achieved, the heater unit is switched off, and the NORS team swiftly proceeds with cold flush, removal of the abdominal organs and SCS similarly to donation after brain death.

More details about the procedure are provided in the national NRP protocol. (https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/29700/uk-protocol-for-normothermic-regional-perfusion-version-110-28-04-2023.pdf [nhsbtdbe.blob.core.windows.net]).

Delivery of the NRP is resource and labour intense. To sustain the service with minimal interruptions and in order to achieve the target patient recruitment, the recruiting site's team will collaborate closely with the NHSBT and negotiate crossover service arrangements to limit any blackouts in the NRP availability.

NRP parameters

The NRP flows vary based on the donor size, but for typical procedures the national NRP protocol suggest the following parameters:

- Pump flow 3 litres/minute
- Temperature 35.5°C - 37.5°C
- Air / O_2 to maintain a venous O_2 saturation (SvO_2) 60-80%
- Arterial pH 7.35-7.45
- Haematocrit > 20%
- Gas flow to maintain arterial pCO_2 4.5 to 6.0 kPa

Assessment of liver suitability for transplantation

During the NRP procedure the team performs every 15 minutes blood gas analyses at a donor hospital a point of care device. The perfusate biochemistry analyses are typically

performed by a point of care device carried by the NRP team. The following biochemical parameters are assessed in real time during the procedure:

- Aminotranferases (ALT): while common practice is to accept livers with a rise in ALT ≤500 IU/L over 2 hours, for the current trial where the DCD liver will be further perfused with end-ischaemic NMP the maximum limit will be increased to ≤1,000 IU/L. This approach has been adopted in UK practice.
- Lactate: the lactate, as liver function marker, should fall over the course of 2 hours but may not reach normal values due to venous return from the upper body and non-perfused limbs. Clamping the intrathoracic IVC may be associated with a greater fall in lactate measured in the circuit.

While these measurement results will be considered, in the current trial the final decision regarding the liver suitability for transplantation will be based on the end ischaemic NMP parameters.

Device safety and maintenance

The Maquet Cardiohelp device is CE marked and is currently used by all the UK NORS teams that have adopted the NRP procedure. To minimise the risk of complications or errors that would prevent a successful organs retrieval, NHSBT has developed a framework for this technology implementation, that specifies the national NRP standards, protocol, liver assessment guidance, a training programme and governance oversight.

Device cleaning and routine maintenance is the responsibility of the local investigator storing the device. Full details for cleaning and routine maintenance required are provided in the relevant section of the Instructions for Use (IFU). The device software and hardware maintenance, annual inspection, staff training and users support are included in the purchase contract and provided by Getinge UK & Ireland company.

5.2.3 Hypothermic oxygenated perfusion

HOPE is applied after SCS by perfusing the liver *ex-situ* for approximately 2 hours with oxygenated preservation fluid at 10°C. The intervention has been demonstrated to reduce the incidence of clinically significant non-anastomotic biliary strictures as compared to SCS. This has been attributed to increased succinate clearance and restoration of mitochondrial integrity before the liver transplant is reperfused in the recipient.

Bridge-to-life VitaSmart device description

The HOPE perfusion will be performed using VitaSmart device (Bridge to Life Ltd, Europe). This device is CE marked and currently used by several UK transplant teams. The device

incorporates a roller pump, an oxygenator, flow probes, pressure sensors, tubing and connector components. The device is comprised of three main components to the set up and running the perfusion:

- A reusable base unit which contains software and hardware
- A disposable perfusion set with cannulae
- Perfusion fluid and additives

Disposable set

A new sterile set of disposables is used for each HOPE perfusion with VitaSmart device. It consists of a tubing set, perfusion lines, an oxygenator and roller pump, pressure sensor lines, and an oxygen inflow line.

Perfusion solution and additives

The HOPE with the VitaSmart device is performed using the Servator M SALF machine perfusion solution, that is provided by the company and accompanies the perfusion each disposable perfusion set.

HOPE procedure logistics and technical aspects

The HOPE using the VitaSmart is a simple procedure and part of the perfusion service offered at the recruiting sites that consists of the liver perfusion via portal vein only. Prior to the commencing HOPE the liver is flushed via the portal vein cannula with 1000 mL cold Belzer MPS UW machine perfusion solution. The perfusion is typically started at the beginning of the liver back-table preparation for transplantation, and is maintained after its completion until the clinical team is ready for the liver implantation. During the connection of the liver to the machine, the perfusion pressure is adjusted manually in the first five minutes after connection so that a minimum of 100 ml/min flow via the portal vein is maintained, but without exceeding a portal vein pressure of 7 mm Hg. Perfusion fluid and liver will be cooled to 12°C by the thermoregulator. The reservoir of the cooling unit must be filled with crushed ice that is regularly replaced. The device registers flow rates and temperature and gives alarms in case of high flow or temperature. A surgeon supervises this procedure and is in the vicinity.

HOPE parameters

HOPE is typically performed with the following parameters:

- Pump flow 2-3 litres/minute
- Temperature 10°C

- Oxygenation kept at PO_2 between 450 and 600 mmHg (60-80 kPa). The recommended initial FiO_2 is 2 litre per minute; after 15 minutes of starting the perfusion the PO_2 needs to be measured and the FiO_2 adjusted as/if needed.

Assessment of liver suitability for transplantation

The is some evidence suggesting that the release of flavin-mononucleotide (FMN) corelates with the liver suitability for transplantation. This parameter will be regularly measured at 15-minute intervals. The auto-fluorescent characteristics of FMN enable the real-time measurement of this molecule from the HOPE-perfusates. While these measurements will be considered, in the current trial the final decision regarding the liver suitability for transplantation will be based on the end-ischaemic NMP parameters.

Device safety and maintenance

The VitaSmart device is CE marked and currently used by several UK transplant teams. Due to the nature of the perfusion, user errors and risk of organ loss are very low. The device software and hardware maintenance, annual inspection, staff training and users support will be provided by the *Bridge-to-Life* Ltd company.

Device cleaning and routine maintenance is the responsibility of the local investigator storing the device. Full details for cleaning and routine maintenance required are provided in the relevant section of the Instructions for Use (IFU).

5.2.4 End- Ischemic Normothermic Machine Perfusion

This trial design incorporates end-ischaemic NMP for livers in all three study arms to enable a robust way to collect trial samples for mechanistic research, and to assess the liver suitability for transplantation to assure safety for trial patients. The perfusion will be performed using OrganOx *metra*™ (OrganOx Ltd, Oxford, UK).

OrganOx *metra* device description

The OrganOx *metra* normothermic perfusion device is CE marked and used routinely by all the UK liver transplant centres. It perfuses the donor liver with blood, oxygen and nutrients, as well as a number of medications, at normal body temperature to replicate physiological conditions and preserve the organ for up to 24 hours. The device provides information as to the haemodynamic, synthetic and metabolic function of the liver during perfusion, which may assist the clinician in assessing the organ's suitability for transplantation.

The OrganOx *metra* incorporates a centrifugal pump, an oxygenator, oxygen concentrator, heat exchanger, reservoir, flow probes, pressure sensors, 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 circuit kit.
- A set of perfusion solutions and additives.

The Disposable Set used with the base unit of the OrganOx *metra* contains all the disposables used with each organ recovery and comprises:

- A disposable tubing set, including a blood reservoir, perfusion lines, a blood oxygenator and centrifugal pump-head together with flow and pressure sensors.
- An organ storage bowl which is pre-connected to the tubing set to contain the organ while on the device.
- Cannulae for the coeliac artery, portal vein and inferior vena cava with easy connection attachment to the perfusion circuit.
- A cannula and connection point for bile collection
- Blood gas sensors for monitoring pO_2, pCO_2 and pH by means of on-line blood gas analysis.
- Perfusion solution and additives.

NMP requires a dedicated oxygen carrier to maintain a physiological liver perfusion, which is provided by the blood-based perfusate. Sodium taurocholate is sourced by OrganOx and included with the disposable set. The other components and additives consist of bolus injections (given at the start of perfusion) and the maintenance infusions (given throughout perfusion).

The perfusion solution is prepared immediately before the organ is attached to the device, and it comprises of:

- Packed red blood cells matched for the liver recipient, supplied by hospital blood bank.
- Gelofusine colloid solution to normalise the haematocrit and osmolarity
- Supplements consisting of boluses of: antibiotic and antifungal agents as per current local protocols (meropenem and fluconazole); heparin 10,000 units to prevent thrombosis in the circuit (due to assumed half-life of ~90 minutes heparin is also given as a maintenance infusion); sodium bicarbonate 8.4% (20mL, with further boluses sometimes required to adjust the pH of the perfusate); calcium gluconate 10%, 10mL to correct the binding of citrate to calcium.

- In addition to the above, during the perfusion the following are infused at a constant rate 1mL per hour: parenteral nutrition solution (a source of amino acids and glucose for liver maintenance); insulin 6.7 units/hr to control the perfusate glucose level; heparin 833 units/hr to maintain anticoagulation; a 2% solution of sodium taurocholate (0.18gr/hr) in isotonic saline to compensate for loss of bile salts; prostacyclin 0.01 mg/hr to optimise micro-perfusion.

NMP procedure logistics and technical aspects

The *metra* device is primed with blood group O (or blood matched to the donor) and prepared for the liver connection. The liver is flushed with 1 litre of HTK solution at room temperature and the perfusate is collected to isolate leukocytes. Once the cannulas are in place the liver is perfused with gelofusine to prevent air in the system before connecting the liver. Once the perfusion is established and the liver stabilised, the NMP oversight and blood sampling is managed by the research team. The organ suitability for transplantation will be assessed 4 hours after having initiated NMP, using the below specified criteria.

Assessment of liver suitability for transplantation

The NMP perfusion parameters control is automated by the Metra device. During the procedure the team will monitor the macroscopic liver appearance and perform sequential perfusate (blood) and bile measurements to assess hepatocyte and cholangiocyte viability (Table 2).

Table 2: Functional assesments performed during NMP

Hepatocyte viability • Perfusate Lactate (mmol/L) • Perfusate pH • Perfusate ALT (IU/L) • Perfusate glucose • Bile production (mls)
Cholangiocyte viability • Bile pH • Δ bile pH • Δ Bicarbonate • Δ Glucose
Macroscopic liver perfusion appearance (homogeneous versus patchy perfusion)

Δ calculated as difference between bile and perfusate values (the Δ does no longer apply if the perfusate glucose is <10)

A liver will be **considered viable for transplantation** if it metabolises perfusate lactate to levels ≤2.5 mmol/L within 4 h of commencing the perfusion, in addition to meeting at least 2 of the following additional criteria: evidence of bile production, maintenance of perfusate pH ≥ 7.30 (with ≤30 mmol bicarbonate supplementation), metabolism of glucose (determined by the a gradual decrease in the perfusate glucose levels), maintenance of stable arterial and portal flows (≥150 and ≥500 mL/min, respectively), and homogenous perfusion (or minimal peripheral patchy perfusion) with soft consistency of the parenchyma.

Given the absence of evidence-based absolute transplantability criteria, in the current trial the ultimate decision to proceed with the transplant operation will be at the discretion of the transplant surgeon in charge of performing the procedure, although any deviation from the criteria outlined above will need to be justified and documented.
It is expected that around 90% of the perfused livers will be deemed suitable and proceed with the transplantation.
The following routine data collected during NMP will be captured in the trial database:
Perfusate and bile samples (the latter when available) should be collected at 30 min, 1h, 2h, 3h, 4h, 6h and at the end of perfusion.

- Perfusate blood gas analysis (lactate, glucose, pH, bicarbonate) at 30min, 1h, 2h, 3h, 4h, 6h and at the end of perfusion
- Perfusate AST, ALT at 1h, 2h, 4h and at the end of perfusion
- Bile production at 1h, 2h, 4h and at the end of perfusion
- Bile pH, bicarbonate, glucose at 30 min, 1h, 2h, 3h, 4h, 6h and at the end of perfusion

Transplant setup logistics

When the transplantation suitability criteria are met, the NMP will continue throughout the recipient liver explant phase of the transplantation, until the receiving transplant team are ready to implant the donor liver. The minimum protocol stipulated NMP duration is 4 hours, with the suggested longest NMP time being 12 hours. The cessation of the NMP timing will be guided by the clinical team readiness to proceed with the graft implantation. The OrganOx *metra* is licenced for NMP up to 24-hour. The reduced suggested preservation within this trial is in order to minimise variability between the livers to assure scientific rigour of the mechanistic research. If clinically indicated, the advised 12-hour NMP duration can be exceeded. Immediately prior to implantation, the research team will remove the liver from the

device, take post-NMP samples, flush the liver with 2 L of cold HTK fluid, place it into a bowl with ice slush and hand it over to the operating team.

Device safety and maintenance

The OrganOx *metra* is CE marked and used by all the UK liver transplant centres for organ preservation and viability testing. From a regulatory standpoint, it is important to note that this device is an organ preservation system that does not involve direct connection to either the donor or recipient at any time, and that the perfusion solution is removed from the organ prior to transplant by flushing the organ with HTK preservation fluid.

Device accountability will be undertaken at each local site throughout the study for the reusable unit 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 site will maintain a log of usage of the retained unit, disposable set and perfusion solutions used throughout the study recording the lot number used against each subject (on the CRF).

At the end of each OrganOx *metra* perfusion procedure, the disposable set and the perfusion solution will be disposed of on site. Device cleaning and routine maintenance is the responsibility of the local transplant team storing the device and is not described in the current protocol. If a device develops a fault during the study, it will be removed from service and a replacement loan device provided as soon as practically possible to allow continuation of recruitment. The device software and hardware maintenance, annual inspection, staff training and users support are included in the lease contract and provided by OrganOx Ltd company.

6 CONSENT

Please refer to section 8.4

7 ELIGIBILITY CRITERIA

The donors comprise of DCD donor livers ≥18 years. The patient population consists of adults listed for elective liver transplantation. Thirty-six donor livers will be enrolled and up to thirty patients will receive transplantation within this clinical trial.

7.1 Inclusion Criteria

7.1.1 Liver Donors inclusion criteria

1. DCD category III donors considered for abdominal organs-only retrieval.
2. Donor age ≥18 years.

3. Retrieval procedure allocated to the recruiting site's NORS team.
4. Donor liver accepted for a patient on the recruiting site's transplant waiting list via the standard offering process.
5. Donor BMI <35kg/m^2.
6. Predicted cold ischaemic time <8 hours.
7. Donor family has given consent to use donated liver for research.

7.1.2 Transplant recipient inclusion criteria

1. Recipients 18 years of age or older.
2. Listed on an elective transplant waiting list.
3. Suitable to receive a DCD graft based on the liver listing MDT.
4. Willing and able to consent for the study participation.

7.2 Exclusion Criteria

7.2.1 Liver donor exclusion criteria (at the time of randomisation)

1. Donor is HIV, hepatitis B (HBV HbsAg) or hepatitis C (HCV RNA) positive.
2. Any medical condition that, in the opinion of the principal investigator, would interfere with safe completion of the trial.

7.2.2 Liver donor exclusion criteria (at the time of retrieval)

1. Liver weight >2.5 kg.
2. Macroscopic evidence of advanced fibrosis.
3. Functional donor warm ischaemia (defined as a period between the systolic blood pressure <50mmHg and aortic cold flush) >30 minutes.
4. Any other clinical issue that in the opinion of the surgical team constitutes a contraindication to proceed with transplantation.

7.2.3 Transplant recipient exclusion criteria

1. High-risk surgical candidates (e.g. presence of extensive portomesenteric thrombosis, previous complex upper abdominal surgery).
2. Patients undergoing liver re-transplantation or multi-organ transplantation.
3. Patients receiving super-urgent transplantation for acute and acute-on-chronic liver failure.

8 RECRUITMENT

8.1 Registration

Due to the study design, there are 2 forms of registration into the trial: Stage 1 (donor liver registration), and Stage 2 (patient registration).

Donor liver registration

The following data will be collected as part of the study:

1. Donor demographics and blood results
    - Age, Sex, Ethnicity
    - Cause leading to withdrawal of treatment (CVA, hypoxia, trauma, other)
    - Height, weight
    - Last biochemistry and haematology results
    - Length of ITU stay
    - Liver risk indexes
    - Inotropic support at withdrawal of supportive care
2. Organ donation timings
    - Withdrawal of support
    - Onset of functional warm ischaemia
    - Cessation of donor circulation
    - Start of cold perfusion
    - Liver removal and placement on ice
3. Retrieval surgeon liver assessment
    - Quality of *in-situ* flush (poor/moderate/good)
    - Degree of liver steatosis (none/mild/moderate/severe)
    - Organ injuries
    - Liver weight
    - Liver photograph
4. Liver preservation and perfusion parameters
    - Static cold storage (time)
    - NRP parameters (NRP passport)
    - HOPE parameters (perfusion timings and flows)
    - NMP parameters

Patient trial entry

1. Recipient demographics and blood results
    - Patient history (medical and cause of liver disease)
    - Standard routine blood tests
2. Liver disease aetiology
3. Comorbidities

8.2 Rationale for selection criteria

Safety

The inclusion and exclusion criteria for the DCD donors correspond to those currently routinely used for transplantation at the recruiting sites. It excludes extremely marginal donors, which have known poor outcomes if preserved by cold SCS (i.e. donor warm ischemia beyond 30 minutes or moderately/severely steatotic DCD livers). The patient safety is ensured by using pre-defined, strict NMP criteria to assess whether to proceed with transplantation. Whilst the study design enforces patients' safety, the inclusion criteria are broad and cover around 50% of the current DCD donor population. This will have a favourable impact on the study recruitment and deliverability. It is worth noting that livers enrolled in the trial but not meeting the transplantability criteria following NMP, will still provide valuable data for the mechanistic research and contribute to the primary endpoint analysis.

Minimisation of bias and confounders in the experimental work

The harmonisation of retrieval technique and transplant procedures across the two participating clinical sites, in combination with the exclusion of high risk recipients, will contribute to the study cohort homogeneity and ensure its scientific rigour.

Real-world relevance and inclusivity

The proposed DCD criteria overlaps with the greatest potential for the future donor pool expansion. The liver transplant waiting lists at the recruiting sites include an extremely diverse patient population, with relative over-representation of underserved communities compared to other UK centres. This will ensure that the study outputs are reproducible and widely applicable to liver patients and the transplant community world-wide.

8.3 Recruitment

All UK DCD liver offers meeting the study inclusion criteria will be eligible for consideration provided they are retrieved by recruting site's NORS team. The offers are managed by NHS Blood and Transplant (NHSBT) Hub Operations using the electronic offering system. Potential donors are identified by the donor hospital intensive care unit staff and referred to the specialist nurse for organ donation (SNOD). The SNOD will obtain consent for donation, arrange any necessary investigations and register the donor with Hub Operations as per standard practice.

Liver offerings will follow standard NHSBT policy that remain unchanged by donor inclusion to the study.

8.3.1 DCD donor screening and eligibility assessment

This clinical trial involves the use of organs from deceased individuals that have been donated for liver transplant through the standard pathways managed and coordinated by the NHSBT. Standard NHSBT organ donation consent, retrieval and allocation processes will be utilised. There is no requirement for specific research consent for the NRP procedure, as this technology has been proven and is currently used for up to 25% of the DCD retrievals in the UK. The NRP availability is restricted by funding, but there are not any regulatory barriers. The details of the organ donation and NRP standard operating procedures are outlined NORS standards (https://www.odt.nhs.uk).

The screening and identification of donors to be included to the trial will be performed in collaboration with the NHSBT Hub. Once a DCD liver is offered to one of the participating sites, the research team will assess the logistic feasibility to attend the retrieval, to perform the NRP procedure, and the presence of a suitable size and blood group-matched recipient on the waiting list who has been consented to participate in the trial (see Trial Schema: 'logistics check'). If these conditions are met, the donor will be randomised. The DCD screening eligibility log will be kept by the research team and used for the auditing the study recruitment progress.

8.3.2 The organ retrieval procedure

The NORS team will be transported with an accompanying researcher to the donor hospital. If the randomisation allocates the donor to the NRP arm, the device will be set-up prior to withdrawal of donor treatment support as outlined below. The retrieval surgery will follow the established protocols. The research tissue biopsies (donor liver and bile duct), and donor

blood samples will be collected at pre-specified time points from every donation procedure (Fig.2). Samples of perfusate fluid and bile are also collected from the donor liver.

8.3.3 Donor data collection

Donor demographics and information about the retrieval procedure are essential to determine the suitability of the liver for transplantation and will be freely available to the clinical team. For research purposes, the donor details will be kept anonymously (specific study identification codes will be used for each study donor) and these data will only be made available to authorised staff of the study sponsor, its authorised representatives and regulatory authorities. Anonymised donor data will be used in future publications arising from the study.

8.3.4 Patient screening and eligibility assessment

The emergency nature of liver transplantation means that once a potential recipient is called in for a transplant there will be a limited time frame for the consent and screening process to occur. This may not allow sufficient time for the potential recipient to consider the implications of participating in the study. This problem is mitigated by the clinical adoption of the different liver perfusion techniques at both study sites. During the assessment process and/or at the time of being listed for transplantation all elective transplant candidates receive detailed education about the organ types, transplant logistics, and specific post-transplant complications, which includes an overview of the different liver perfusion techniques and any new technologies. All patients who fulfil the study inclusion criteria and are considered candidates for DCD donor livers will be given the trial Patient Information Sheet (PIS). This will take place during the transplant assessment or at waiting list clinics when the recipients are consented for their transplant. This will provide all patients who are suitable to participate in the trial ample time to consider their involvement into the study and to discuss their participation with others outside of the site research team. The patient screening eligibility log will be kept by the research team and used to audit of the study recruitment.

8.4 Informed consent

The trial consent will be taken once the potential patient is activated on the liver transplant waiting list. A patient information sheet (PIS) will be provided to facilitate this process. Investigators will ensure that they adequately explain the aims, trial interventions, anticipated benefits and potential hazards of taking part in the trial to the patient. Potential study participants will be given an opportunity to ask questions, which should be answered to their

satisfaction. Furthermore, the investigators will also stress that the patient is completely free to refuse to take part without giving a reason or withdraw from the trial at any time after consent. Full written consent will be obtained during a face-to-face meeting by one of the study team members during an outpatient appointment or inpatient admission and will be confirmed on the day of the liver transplant operation. Due to the nature of the study, in cases when it has not been possible to obtain consent beforehand, it can also be obtained immediately prior to / on the day of the liver transplant operation. It is the responsibility of the Principal Investigator (PI) or delegate, as captured on the site delegation log, to obtain written informed consent for each patient prior to performing any trial related procedure.

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

1. Suitably qualified and capable of providing information about the study.
2. Capable of answering questions about the study or ensuring that such questions are answered by a suitably qualified individual.
3. Authorised to do so by the local participating centre PI – detailed on site delegation log.

A copy of the PIS and the signed and dated Informed Consent Form (ICF) will be given to the participant. The original signed form will be retained at the study site and a copy will be placed and/or electronically scanned in the medical notes.
Once the patient is entered into the trial and is allocated a liver, the patient's trial number will be entered on the Informed Consent Form maintained in the ISF.

Details of all informed consent discussions (initial contact, verbal consent (if applicable) and written consent) will be recorded in the patient's medical notes (within 24 hours of the event). This should include date of, and information regarding the initial discussion, the date consent was given, with the name of the trial and the version number of the PIS and ICF.

Throughout the trial the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient, in which case the process described above will be followed and the patient's right to withdraw from the trial respected.

Electronic copies of the PIS and ICF will be available from the Trials Office and will be printed or photocopied onto the headed paper of the local institution. Details of all patients

approached about the trial should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent.

8.5 Randomisation

The study is going to randomise donor livers rather than transplant recipients. However, due to the rapidly evolving situation concerning the use of liver preservation strategies, with significant disparities of what different clinical units consider as standard-of-care, not all participating centres will randomised donor livers to all of the 3 MP strategies being evaluated in the current study. To accommodate this and maintain a balanced allocation of livers to the 3 arms of the study, each participating clinical site will have a different randomisation system that will take into account their estimated recruitment rate and MP strategies availability.

8.5.1 Randomisation eligibility

- Donor meeting the study inclusion criteria and none of the exclusion criteria at the time of randomisation
- Suitable consented liver transplant recipient
- Logistical feasibility to attend the retrieval and perform the NRP procedure
- Availability of HOPE and NMP equipment

8.5.2 Randomisation procedure

Randomisation of participants will be conducted by an online system hosted by the King's CTU, using stratification by centre and minimisation. Minimisation dynamically allocates participants based on the current balance across arms and strata. This inherently minimises both local and global imbalances and is well-suited to trials with uneven dropout. A Patient Identification Number (PIN) will be generated by registering the patient on the MACRO eCRF system (InferMed Macro), after consent has been signed. This unique PIN will be recorded on all source data worksheets and used to identify the patient throughout the study. Authorised site staff will be allocated a username and password for the randomization system.

8.6 Participant retention and withdrawal

All consented patients who receive transplantation and complete the 30-day follow up assessment (Study Visit 2) will be regarded as having completed the primary study. All patients will be encouraged to continue and complete the trial follow-up. The study visits

overlap with the routine hospital appointments, and all efforts will be made to ensure completeness of 12-month follow-up (Study Visit 5).
The measures to encourage patients' retention in the study include arrangements to minimise patients' time burden and extra financial costs (e.g. travelling) related to this trial. Of note, after hospital discharge following transplantation (Study Visit 1), there are no additional scheduled interventions as compared what is a standard clinical follow up).
It is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. The investigators may also withdraw a recipient from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures. We will keep all data accrued to the point of withdrawal, as is stipulated in the trial consent form.
Anonymised data collection regarding transplant centres outcome monitoring, consisting of information regarding each patient's status (alive or death), and graft function (yes or no) are required by the NHSBT, and these will continue to be recorded and used for the overall patient cohort outcome assessment.

8.7 Early discontinuation/withdrawal of participants

All patients completing the 30-day follow-up assessment will be regarded as having completed the primary study. All patients will be encouraged to complete the 12-month long-term follow-up study visit (visit 5), and all reasonable efforts will be made to ensure completeness of follow-up. Measures will include ensuring that sample collection and 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.
It is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. The investigators may also withdraw a recipient from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures. We will keep all data accrued to the point of withdrawal, as is stipulated in the trial consent form.
Possible reasons for investigator-led withdrawal of a participant from the trial include:

- Major protocol deviation
- Withdrawal of consent
- Loss to follow-up
- SAE/SUSAR

- Early termination of the study

In the event of a patient withdrawing from the trial, the reason for withdrawal will be documented on the eCRF. Such patients will be asked whether they consent to the use of ongoing data collected as standard in the national transplant registry for the purposes of this study.

All efforts will be made to report the reason for withdrawals as thoroughly as possible.

8.8. Replacement of study participants

Donor livers found not to meet eligibility criteria for participation in the trial at the time of retrieval will be withdrawn from the study and replaced. These include (livers exhibiting macroscopic evidences of advanced fibrosis, weighting >2.5kg, those who undergo functional warm ischemia time >30 minutes, or those showing any other clinical issue that according to the surgical team constitutes a contraindication to transplantation)..

Furthermore, donors randomised to NRP or HOPE in whom these interventions cannot be performed due to logistical or technical reasons (e.g. failure of the pump/circuit, failure of cannulation) will also be replaced.

Donor livers that are found not to meet the study eligibility criteria will be offered out to other centres as part of the national fast-track process. If this occurs, a surgeon to surgeon handover regarding the donor liver will occur. This will include information detailing that the liver was part of the iMAPS trial and may have had additional biopsies taken as part of this process. Of note this trial has been discussed with the NHSBT liver advisory group and presented at a British Transplantation Society meeting on organ preservation. All liver transplant centres in the UK are therefore aware of the details of the iMAPS trial.

9 Definition of end of trial

The end of the trial will be deemed to occur after database lock (following completion of monitoring of the last patient last visit). End of the trial will be defined by the database lock.

10 SAMPLE COLLECTION AND HANDLING

10.1 Research sample collection and handling for trial purposes

The trial clinical research fellow and/or a member of investigator team will be responsible for collection of all research samples perfusate and peri-operative samples as described in Table 2. For mechanistic studies, specimens will be collected at 5 key timepoints:

- After NRP/HOPE/SCS (immediately before NMP reperfusion).
- 30 minutes and 4 hours after initiating NMP
- At the end of NMP
- 30 min and 2 hours after reperfusion in the transplant recipient
- Post-transplant during patient follow-up

Liver biopsies: A total of 4 liver biopsies will obtained as part of the study (before NMP, 30 minutes and 4 hours after NMP, and 2 hours after reperfusion in the recipient). Two cores of liver tissue will be obtained at the specified timepoints employing a 16G Menghini needle (we expect to collect 15-20mg tissue in each pass). Experiments requiring liver tissue will be prioritised as follows: 1) mitochondrial enzyme activity (15 mg; to be inserted into cryotube and immediately frozen in dry ice in theatre; 2) targeted metabolomics (10mg; frozen in dry ice); 3) RNASeq, mtDNA and gDNA (5mg; preserved in RNAlater and subsequently frozen); 4) Histology (10mg; in formalin).

Collection of blood samples from recipient: in addition to the liver samples collected during the peri-transplant period, blood samples will be collected from the recipient before transplantation (at the anesthesia induction), 30 minutes after reperfusion, and on days 1, 3, 6, months 1, 3, 6 and 12. These samples will include: 60mL in EDTA tubes, 10mL serum separator tube, 10mL plasma tube.

Laboratory Manual: a laboratory manual describing the detailed procedures for sample collection, processing, storing and transportation will be provided to all investigators.

All research samples will be stored for future research and the mechanistic studies described in the study protocol. Overall, the trial ID will be used as an identifier for all stored samples. Only personnel authorised by the Chief Investigator will be responsible for the storage, access and release of these samples for analysis.

Table 3: Specimen collection for mechanistic studies

Assays	Prior NMP		NMP								Transplant procedure				Post Transplant Days 0,1,3,6 Days 30, 90,180 Year 1
			30 mins			4 hours			End NMP			Pre-Tx	Post-R			
	LT	BT	LT	Pe	Bi	LT	Pe	Bi	Pe	Bi	BT	Blood	LT (2h)	Blood (30 min & 2h)	BT (prior to BA)	Blood
Mitochondrial resp enzyme activity	x		x			x							x			
RNA Seq	x	x	x			x					x		x		x	
ScRNA Seq (3 patients per arm)	x					x							x			
Flow cytometry immune phenotyping				x			x		x							x
Cytokine analysis				x	x		x	x	x	x		x		x		x
Targeted metabolomics	x		x			x							x			
Biomarker analyses (Elisa & untargeted metabolomics)				x	x		x	x		x						
MtDNA / gDNA / cfDNA	x	x	x	x		x	x		x		x	x	x		x	x
Histology	x	x	x			x					x		x		x	

Abbreviations: Tx (transplantation), LT (liver tissue), BT (bile tissue), Pe (perfusate), Bi (bile fluid), post-R. BT will include 2 rings of 2mm distal bile duct at each time point. All liver biopsies will include 2 tissue cylinders obtained employing a 16G Menghini needle.

10.2 Standard of care sample handling

Routine blood samples taken for this study (donor and recipients) are part of standard clinical care and will be processed in local laboratories for clinical purposes as per normal protocols. For study purposes, the results of these investigations will be documented.

11 STATISTICAL METHODS

11.1 Statistical Analysis Plan (SAP)

The statistical analysis plan (SAP) will be finalised prior to first patient enrolment, and it will include a detailed technical description of the statistical analyses described in section 11.2.

11.2 Statistical analyses

Our RCT has been specifically designed to: i) allow investigation of the early molecular events eliciting IRI in human livers under controlled experimental conditions (i.e. during ex-situ NMP); ii) eliminate the confounding effects of arbitrary donor acceptance criteria, recipient heterogeneity, and liver transplant surgery; iii) establish correlations between mechanistic data, functional endpoints and relevant post-transplant clinical endpoints.
Our HYPOTHESIS is that the most effective MP strategy will be that which decreases IRI. In turn, this protection will be determined by modifying the metabolic changes induced by ischaemia that lead to ROS-induced mitochondrial damage, and/or by enhancing endogenous cellular anti-oxidant defence pathways.
Utilising available literature to identify the most promising MP regimen among the 3 strategies currently employed in the clinic is problematic, given the confounding effects of patient/donor selection, lack of standardized MP protocols, and differences in surgical technique and medical care. Our goal is to ensure that if one of the 3 MP strategies is superior, then there is a high probability that we will select it. This requires controlling type II rather than type I error. To this end, we have chosen a three-arm phase II randomized selection clinical trial design ('pick the winner' or screening design). This design is underpowered for performing formal hypothesis testing comparing clinical efficacy endpoints across the arms, but is optimal to select the most promising intervention based on

mechanistic endpoints, while reducing the unknown effects of confounders that bias most liver MP trials.

Given the study design, all analyses will be based on descriptive data without testing. Continuous variables will be summarised using means and standard deviations or medians and interquartile ranges. Categorical data will be reported as numbers and frequencies. The 3 study arms will be compared for all primary and secondary outcomes. In each group we will report baseline characteristics of donor and recipients, as well as outcomes with 95 % confidence intervals. SCS corresponds to the current standard-of-care in the recruiting sites and will constitute the control arm. HOPE and NRP are the 2 MP modalities currently employed in the clinic to improve DCD livers and will be considered as the experimental groups. We will report upon the proportion of eligible donors who are randomised, perfused and transplanted within each group.

The primary analysis will be based on the primary mechanistic endpoint, which has been selected based on literature highlighting the key role of mitochondrial complex I in the initiation of the IRI responses (9). However, given that the mechanisms of action of MP strategies have not been adequately investigated employing human donor livers suitable for transplantation, we will use the list of secondary mechanistic endpoints to support and complement the results of the primary endpoint, and to explore if they can constitute better markers of biological activity and/or safety. In addition, we will analyse the interactions between functional endpoints and mechanistic endpoints using regression models, to identify the key biological pathways contributing to tissue damage during IRI. Finally, we will analyse the relationships between the various functional, mechanistic and biomarker endpoints assessed during NMP and between these endpoints and all post-transplant parameters independently from the allocated treatment arm to identify predictors of post-transplant outcomes (this will be restricted to livers that proceed to transplantation within 12 hours of NMP).

11.3 Sample size determination

We have optimized sample size using previously published data on perfusate flavin mononucleotide (FMN) release as a marker of mitochondrial complex I inactivation (2), showing that: i) the magnitude of FMN released within 30 minutes of initiating HOPE predicts the development of liver failure (primary non-function or severe ischemic cholangiopathy) post- transplantation; and ii) the amount of FMN released after 30 minutes of NMP is 3-fold higher than after HOPE. We have employed the selection theory approach (21) and used the Centre for Clinical Research and Biostatistics software

(https://www2.ccrb.cuhk.edu.hk/stat/phase2/Randomized.htm) with the following parameters: p=0.29, D=0.35, and k=3; where p= the estimated percentage of livers with very high FMN levels after HOPE perfusion, D= difference in the percentage of livers with very high FMN between HOPE and NMP, and k= number of treatment arms. Of note, although we should expect a difference between HOPE and NMP of 0.50 (2), we have chosen a conservative effect size of 0.35. 10 livers in each arm would be required to correctly rank the 3 MP strategies with 90% probability. We will enrol in the study a total of 36 donor livers to account for the livers that do not meet viability criteria during NMP and/or for the transplanted recipients who do not complete follow-up.

11.4 Analysis populations

Being a mechanistic trial, the primary analysis will be as per protocol and include all livers that are perfused under NMP conditions for at least 4 hours. In addition, a secondary analysis including recipient outcomes will include only livers actually transplanted.

11.5 Decision Points and Stopping Rules

Data will be reviewed by the Data Safety Monitoring Committee (DMC) after the first 10 liver perfusions. If there are no safety concerns recruitment will continue as per the study protocol. There are no formal stopping rules.

12 PATIENT AND PUBLIC INVOLVEMENT (PPI)

The grant application was discussed with 3 members of the LISTEN group. The trial protocol and all patient related documents have been reviewed by the LISTEN group steering committee, composed of 6 members. PPI group has provided input into clarifying aspects of the IC and PIS that were considered not clear enough for a non-medical audience.

13 FUNDING AND SUPPLY OF EQUIPMENT

The study funding has been reviewed by the KCH R&I Office and deemed sufficient to cover the requirements of the study.

The research costs for the study have been supported by MRC grant number MR/X019470/1.

14 DATA HANDLING AND MANAGEMENT

14.1 Data management

For data collected, source data verification (SDV) worksheets will be prepared for each patient and data will be entered onto the MACRO eCRF database. The CI will act as a custodian for the data and any data queries will be raised with the trial manager or CI.
The CI will undertake, on behalf of the Sponsor, independent administrative audits of the trial master file and monitoring at all sites periodically during the trial to ensure compliance with the Medicines for Human Use (Clinical Trials) Regulations 2004 and its subsequent amendments.

Worksheets as Source Documents

Data will be entered directly onto the paper worksheets which will be considered the source document. Patients' electronic records will also be considered a source document. The source documents will be transcribed into MACRO database. The trial site will retain a copy to ensure that the PI has an independent account from the sponsor as to what has occurred during the trial at his/her site including in signed consent forms. Additional information can be found in ICH E6, section 6.4.9.
The worksheets will consist of multisource data including a standardised self-report or clinician rated tools, procedure (venepuncture and saliva collection) and data extraction from routine medical records.

14.2 Direct Access to Source Data and Documents

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g. Participants' case sheets, blood test reports, X-ray reports, histology reports etc.).

14.3 Data Handling

The CI will act as custodian for the trial data. The following guidelines will be strictly adhered to:

1. Participant data will be pseudo-anonymised.
2. All pseudo-anonymised data will be stored on a password protected computer at each NHS site.
3. All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006, The General Data protection regulation (GDPR) and the Data

Protection Act 2018 and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the KHP-CTO and/or KCH R&I Archiving Standard Operating Procedure (SOP).

15 MATERIAL/SAMPLE STORAGE

The study involves sequential measurements of immune and metabolic parameters, which will be conducted in the laboratories of the study co-investigators at KCL, UCL and University of Birmingham. The samples obtained as part of the trial will be transferred to these laboratories under the appropriate collaboration agreement, as well as the Material Transfer Agreement.

In the study, blood and liver tissue will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them. Samples will be processed, stored and disposed in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereafter.

16 PEER AND REGULATORY REVIEW

The study has been peer reviewed in accordance with the requirements outlined by KCH R&I. The Sponsor considers the procedure for obtaining funding from the Medical Research Council to be of sufficient rigour and independence to be considered an adequate peer review.

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including UK Policy Framework for Health and Social Care v3.3 07/11/17 and any subsequent amendments.

This protocol and related documents will be submitted for review to Health Research Authority (HRA) and REC (Research Ethics Committee).

The CI will submit a final report at conclusion of the trial to the KCH R&I and the REC within the timelines defined in the Regulations.

17 ADVERSE EVENTS AND INCIDENT REPORTING

17.1 Definitions of Adverse Events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant, which does not necessarily have a causal relationship with the intervention/treatment/procedure involved.
Serious Adverse Event (SAE).	Any adverse event that: • results in death, • is life-threatening*, • requires hospitalisation or prolongation of existing hospitalisation**, • results in persistent or significant disability or incapacity, or • consists of a congenital anomaly or birth defect
*A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. ** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE.	

17.2 Assessments of Adverse Events

Each adverse event will be assessed for severity, causality, seriousness and expectedness as described below.

Severity

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require further procedure; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the participant's routine, or requires further procedure, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

Causality

The assessment of relationship of adverse events to the procedure is a clinical decision based on all available information at the time of the completion of the case report form.

The following categories will be used to define the causality of the adverse event:

Category	Definition
Definitely:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Possibly	There is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the study procedure). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant events).
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the study procedure). There is another reasonable explanation for the event (e.g. the participant's clinical condition).
Not related	There is no evidence of any causal relationship.

Expectedness

Category	Definition
Expected	An adverse event which is consistent with the available information about the intervention/treatment/procedure in use in this study.
Unexpected	An adverse event which is not consistent with the available information about the intervention/treatment/procedure in use in this study*

* this includes listed events that are more frequently reported or more severe than previously reported

17.3 Procedures for recording adverse events

- All adverse events will be recorded in the CRF and medical records starting from the time the liver transplant procedure is initiated.

- All adverse events will be recorded with clinical symptoms and accompanied with a simple, brief description of the event, including dates as appropriate.

17.4 Procedures for recording and reporting Serious Adverse Events

All serious adverse events will be recorded in the medical records and the CRF.

All SAEs (except those specified in section 17.5 as not requiring reporting to the Sponsor) must be recorded on a serious adverse event (SAE) form. The PI or designated individual will complete an SAE form and the form will be emailed to both the Trial Manager and the CI within 1 working day of becoming aware of the event. The Chief Investigator will submit all SAE reports to the R&I Office (kch-tr.research@nhs.net).

Where the event is unexpected and thought to be related to the intervention/treatment/procedure this must be reported by the Investigator to the REC and Health Research Authority, using the SAE Report form for non-CTIMPs (available from the HRA website) within 15 days.

17.5 Serious Adverse Events that do not require reporting

Liver transplantation is a major surgical procedure commonly associated with clinically significant complications, including:

- Infection (chest, urine, blood, bile, wound, abdominal)
- Fluid collection (abdominal, pleural)
- Rejection
- Renal dysfunction
- Hepatic dysfunction
- Cardiac failure
- Respiratory failure
- Neurological complications (delirium, seizures)
- Clinically significant abnormal laboratory findings (leukocytosis, cytopenias, electrolyte abnormalities)

The investigator will exercise his/her medical judgment in deciding whether the frequency or severity of these events is greater than expected, in which case they will need to be reported as SAEs.

17.6 Reporting Urgent Safety Measures

If any urgent safety measures are taken the CI/ PI shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC, Health Research Authority and R&I office of the measures taken and the circumstances giving rise to those measures.

17.7 Protocol deviations and notification of protocol violations

A deviation is usually an unintended departure from the expected conduct of the study protocol/SOPs, which does not need to be reported to the sponsor. The CI will monitor protocol deviations.

A protocol violation is a breach which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the participants of the study; or

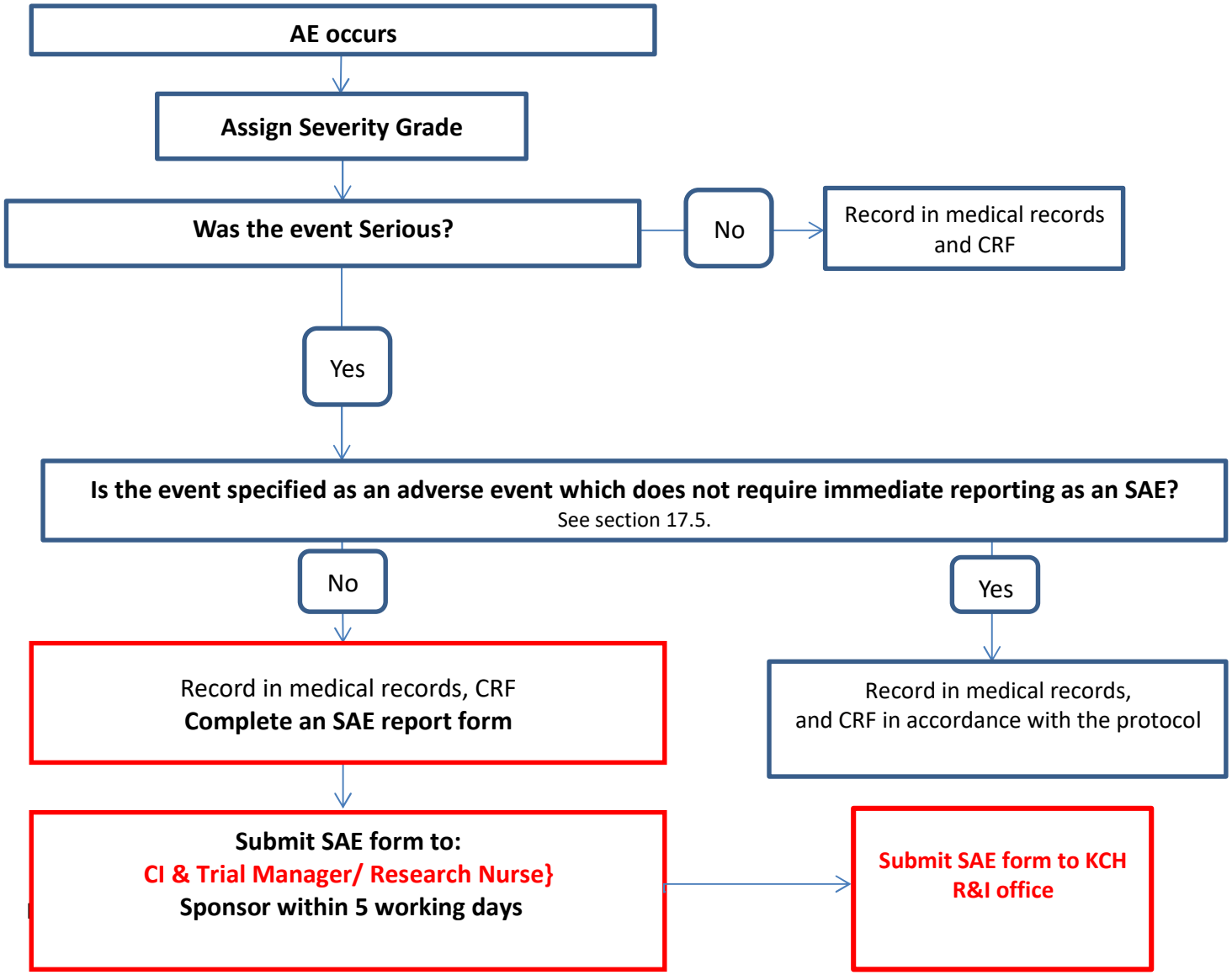
(b) the scientific value of the study.

The CI and R&I Office should be notified immediately of any case where the above definition applies during the study conduct phase.

King's College Hospital NHS
NHS Foundation Trust

KING'S College LONDON
University of London

Flow Chart for SAE reporting



17.8 Trust incidents and near misses

An incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a. It is an accident or other incident which results in injury or ill health.
b. It is contrary to specified or expected standard of patient care or service.
c. It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d. It puts the Trust in an adverse position with potential loss of reputation.
e. It puts Trust property or assets in an adverse position or at risk.

Incidents and near misses must be reported to the Trust through DATIX as soon as the individual becomes aware of them.
A reportable incident is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a) It is an accident or other incident which results in injury or ill health.
b) It is contrary to specified or expected standard of patient care or service.
c) It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d) It puts the Trust in an adverse position with potential loss of reputation.
e) It puts Trust property or assets in an adverse position or at risk of loss or damage.

18 MONITORING AND AUDITING

The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting and ensure adequate data quality.

The Chief Investigator will inform the sponsor should he/she have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

18.1 Trial committees

The following committees will be involved with the oversight of the trial:

18.1.1 Trial Management group (TMG)

A TMG comprising the CI, local principal investigators and other lead investigator, and the trial will be responsible for the day to day running and management of the trial and will meet at least once a month.

18.1.2 Trial Steering Committee (TSC)

The role of the TSC is to provide overall supervision for a project on behalf of the Project Sponsor and Project Funder and to ensure that the project is conducted to the rigorous standards set out in the Department of Health's Research Governance Framework for Health and Social Care and the Guidelines for GCP, and that there are no safety or ethical reasons why the trial should not continue.

- The TSC will operate independently from the Trial Management Group (TMG), the study Funder (Medical Research Council), and the Co-sponsors (King's College London and King's College Hospital).
- The TSC's key purpose will be to ensure the overall integrity of the study. Committee membership will be documented in the first (joint) minutes of the TSC.

Composition of the TSC:

• An Independent Chair (UK based and/or holding a substantive UK based appointment)

• Independent statistician and/or clinician(s) and any others with expertise relevant to the project

• At least one individual who is able to contribute a patient and/or wider public perspective

• Ideally, the TSC should invite observers, including a representative of the sponsor and a representative from the research network to meetings

• TSC meetings will be scheduled to follow shortly after DMC meetings so that reports from that group can be considered if appropriate

• Minutes of meetings will be sent to all members, the sponsor, and the funder and will be retained in the study master file. The responsibility for calling and organising TSC meetings lies with the CI, in association with the Chair.

18.1.3 Data Safety Monitoring Committee (DMC)

The DMCs main role is as follows:

1. It is the only body involved in a trial that has access to the unblinded comparative data
2. The role of the DMC is to monitor the trial data and make recommendations to the TSC on whether there are any ethical or safety reasons why the trial should not continue.
3. The DMC considers the need for any interim analysis advising the TSC regarding the release of data and/or information
4. The DMC may be asked by the TSC, Project Sponsor or Project Funder to consider data emerging from other related studies
5. There are also rare occasions when the DMC chair might be asked by the Project Funder to provide a confidential interim or futility analysis if serious concerns are raised about the viability of the study or if the research team are requesting significant extensions.

Independence is a key characteristic of a DMC where the committee members are completely uninvolved in the running of the trial.

Composition of the DMC

The DMC will include 2 members (one expert trial statistician and one clinician with experience in the field) who will be independent. The roles will be defined in the DMC charter. Reports to the DMC will be prepared and presented by the Trial Statistician. We will be using DAMOCLES design.

19 TRAINING

The Chief Investigator will review and provide assurances of the training and experience of all staff working on this study. Appropriate training records will be maintained in the study files.

20 INDEMNITY ARRANGEMENTS

King's College London holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that KCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. King's College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

21 ARCHIVING

At the end of this trial, all trial hard copies will be stored in a locked cupboard within the Liver R&D archive (Coldharbour Works storage room), while electronic data will be archived on the Liver Research shared Q Drive, in line with local SOPs from the Liver R&D team at KCH.

22 PUBLICATION AND DISSEMINATION POLICY

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. No patient identifiable data will be contained in any publication related to this trial.

23 REFERENCES

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24 APPENDICES

24.1 Appendix 1: PROTOCOL VERSIONS

Versions No	Version Date	Status
v0.2	23.05.2024	Superseded
v0.3	03.01.2024	Superseded
v.04	21.01.2024	Superseded
V1	14.05.2024	Superseded
V2	03.09.2024	Superseded
V3	09.04.2025	Current
V3.1	26.06.2025	Pending approval

24.2 Appendix 2: Clavien-Dindo Classification of Surgical Complications

The Clavien-Dindo Classification

The therapy used to correct a specific complication is the basis of this classification in order to rank a complication in an objective and reproducible manner.

It consists of 7 grades (I, II, IIIa, IIIb, IVa, IVb and V). The introduction of the subclasses a and b allows a contraction of the classification into 5 grades (I, II, III, IV and V) depending on the size of the population observed or the of the focus of a study.

Complications that have the potential for long-lasting disability after patient's discharge (e.g.: paralysis of a voice cord after thyroid surgery) are highlighted in the present classification by a suffix ("d" for disability). This suffix indicates that a follow-up is required to comprehensively evaluate the outcome and related long-term quality of life.

Grades	Definition
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusionsand total parenteral nutritionare also included.
Grade III	Requiring surgical, endoscopic or radiological intervention
- IIIa	Intervention not under general anesthesia
- IIIb	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)* requiring IC/ICU-management
- IVa	single organ dysfunction (including dialysis)
- IVb	multiorgandysfunction
Grade V	Death of a patient

**brain hemorrhage, ischemic stroke, subarrachnoidalbleeding,but excluding transient ischemic attacks (TIA);IC: Intermediate care; ICU: Intensive care unit.*

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Appendix 3: MEAF, L-GRAFT and UKELD scores

The UKELD score (Cholongitas E et al. Nat Rev Gast & Hepatol 2010; PMID 21045793) is calculated from the patient's INR, serum creatinine, serum bilirubin and serum sodium, according to the formula:

(5.395 x ln INR) + (1.485 x ln creatinine) + (3.13 x ln bilirubin) - (81.565 x ln Na) + 435

Units: creatinine umol/L; bilirubin uml/L; sodium mmol/L.

The MEAF score (Pareja E et al. Liver Transpl 2014; PMID 25204890) is calculated according to the following formular:

$$\text{MEAF} = (\text{score ALT}_{\text{max.3POD}} + \text{score INR}_{\text{max.3POD}} + \text{score bilirubin}_{\text{3POD}})$$

$$\text{Score ALT}_{\text{max.3POD}} = \frac{3.29}{1+e^{-1.9132}(\ln(\text{ALT}_{\text{max.3POD}})-6.1723)}$$

$$\text{Score INR}_{\text{max.3POD}} = \frac{3.29}{1+e^{-6.8204}(\ln(\text{INR}_{\text{max.3POD}})-0.6658)}$$

$$\text{Score bilirubin}_{\text{3POD}} = \frac{3.4}{1+e^{-1.8005}(\ln(\text{bilirubin}_{\text{3POD}})-1.0607)}$$

The L-GRAFT score (Agopian V et al. JAMA Surg 2018; PMID 29261831) is calculated according to the following formula:

Risk Score = 6.96 - 0.58 * (AUC log AST) + 0.008 * (AUC log AST squared) + 5.25 * (slope log AST) + 4.65 * (slope log AST squared) + 1.14 * (log AUC INR) -0.0345 * (AUC log TBIL) + 0.006 * (AUC log TBIL squared) + 4.31 * (slope log TBIL) + 5.85 * (slope log TBIL squared) -0.05* (AUC log PLT)