Perfused Liver Utilisation Study (PLUS)

Utilisation of normothermic machine preservation in extended criteria livers – a national threshold-crossing study

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Funder:	NIHR Invention for Innovation Challenge Awards (NIHR201003) Funding in-kind from OrganOx Ltd
Chief Investigator Signature:	Electronically approved by Professor Peter Friend
Date:	27th July 2024
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Date:	29th July 2024

Conflicts of Interest

Peter Friend and Constantin Coussios are co-founders and shareholders in OrganOx (a University of Oxford spinout company). They receive consultancy payments as non-executive medical and technical directors of the company.

Simon Knight, David Nasralla and Reena Ravikumar have received consultancy income from OrganOx for assisting with the design and conduct of previous trials.

Peter Friend, Constantin Coussios and Simon Knight will be not be involved in approaching, consenting, recruiting, or in the clinical management of patients in the proposed trial (the Oxford Transplant Centre is not a liver transplant unit).

Craig Marshall is CEO of OrganOx Limited, SME partner for this study. He has had no direct involvement in the design of the study, and will not be involved in the recruitment or clinical management of patients in the study.

Protocol signatures continued

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The undersigned has read and understood the trial protocol detailed above and agrees to conduct the trial in compliance with the protocol.

Principal Investigator (Please print name)	**Signature**	**Site name or ID number**	**Date**

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1. KEY TRIAL CONTACTS

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Data Monitoring Committee (DMC)	Members: • Independent Chair: James Neuberger, Retired Hepatologist • Independent Statistician: Kerri Barber, PRA Health Sciences • Independent Clinical Expert: Chris Callaghan, Guy's and St Thomas' NHS Foundation Trust
Trial Management Committee (TMG)	All lead investigators will be trial management committee members, along with a local PI from each participating site. These individuals are: • Professor Peter Friend (Chair) • Professor Simon Knight • Professor Brian Davidson • Professor Chris Watson • Professor Constantin Coussios • Mr David Nasralla • Miss Reena Ravikumar • Dr Kerrie Brusby • Mrs Helen Thomas • Professor Steve Morris • Mr David Radford • Mr Craig Marshall • Miss Barbara Fiore • Mr Ahmed Sherif • Mr Colin Wilson • Mr Joergen Pollock • Ms Rebeca Mateos • Mr Wayel Jassem

	• Mr Rohit Gaurav • In addition, the trial manager, trial data manager and trial statistician will sit on the TMG

2. LAY SUMMARY

Liver transplantation is a highly effective treatment, but the supply of suitable donor organs is greatly exceeded by the number of patients who would benefit. More than 10% of patients on the waiting list die before receiving a transplant and many others are never placed on the list because access is restricted to patients with the best chance of success.

Less than 2/3 of deceased donors in the UK result in a liver transplant, because the livers from many donors are less suitable, due to older age, medical conditions or circumstances of death, and are much more likely to cause complications. To use these higher-risk livers safely, we need to find better ways to preserve, repair and test livers so that more of the available donor organs can be transplanted without compromising the survival rate.

Normothermic machine perfusion (NMP) is a novel method of organ preservation which replaces the conventional icebox, using a machine which restores the flow of blood at body temperature allowing the liver to function during storage. This results in (i) better preservation of the liver (less injury), (ii) repair of the donor organ (reconditioning), and (iii) assessment of the organ's functional state (viability assessment).

Previous studies, from the UK and elsewhere, showed a substantial reduction in injury. They also suggested that transplant surgeons could accept higher risk organs with confidence, mainly due to the ability to assess function prior to transplant. However, these studies were not primarily designed to test the effects of NMP on organ use, and there remains a crucial need for high-quality evidence as to whether this more complex and expensive technology should become the standard of care.

The UK-designed and manufactured NMP device proposed in this study has already been used in small numbers of transplants in all of the UK's seven liver transplant units. This has mostly relied upon charitable funding, because this technology has not yet been funded by NHS commissioners.

In this study, we will identify offers of those donor livers less likely to be used and make the NMP machine available for storage and assessment. We will compare the proportion resulting in successful transplants with a group of offers where NMP was not used to see if a pre-defined threshold for increased use is met. We will also collect information about the overall cost of the new technology compared to the old, so that the NHS can decide whether NMP is good value for money.

3. SYNOPSIS

Trial Title	Utilisation of normothermic machine preservation in extended criteria livers - a national threshold-crossing study		
Internal ref. no. (or short title)	Perfused Liver Utilisation Study (PLUS)		
Trial registration	ISCRTN 11552402		
Sponsor	University of Oxford		
Funder	NIHR Invention for Innovation Challenge Awards (NIHR201003)		
Clinical Phase	Phase III		
Trial Design	Threshold-crossing design with a prospectively-defined efficacy threshold. *Control cohort (static cold storage; SCS):* A priori defined real-world control cohort meeting study inclusion criteria, identified from UK Transplant Registry held by NHS Blood and Transplant (referred to as 'the registry' throughout the protocol. *Study cohort (normothermic machine perfusion; NMP):* Prospectively identified, consecutive liver offers with a donor utilisation index (DUI) > 0.27 (appendix 3), in which NMP is made available		
Trial Participants	Extended criteria (DUI > 0.27) liver offers made to participating centres through the NHSBT offering system		
Sample Size	Control cohort: 2465 (retrospective) Study cohort: 1035 liver offers (prospectively recruited in this study)		
Planned Trial Period	Total study: 01/10/2021 – 30/09/2026 (60 months) Participant follow-up: 12 months. Long-term patient and graft survival will be assessed using registry data at 5 years post-transplant		
Planned Recruitment period	11/04/2022 – 11/10/2023 (18 months)		
	Objectives	Outcome Measures	Timepoint(s)
Primary	To assess whether NMP can increase the availability of livers for transplantation without compromising outcome	Functional utilisation – transplantation of the liver with 12-month patient and graft survival	12 months post-transplant
Secondary	To assess the cost-effectiveness of NMP in the context of NHS commissioning guidelines	Intervention costs NHS Resource Use Quality of Life	Pre-transplant, Day 7, Months 3 and 12
	To assess biochemical liver function in the study cohort	Biochemical liver function (ALT, GGT, INR, Bilirubin) Daily serum lactate (whilst on ITU/HDU) Model for Early Allograft Function score (MEAF)	Days 1-7, Month 12

	To compare graft and patient survival between study and control cohorts.	Primary non-function Graft survival (death censored) Graft survival (non-censored) Patient Survival	Day 7, Months 3 and 12. Long-term (5 year).
	To compare the use of hospital resources between study and control cohorts	Length of ITU/HDU stay Length of hospital stay Need for renal replacement therapy	Days 1-7, discharge from hospital.
	To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation.	Perfusion parameters (pressures, flows, bile production, pH, pO_2, pCO_2 blood temperature, glucose) Perfusate ALT Perfusate lactate Perfusate cell-free DNA levels (cfDNA) Post-reperfusion liver histology, including degree of macrosteatosis	During preservation (refer to section 9.7.2)
	To assess safety in the study and control cohorts	Organ discard rate Perfusate cultures Adverse outcome rates	Days 1-7, Months 3 and 12

4. ABBREVIATIONS

AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
cfDNA	Cell-free DNA
CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DLI	Donor Liver Index
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DUI	Donor Utilisation Index
EAD	Early Allograft Dysfunction
ECD	Extended Criteria Donor
EQ-5D-5L	EuroQoL 5 dimension 5 level health-related quality of life instrument
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GP	General Practitioner
GST	Glutathione S-Transferase
HD	Haemodialysis
HDF	Haemodiafiltration
HEAP	Health economics analysis plan
HF	Haemofiltration
HRA	Health Research Authority
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IFU	Instructions For Use
INR	International Normalised Ratio
IRB	Independent Review Board
ITU	Intensive Care Unit

IVC	Inferior Vena Cava
MEAF	Model for Early Allograft Function
MAP	Mean Arterial Pressure
MELD	Model for End-stage Liver Disease
NHS	National Health Service
NHSBT CTU	NHS Blood and Transplant Clinical Trials Unit
NMP	Normothermic Machine Preservation
NRP	Normothermic Regional Perfusion
PGD	Primary Graft Dysfunction
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PNF	Primary Non-Function
QALY	Quality-adjusted life year
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SDV	Source Data Verification
SNOD	Specialist Nurse for Organ Donation
SOP	Standard Operating Procedure
TMF	Trial Master File
TSC	Trial Steering Committee
UW	University of Wisconsin

5. BACKGROUND AND RATIONALE

5.1. Liver transplantation in the United Kingdom

The dominant challenge in current-day clinical organ transplantation is the shortage of deceased donor organs. Although the number of organ donors has increased by 95% over 10 years (a result of concerted public education, NHS investment and political commitment), there remains a major discrepancy between demand and supply. The liver transplant waiting list has remained largely static over the past 10 years, with 432 patients waiting at 31st March 2019 (1).

Liver disease is increasing and contributed to 13,417 deaths in England and Wales in 2017: as a result, the demand for liver transplantation is growing. Meanwhile donor livers being offered for transplant are progressively more marginal, in terms of hypoxic injury, obesity, cardiovascular disease and age: the proportion of donors with high-risk factors known to be associated with inferior outcomes and higher discard rates has increased from 8% (2007) to 39% (2018). Because of this, only 62% of deceased donors in the UK result in a liver transplant.

Around 3% of patients on the national waiting list die before a suitable donor organ becomes available. Indeed, this under-estimates the problem because access to the liver transplant waiting list is restricted to only those patients most likely to benefit. Many more patients who would nonetheless benefit from a transplant, such as some with primary or metastatic cancer, are not placed on the waiting list in order to focus scarce donor resources on patients with the best prognosis.

5.2. Utilisation of extended-criteria livers

A recent study based on UK registry data developed a Donor Liver Index (DLI) to predict graft survival from donor demographic factors (2). Recent analysis shows that livers in the upper half of DLI (> 1.61) demonstrate not only worse graft survival, but also lower utilisation than those in the lower half of DLI (19% vs 77%).

Whilst this study demonstrates a clear link between donor risk and utilisation, the DLI was not primarily intended as a score for utilisation (proportion of livers offered that are transplanted). Further analysis of NHSBT data has demonstrated factors leading to poor utilisation of DBD and DCD livers, leading to the development of a Donor Utilisation Index (DUI; Appendix 3). Many of the factors linked with poor graft survival are also associated with poor utilisation, although there are some differences and these factors differ between DBD and DCD livers. Analysis of historical data (from 01/02/2016 to 31/01/2019) demonstrates that utilisation of livers in the top 60% of DUI (represented by a DUI > 0.27) is just 26% compared with 85% for livers in the lower 40% of DUI (DUI<=0.27). These data exclude livers not eligible/suitable for NMP (donors from outside of the UK, HIV or hepatitis C positive donors, and paediatric donors).

5.3. Normothermic Machine Preservation (NMP)

Preservation of donor organs for transplantation is currently based on cooling to ice temperature with a specialist solution and storage in an ice box (static cold storage; SCS). Whilst satisfactory for organs of

optimal quality, it is a major limitation in the context of the suboptimal donor organs that form an increasing proportion of current transplant practice.

An extensive experimental programme of development of the NMP technology showed this to be a superior means of liver preservation, which also allowed accurate viability prediction by means of functional testing during perfusion (3). The first patient to receive a liver transplanted after normothermic preservation underwent surgery in 2013 (4).

This Phase 1 study was followed by a randomised controlled trial, comprising 220 transplanted livers across seven European liver transplant centres, providing a direct comparison between conventional (ice-box) static cold storage (SCS) and normothermic perfusion (5). This demonstrated a significant reduction in biochemical markers of transplant-associated liver injury (the primary endpoint of the trial and a surrogate marker of graft survival), as well as a 50% reduction in the proportion of organs that were discarded (12% versus 24%). Notably livers in the normothermic arm were preserved for 54% longer than those in the control arm, implying considerable logistic benefits. NMP was safe, with no excess of adverse events in either arm.

The OrganOx metra NMP device, used in the studies described above, is now commercially available and has been used outside the trial environment in all of the UK's seven liver transplant centres. In January 2019, NICE approval was granted for oxygenated machine perfusion of the liver to be used 'with special arrangements for clinical governance'. However, at present NMP of the liver is not routinely funded by NHS commissioners, and liver transplant units wishing to use this technology are essentially dependent upon charitable funding.

5.4. Liver utilisation with NMP

The phase III study described above provide strong circumstantial evidence that NMP increases the utilisation of high-risk donor organs. Experience during the trial suggested that surgeons were able to transplant a higher proportion of high-risk organs (i) by selecting viable organs by having access to functional parameters and (ii) because of improved viability of NMP organs. However this trial was not designed or intended to test the impact of NMP on organ discard: not least because all organs accepted for the trial had to be deemed transplantable following either method of preservation. High-risk livers that were not suitable for cold storage were excluded from the trial, although these are the very livers that may benefit the most from improved preservation and viability assessment.

Other, non-randomised, studies have since corroborated the evidence of improved organ utilisation. In a Phase-2 study from Birmingham (VITTAL), 22 of 31 perfused livers, previously declined by every UK liver transplant unit, were deemed transplantable based upon functional testing during perfusion, and were transplanted with immediate function (6). Evidence from Cambridge shows a 25% increase in overall liver transplant rate following introduction of NMP for high-risk donor organs (personal communication C Watson).

There are several mechanisms by which NMP may be able to improve organ utilisation:

- NMP allows longer preservation without detriment to organ quality. Transplantation of marginal organs currently necessitates very short storage times: transport and other logistic factors often

render this unfeasible. Transplantation of high-risk donor organs after preservation of 24 hours (allowing daytime surgery) has been shown to be feasible using NMP by several groups (7)

- Reconditioning of damaged organs during perfusion. NMP restores cellular energy following hypoxia, reducing ischaemia-reperfusion injury at the time of implantation. Similarly, NMP mitigates the severe ischaemia-reperfusion associated with transplantation of steatotic (fatty) livers (such organs are very often declined) (personal communication C Ceresa).
- NMP provides the transplant surgeon with viability data. Real-time information about the metabolic function of the liver immediately before the transplant (e.g. clearance of lactate and biochemistry of the bile produced) correlates with post-transplant performance (8). This has resulted in the successful transplantation of livers that would not otherwise have been transplanted (6).
- NMP provides the surgeon with more time. In contrast to the maximum urgency with which high-risk organs are currently transplanted in order to minimise cold preservation injury, NMP allows the final decision to transplant a high-risk organ to be deferred until more information is available.

5.5. Summary

Whilst previous studies have demonstrated efficacy and safety of NMP and suggested an increase in the proportion of livers transplanted, none have been specifically designed to quantify the impact of availability of NMP on organ utilisation. Given the additional cost and logistical burden that NMP brings over the simplicity of static cold storage, it is important to quantify the clinical and health economic impact of NMP in the NHS setting. Given the very low utilisation in extended-criteria livers with a high DUI, it would make sense to target these liver offers as the potential yield of additional transplants will be highest.

This study will achieve this by recruiting livers at the point of offering and assessing the impact of availability of NMP on the entire transplant pathway. The use of functional utilisation as a novel endpoint will assess the impact of NMP on organ utilisation and transplant rate, whilst ensuring that the additional transplants do not come at the cost of inferior clinical outcomes.

6. OBJECTIVES AND OUTCOME MEASURES

6.1. Primary Objective and Outcome Measure

Objective	Outcome Measures
To assess whether NMP can increase the availability of livers for transplantation without compromising outcome	Functional utilisation – transplantation of the liver where the patient is alive, without the need for a retransplant, 12 months postoperatively.

6.2. Secondary Objectives and Outcome Measures

Objective	Outcome Measures
To assess the cost-effectiveness of NMP in the context of NHS commissioning guidelines	1. NMP versus SCS intervention costs 2. NHS resource use, measured by documentation of inpatient and outpatient episodes and treatments at study timepoints, data collected for patients on the transplant waiting list, historically published data and hospital episode statistic (HES) data; valued using standard NHS unit costs 3. Health-related quality of life for patients on the waiting list and post-transplant, assessed by completion of the EQ-5D-5L questionnaire
To assess biochemical liver function in the study cohort	1. Daily serum bilirubin, GGT, ALT and INR at days 1-7 following transplantation. 2. Daily serum lactate at days 1-7 whilst in high level (ITU/HDU) care 3. Serum bilirubin, ALP, ALT and AST at month 12 following transplantation. 4. Model for Early Allograft Function (MEAF) score (9): a composite score based upon the maximum values of ALT, INR and bilirubin during the first 3 days postoperatively.
To compare graft and patient survival between study and control cohorts	1. Primary non-function: graft failure during the first 10 days after liver transplantation, documented by the site team as PNF with no initial function 2. Graft survival (death censored) at 7 days, 3 and 12 months and 5 years following transplantation (defined as a functioning transplant in the absence of death and re-transplantation). 3. Graft survival (non-censored) at 7 days, 3 and 12 months and 5 years following transplantation (graft loss includes death or retransplantation) 4. Patient survival at 7 days, 3 and 12 months and 5 years following transplantation.
To compare the use of postoperative hospital	1. Length of stay in high level (HDU/ITU) care post-transplant 2. Length of initial hospital stay post-transplant

resource between study and control cohorts	3. Need for renal replacement therapy (haemodialysis, haemofiltration, haemodiafiltration)
To assess the ability of perfusion parameters, biomarkers in perfusion fluids and graft histology to predict clinical outcomes following transplantation.	1. Perfusion parameters (logged automatically by the device): a. Arterial and caval pressures (in mmHg) b. Arterial, portal and caval flow rates (in L/min) c. pO_2, pCO_2 and pH d. Blood temperature (^oC), Glucose (mmol/L) and bile production (ml/h) 2. Perfusate ALT at time zero, 2 and 4 hours, and the end of NMP 3. Perfusate lactate at time zero, 15 min, 1, 2 and 4 hours, and end of NMP Note: time zero is when the liver is put onto the OrganOx metra 4. Perfusate cell-free DNA (cfDNA) levels during perfusion at 2 and 4 hours 5. Graft histology, including degree of macrosteatosis, following reperfusion
To assess safety in the study and control cohorts	1. Organ discard rate 2. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture (NMP livers only). 3. Adverse outcome rates. Severity will be graded according to the Clavien-Dindo classification for any reported as SAEs for the study cohort (10) a. Recipient infection b. Biopsy proven rejection c. Biliary complications (biliary strictures or bile duct leaks) requiring intervention d. Vascular complications requiring intervention (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis) e. Reoperation rate

7. TRIAL DESIGN

Whilst a randomised-controlled trial (RCT) design is the gold standard for comparing interventions, current use of machine perfusion technologies mean that the majority of centres would not consider randomising certain groups of high-risk livers to static cold storage. Given this loss of equipoise, an RCT would be considered unethical and unlikely to successfully recruit. Despite this, the effect of NMP on utilisation and overall cost remains unknown and further evidence is required to determine if the technology should be routinely funded.

In order to determine the effect of routine availability of NMP on the utilisation of these livers, the current study will employ a "threshold-crossing" design as described by Eichler et al. (11). A real-world control cohort will be identified *a priori* from consecutive liver offers in the NHSBT transplant registry meeting trial inclusion criteria to determine functional utilisation where normothermic machine preservation was not routinely available. Using these data, a threshold will be set above which availability of NMP would be considered to have a significant clinical impact on utilisation. Once defined, a single-arm prospective trial will recruit participants with NMP routinely available, and analysis performed to see if the pre-defined threshold is crossed in the study cohort.

Definition of the control cohort and threshold in advance of recruitment minimises the risk of bias in selecting controls. Use of identical inclusion criteria in both study and control cohorts will ensure comparability of groups, which will also be confirmed in a secondary propensity-matched analysis.

Identification of eligible liver offers will be undertaken by the NHS Blood and Transplant (NHSBT) Hub Operations at the point of organ offer, before the recipient surgeon has any clinical information about the donor or liver being offered. This allows assessment of the impact of NMP on the whole of the transplant pathway including organ acceptance, assessment and subsequent transplantation.

All seven UK liver transplant centres will participate in the study. Upon identification of an eligible liver, the recipient centre will decide whether to take the NMP device to the donor hospital (device-to-donor), or to transport the liver under SCS and perfuse it on arrival at the implant centre (back-to-base). In either case, the implanting surgeon will make a decision whether to transplant based upon all available information (donor characteristics, organ appearance, histology and NMP parameters).

Liver offers that are declined by the initial centre will follow the existing NHSBT offering pathway. Inclusion in the study will be maintained when the liver is offered to subsequent transplant centres. If the accepting centre is unable to follow the study protocol because the intervention is not available, the liver will be excluded from modified intent-to-treat analysis.

Transplant recipients in the study cohort will be consented for use of their data and followed for 12 months post-transplant. Data collection timepoints will be pre-transplant, and at days 1-7, months 3 and 12 post-transplant. All study assessments and investigations are part of standard care other than quality of life questionnaires. Long-term patient and graft survival will be assessed using registry data at 5 years post-transplant.

Some eligibility criteria cannot be assessed until after recruitment, when the liver recipient and transplant type are known. In these cases (where patients are aged <18 years, have not agreed to use of NMP according to local consent policy, or do not consent to use of their data, or for split liver, multi-organ or non-UK transplants) the recipient will not be included in the study and the liver will be transplanted into

the recipient as per standard care. If preservation has started prior to the consent process, the liver will continue to be preserved in the same manner until transplant. If preservation has not yet commenced, the liver will be preserved according to usual care in that centre.

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

Primary outcomes will be analysed 12 months following enrolment of the last liver offer to the study. Participant involvement in the study will complete at the last follow-up assessment of the last participant, 12 months post transplant, and the study will close. Collection of 5-year survival outcomes (beyond the end of the study) will be from the registry.

Anticipated flow of liver offers through the trial is depicted in *Appendix 1*.

8. STUDY COHORTS

8.1. Real world control (RWC) cohort

The real-world control cohort was defined in advance of recruitment of the prospective study cohort in order to minimise risk of bias in selection, and to allow the pre-defined efficacy threshold to be set.

All consecutive liver offers meeting the study inclusion and exclusion criteria defined in sections 8.3 and 8.4 between 1st January 2018 and 31st December 2019 were identified in the NHSBT transplant registry. This time period is contemporary enough to ensure stable utilisation over time, but excludes livers offered during the peaks of the coronavirus pandemic in the UK where offering and utilisation were affected by hospital capacity.

Broadly speaking, all livers with a donor utilisation index (DUI) greater than 0.27 from donors aged 16 years or over, offered to the seven participating UK liver transplant units, were eligible for inclusion. Those originating from or transplanted outside of the UK, or from Hepatitis C or HIV positive donors, were excluded. Livers that were split or reduced, that were transplanted as multi-organ transplants or where the recipient was aged <18 years were also excluded. Donor Utilisation Index was calculated using the formulae shown in Appendix 3, which differ for DBD and DCD donors. The DUI is a clinical risk score using eleven (DBD) or seventeen (DCD) donor demographic variables to predict graft utilisation. Recent analysis of UK donor data suggests that a cut-off of 0.27 represents the most poorly-utilised 60% of livers offered.

The RWC cohort included 2465 liver offers that met all these inclusion and exclusion criteria. Records maintained by the seven participating transplant centres allowed the identification of 118 livers from this cohort (5%) in which any form of *ex-vivo* machine preservation was used (including NMP and hypothermic machine preservation). These livers had lower DUI than the rest, and so excluding them would bias the cohort by excluding some lower risk livers. Instead, these livers remained in the control cohort, but their outcomes were imputed to match those of other livers that were retrieved for transplantation, which had a more similar risk profile. The remaining transplanted livers in the RWC cohort would therefore have been retrieved, preserved and transported according to NHSBT national protocols. This includes placement in ice-cold perfusion solution on the back-table, and subsequently stored in cold perfusion solution within an icebox. The organ will have been transported to the recipient centre and removed from storage prior to implantation for standard back-table preparation. The duration of cold storage will have been dictated by logistics and local policy.

8.2. Study cohort

The study cohort will be prospectively recruited in a single-arm trial in the seven participating transplant centres. Inclusion and exclusion criteria will be identical to those used to define the RWC cohort, as defined in sections 8.3 and 8.4.

All eligible offers to the participating centres will be identified (by means of DUI calculation) by NHSBT Hub Operations, and eligibility for the study confirmed with the offer when made to the centres. All consecutive offers meeting the criteria will be included and outcomes analysed, other than where NMP is not available in the accepting centre for logistical reasons (eg. Lack of appropriate personnel or device availability) In these cases, the liver will be excluded from modified intent-to-treat analysis.

Full details of recruitment procedures are defined in section 9.

8.3. Inclusion Criteria

Liver offers:

- Deceased organ donors aged 16 years or over
- Offered through the national offering scheme
- Donor Utilisation Index (DUI) greater than 0.27

Liver transplant recipients:

- Recipients 18 years of age or above
- Elective and super-urgent
- Participant is willing and able to give informed consent for participation in the study

Livers undergoing normothermic regional perfusion (NRP) will be included in the study and use of NRP corrected for in analysis.

8.4. Exclusion Criteria

Liver offers:

- Donors falling outside national offering scheme
- Donors from outside of the UK
- Donor is HIV or hepatitis C positive
- Donor Utilisation Index ≤ 0.27
- Donor not DBD or DCD
- Donors aged < 16 years
- Livers undergoing any other form of ex-situ machine preservation
- Participating centre cannot offer NMP due to logistical reasons (eg. Lack of appropriate personnel or device availability)

Liver transplant recipients:

- Have not agreed to use of NMP according to local consent policy
- Receipt of a split liver or reduced liver transplant
- Receipt of a multi-organ transplant
- Transplanted outside of the 7 participating centres

9. TRIAL PROCEDURES (STUDY COHORT)

All information in this section relates to the prospective study cohort only.

All trial procedures are summarised in *Appendix 2 – Schedule of procedures.*

9.1. Recruitment

All UK liver offers meeting the inclusion criteria will be eligible for consideration. Offers are managed by NHS Blood and Transplant Hub Operations using the electronic offering system (EOS). Potential donors are identified by the donor hospital ITU 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 offering will follow standard NHSBT policy, and offering will not be altered in any way by participation in the study (12).

All seven UK liver transplant centres will participate in the study:

- Addenbrooke's Hospital, Cambridge, UK
- King's College Hospital, London, UK
- Queen Elizabeth Hospital, Birmingham, UK
- Royal Free Hospital, London, UK
- Edinburgh Royal Infirmary, Edinburgh, UK
- St James's University Hospital, Leeds, UK
- Freeman Hospital, Newcastle, UK

9.2. Screening and Eligibility Assessment

NHSBT Hub Operations will initiate a matching run as per standard procedure. In running this, a tool is automatically run in the background which will calculate the Donor Utilisation Index (DUI) from donor variables documented in the EOS system (as described in Appendix 3).

Where a liver is eligible for inclusion in the study, the matching run will display a message to the Hub Operations team directing Hub Operations to communicate eligibility of the liver when offering. The matching run itself, offering and transplantation will follow standard practice.

9.3. Organ offering

Following screening, Hub Operations will offer through the matching run as per standard practice. The top ranked recipient transplant centre will be informed that the offer is eligible to be included in the study and that NMP is available.

If the liver is accepted, the liver will be retrieved by the NORS team as per standard NORS guidelines (13). If the accepting centre wish to perfuse the liver at the donor hospital ("device-to-donor"), the accepting centre will be responsible for providing personnel and transport for the device. Otherwise, the liver will be transported to the recipient hospital under static cold storage, and on arrival at the recipient centre, the organ will be preserved by NMP.

If the initial centre refuses the offer, the offer will be passed down the matching run to other centres as per standard liver offering policy. Each offer will include details of study inclusion. If accepted, the organ will remain in the study and preservation in the accepting centre will follow study protocols (utilising NMP where feasible). If all centres decline an organ, it will be marked as a discard.

9.3.1. Reoffering

If a liver is accepted by a centre and then subsequently declined for any reason (organ or recipient), it will continue down the offering chain as per normal NHSBT practice. Every attempt will be to maintain the use of NMP where feasible.

If a liver had already commenced NMP in the initial accepting centre and is subsequently reoffered, the liver will remain on the NMP device where possible and both the device and liver will be transported to the accepting implant centre. The accepting centre will be responsible for arranging transport and sending a trained member of staff to accept handover and supervise the perfusion during transport. A replacement device will be delivered to the original centre as soon as is possible.

9.4. Crossovers

There may be circumstances where it is not possible to undertake NMP, such as:

- Unforeseen device issues or lack of trained operating staff
- Anatomical variation making cannulation of the liver for NMP impossible
- Unavailability of NMP due to lack of resources/staff

In these circumstances, the liver will cross over to static cold storage, and the reason for crossover will be documented.

Where a liver cannot be perfused for logistical reasons (e.g. lack of device or staff), it will be excluded from analysis. Otherwise, all livers will be analysed in the study group (modified intent-to-treat).

9.5. Informed Consent

9.5.1. Patients with capacity

All seven participating centres have an OrganOx metra NMP device available for routine clinical use. Patients in all centres are informed of different methods of preservation at the time of listing and/or explicitly consented for the use of preservation technologies. Therefore, as part of standard care recipients may receive a liver preserved by either NMP or SCS. Currently less than 10% of livers in the UK undergo NMP. A record will be made in the study recruitment log of patients who have not agreed to the use of NMP and these patients will not be invited to take part in the study.

The proposed prospective study recruits offered livers, rather than patients. As recruitment occurs before the eventual recipient has been identified, consent of recipients prior to recruitment and preservation of the liver will not be possible. Study information will be provided to all patients on the transplant waiting lists in participating centres, and patients will be informed that either of the preservation methods in use in their centre may be employed.

When the patient is admitted to the recipient centre, they will be informed that the organ offered to them is part of the study and consent will be sought for their participation in the study. The participant must personally sign and date the latest approved version of the Informed Consent form before any trial data are collected.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: (i) the exact nature of the trial; (ii) what it will involve for the participant; (iii) the implications and constraints of the protocol; (iv) the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as possible to consider the information, and the opportunity to question the Investigator or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of dated signatures of the participant and the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief or Local Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be placed in the patient notes, and a copy retained at the trial site.

If a patient refuses consent for use of data, the liver will be stored as per usual centre practice and the recipient will not be enrolled in the study. If preservation has commenced, perfusion will continue. The liver will then be transplanted to the patient and the recipient will not be enrolled in the study.

Whether or not a patient agrees to participate in the study does not affect organ offering or the chances of receiving a transplant in any way. Once a liver has been offered to the recipient, this offer will be maintained unless the recipient surgeon feels that the liver is not suitable for transplant or the recipient is not medically fit to undergo the procedure.

In circumstances where there is insufficient time for the recipient to consider participation in the study prior to transplant, consent may be deferred to after the transplant procedure. In these cases, no data will be collected until after informed consent has been provided.

Obtaining informed consent post-transplant may not be feasible in-person due to PLUS liver transplant recipients being discharged back to their local referring specialists, for example. In this situation, the approach can be made by telephone and the written study information/Informed Consent Form (ICF) will be provided via post or email. In order to allow sufficient time for considering participation in the study, this initial approach will be followed up by a further phone call or clinic visit at which point a consent discussion will take place and consent will be requested. If followed-up by phone call, the patient will be asked to confirm consent by signing the ICF and posting or emailing the signed ICF back to the site team. In cases where email is used, the patient will be requested to scan/photograph and send the signed ICF back by replying to an email sent by the local site team/consenter.Guidance on obtaining remote consent is provided in the PLUS Remote Consent Process Guidance document.

9.5.2. Patients lacking capacity

Where the recipient lacks the capacity to provide consent (e.g. a super-urgent recipient on the ITU), which is anticipated in a minority of participants in the trial, guidance is provided by the Mental Capacity Act (England and Wales; 2005) and Adults with Incapacity Act (AWI) (Scotland; 2000).

In England, attempts will be made to consult a person who knows the recipient well (e.g. a family member) to advise on participation on the recipient's behalf, in accordance with the Mental Capacity Act 2005. The consultee will be provided with study information and asked to indicate the recipient's likely wishes. If the

consultee refuses research participation, the liver will be stored as per usual centre practice and the recipient will not be enrolled in the study. If preservation has commenced, perfusion will continue. The liver will then be transplanted to the patient and the recipient will not be enrolled in the study.

In Scotland, if the patient has incapacity the relative/welfare attorney will be approached, and consent requested. All patients who had mental incapacity initially will be approached for consent to remain in the trial at the earliest opportunity once they regain capacity. If a patient (or Guardian/welfare attorney/closest family member) refuses research participation, the patient will not be enrolled in the trial according to Scottish legislation. The liver will be stored as per usual centre practice and the recipient will not be enrolled in the study. If preservation has commenced, perfusion will continue. The liver will then be transplanted to the patient and the recipient will not be enrolled in the study. If the Guardian/welfare attorney/closest family member has provided the consent to participate, but the patient subsequently dies or does not regain mental capacity before the end of the follow up period, patients will be included in the analysis based on the consent provided by the Guardian/welfare attorney/closest family member.

Ultimately, the interests of the person have greater importance than any potential benefits to others/research.

Following transplant, every attempt will be made to obtain retrospective consent from the recipient themselves once capacity has been regained. If the patient regains capacity after transplant but decides they do not wish to remain in the trial, we will use the data collected up to that point in the analysis based on the consent provided by the relative/welfare attorney unless explicitly refused by the participant.

9.5.3. Patients who lack understanding of verbal or written English

Patients and parents/carers with an insufficient understanding of the English language will not be approached to discuss trial participation unless there are adequate arrangements at the site for translation or interpretation of the trial documents. The research team is unable to cover the cost of translation due to financial constraints. However, most participating sites will make use of translation services for communication and procedure consent and use of these services is permissible if feasible.

9.5.4. Samples for central analysis

Biological samples (perfusate and liver biopsy) will be obtained to be analysed centrally to allow assessment of potential viability markers. Optional consent from the recipient will be obtained to specifically address the collection and transfer of these perfusate and histological specimens, and for their use in future studies. Refusal to consent for the storage of these samples will not preclude inclusion in the trial.

9.6. Baseline Assessments

9.6.1. Donor Demographics

Donor demographics are routinely collected in the registry and will include the following:

- Age
- Sex
- Ethnic origin

- Cause of death (CVA, hypoxia, trauma, other)
- Type of donor (DBD, DCD)
- Donor height
- Donor weight
- Donor smoking history
- Last and peak serum AST
- Last and peak serum ALT
- Last and peak serum bilirubin
- Last and peak serum sodium
- Last and peak GGT
- Length of ITU stay
- Donor Utilisation Index (DUI)

9.6.2. Recipient Demographics

Recipient demographics that are routinely recorded in the registry will include the following:

- Age
- Sex
- Aetiology of liver disease
- Indication for transplant
- Pre-transplant INR
- Pre-transplant creatinine
- Pre-transplant bilirubin
- Pre-transplant sodium
- Weight
- Height

A baseline assessment of quality of life using the EQ-5D-5L will be performed prior to transplant, on admission to hospital, for the study cohort (*Appendix 4)*.

9.7. Intervention

9.7.1. Normothermic machine preservation

It is the decision of the recipient centre as to whether the device is transported to the donor hospital (device-to-donor) or the liver is transported under SCS to the implanting centre prior to NMP (back-to-base). If the centre wishes to use the device-to-donor model, they will be responsible for arranging transport and staff to ensure safe preparation of the liver, perfusion and transport.

Whichever model is used, the recipient surgical team will request 3 units of donor-type (or O-negative) red blood cells to be cross-matched at the centre where NMP is to be initiated. The liver will be placed in ice-cold perfusion solution (according to local protocol) on the back-table and prepared for cannulation. The procedure for preparing the device for use and placing the organ on the device is described in detail in the instructions for use (IFU) document (L300-0437ReV1.0 RoW Version 25/09/2017). The duration of

machine perfusion will be dictated by logistics and local policy but should not be less than 4 hours or more than 24 hours.

The procedure for removing the liver from the device is also described in the IFU. Implantation and reperfusion of the liver proceed as per the usual practice of the implanting centre.

If cannulation proves impossible, the liver will be preserved using standard static cold storage as described below and transplanted as soon as logistically feasible. Results will be analysed in the study group (modified intention-to-treat).

9.7.2. Recording of operative and perfusion parameters

The retrieving and implanting research teams will record the following data:

Donor timings

These are all routinely collected at the time of retrieval and will be obtained from the NHSBT database.

The parameters to be recorded include:

- Timings:
    - Withdrawal of support (DCD donors only)
    - Onset of functional warm ischaemia (DCD donors only)
    - Cessation of donor circulation (asystole in DCD donors)
    - Start of cold perfusion
    - Liver removal from the donor (hepatectomy)
    - Liver packed for transport time
- Perfusion solution used for aortic perfusion
- Perfusion solution used for storage and transport
- Degree of steatosis (graded mild, moderate, severe) – surgeon's assessment
- Quality of *in-situ* perfusion (graded poor, moderate, good)

Preservation parameters

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

- Time of initiation of normothermic machine preservation
- Time of cessation of normothermic machine preservation (cold flush)
- Cold flush solution (UW, HTK, other)
- Perfusion parameters (logged automatically by the device):
    - Arterial, and caval pressures (in mmHg)
    - Arterial, portal and caval flow rates (in L/min)
    - pO_2, pCO_2 and pH
    - Blood temperature (^oC), Glucose (mmol/L) and bile production (ml/h)
- Perfusate biochemistry
    - Perfusate lactate at time 0, 15 minutes, 1, 2 and 4 hours and the end of NMP
    - Perfusate ALT at time 0, 2 and 4 hours and end of NMP

- Glucose levels at time 0, 2 and 4 hours and at the end of NMP
- Bile pH and glucose at 2 and 4 hours and at the end of NMP
- Bicarbonate use (time and dose of each bolus)

Note: time zero is when the liver is put onto the OrganOx metra

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

Perfusate samples

Optional perfusate samples will be collected at 2 and 4 hours and at the end of perfusion. 2 samples will be taken at each timepoint:

- 1x EDTA 6 ml separator tube
- 1x Serum 6ml separator tube

To ensure minimal sample degradation and pre analytical variability, perfusate samples should be kept at room temperature prior to separation of plasma from cellular parts. Separation of cells from plasma and serum should be achieved by centrifugation at 1500g for 10 min at room temperature as close as possible to blood collection. After centrifugation plasma and serum samples should kept at 4°C.

These samples will be transported to the Oxford Radcliffe Bioresource using logistical arrangements already in place in all centres for the Quality in Organ Donation (QUOD) biobanking project. They will be analysed centrally for biomarkers for organ viability, including cell-free DNA levels.

Recipient Operative parameters

The parameters routinely collected from the registry will include:

- Cold ischaemic time
- Veno-venous bypass time
- Operative reperfusion time
- Intraoperative transfusion of blood products measured in units.

Additionally, data that will be collected as part of the study will include:

- Total operative time: defined as time from knife-to-skin to skin closure.
- Time of removal from ice (recipient operation)
- Portal reperfusion time
- Arterial reperfusion time
- Type of caval anastomosis (standard end-end, piggyback (end-side or side-side))

Post-reperfusion biopsy

Optional liver biopsy will be obtained following reperfusion in the recipient, prior to skin closure. Biopsy segments will be divided into two and stored in pre-prepared formalin or RNAlater tubes. Samples stored in RNAlater will be frozen in liquid nitrogen and stored at -80°C for long-term storage. These samples will be transported to the Oxford Radcliffe Bioresource using logistical arrangements already in place in all centres for the Quality in Organ Donation (QUOD) biobanking project. They will be used to assess for the ability of graft histology to determine viability and post-transplant outcome, in particular the degree of macrosteatosis.

Declines and discards

If a decision is made to decline an organ at any point after retrieval, any donor and preservation data recorded will be kept, and the reason for decline clearly documented in the eCRF. If deemed appropriate by the OTDT Hub, the organ may be offered to other centres on the matching run, maintaining the method of preservation.

If all centres subsequently decline an organ, the organ will be documented as a discard and it will be offered for research or disposed of as per standard procedures.

9.7.3. Concomitant care

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

9.8. Subsequent Follow-up

9.8.1. Inpatient stay

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

Outcome assessment

All investigations while an inpatient are performed as part of routine clinical care. No additional procedures are required as part of the study protocol while participants are inpatients.

The following biochemical outcomes will be collected as part of the study:

- Daily serum samples for the first 7 days post-transplant, to include:
    - Serum bilirubin (measured in µmol/l)
    - Serum gamma-glutamyl transferase (GGT; measured in IU/L)
    - Serum alanine transaminase (ALT; measured in IU/L)
    - International normalised ratio (INR)
- Daily serum lactate (measured in mmol/L) whilst on ITU/HDU.

The first measurements should be taken at 12 to 24 hours post-transplant. For subsequent measurements, in the event that more than one measurement is taken in a 24 hour period, the measurement taken closest to the specified follow-up time-point should be used.

Other outcomes include data routinely collected in the registry immediately post-transplant:

- Length of stay in ITU (days)
- Number of days ventilated
- Total length of hospital stay (days)
- Requirement for renal replacement therapy during transplant admission:
    - Transient renal filtration

    - Short-term dialysis
    - Long-term dialysis
- Graft and patient survival at day 7 post-transplant
- Primary non-function: graft failure during the first 10 days after liver transplantation, documented by the site team as PNF with no initial function, as documented in the transplant registry.

Safety outcomes

These safety outcomes are data routinely collected in the registry immediately post-transplant:

- Recipient infection (defined as both clinically diagnosed treated infection and infection with a positive microbiological culture result):
    - CMV infection
    - Fungal infection
    - Postoperative sepsis and site
- Biopsy-proven rejection episodes
- Biliary complications
    - Biliary tract strictures requiring intervention - anastomotic and non-anastomotic. Defined as those requiring surgical or radiological intervention
    - Bile tract leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Haemorrhage. Defined as bleeding requiring reoperation.
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - IVC/hepatic vein occlusion. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
- Reoperation rate

Severity for any adverse events reported as SAEs will be graded according to the Clavien-Dindo classification (10) – *Appendix 5*.

Immunosuppression

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

9.8.2. Month 3

For efficiency, the majority of 3-month data will be collected from the NHSBT transplant registry. As the only available outcome data for the RWC cohort will come from the registry, this will also ensure comparability between the control and study cohorts.

In addition to clinical data, patients will be asked to complete the EQ5D-5L quality of life questionnaire at 3 months. The quality of life questionnaire (EQ-5D-5L) will be delivered either during a routine hospital visit, or remotely via telephone or internet.

Outcome assessment

Outcomes to be included from routinely collected data in the registry:

- Graft and patient survival at month 3 post-transplant
- Lifestyle activity score (see *Appendix 6*)

Outcomes to be collected and which are not routinely collected data in the registry:

- Healthcare resource use (including days in ITU, days ventilated and length of initial hospital stay). Hospital Episode Statistic (HES) data linkage will be used as a backup to verify resource use.
- Quality of life (ED5D-5L questionnaire).

Safety outcomes

Safety outcomes that are not part of routine registry collection and will be collected for the study:

- Biopsy-proven rejection episodes and treatment
- Biliary complications
    - Biliary strictures – anastomotic and non-anastomotic. Both radiologically diagnosed and clinically apparent strictures
    - Bile duct leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Haemorrhage. Defined as bleeding requiring reoperation
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - IVC/Hepatic vein occlusion. Defined as venous occlusion resulting in graft dysfunction or loss, or requiring radiological or surgical intervention.
- Reoperation rate

Immunosuppression

Details of maintenance immunosuppression at 3 months post-transplant will be based on data routinely collected by the registry.

9.8.3. Month 12

For efficiency, most 12-month data will be collected from the NHSBT transplant registry. As the only available outcome data for the RWC cohort will come from the registry, this will also ensure comparability between the control and study cohorts.

Some additional 12-month data will be collected at a routine clinic visit. This will collect more detailed information on complications, including biliary complications and vascular complications, than is

documented in the registry. The data collected at the 12 month time point are to supplement the data collected at during the inpatient stay listed in section 9.8.1.

In addition, patients will be asked to complete an ED5D-5L questionnaire to assess quality of life. The quality of life questionnaire (EQ-5D-5L) will be delivered either during a routine hospital visit, or remotely via telephone or internet.

Outcome assessment

Outcomes to be recorded include in addition to data recorded in registry:

- Graft and patient survival at month 12 post-transplant
- Healthcare resource use in past 12 months (including inpatient days, days in ITU/HDU). Hospital Episode Statistic (HES) data linkage will be used as a backup to verify resource use.
- Quality of life (EQ5D-5L)

The following outcomes are included from routinely collected data in the registry at month 12 post-transplant:

- Number of readmissions in past 12 months (and reasons for readmission)
- Presence of transplant-related renal dysfunction
- Lifestyle activity score (see *Appendix 6*)
- Serum bilirubin (measured in µmol/l)
- Serum alanine transaminase (ALT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L)
- Serum alkaline phosphatase (ALP; measured in IU/L)
- Blood urea (mmol/L)
- Serum creatinine (µmol/l)

Safety outcomes

Safety outcomes that are not part of routine registry collection and will be collected for the study:

- Biopsy-proven rejection episodes and treatment
- Biliary complications
    - Biliary strictures – anastomotic and non-anastomotic. Both radiologically diagnosed and clinically apparent strictures
    - Bile duct leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Haemorrhage. Defined as bleeding requiring reoperation
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - IVC/Hepatic vein occlusion. Defined as venous occlusion resulting in graft dysfunction or loss, or requiring radiological or surgical intervention.
- Reoperation rate

Immunosuppression

Details of maintenance immunosuppression at 12 months post-transplant will be based on data routinely collected by the registry.

9.8.4. 5-year outcomes

Whilst the end-point for trial participation will be 12 months, patients will also be consented for ongoing follow-up by linkage to outcomes recorded by NHS Digital and in the UK Transplant Registry. Registry data will be used to collect:

- Patient survival at 5 years post-transplant.
- Graft survival (death censored) at 5 years post-transplant (defined as a functioning transplant in the absence of death and re-transplantation).
- Graft survival (non-censored) at 5 years post-transplant (graft loss includes death or re-transplantation)

9.9. Sample Handling

9.9.1. Routine sampling

All samples taken as part of routine care (donor and recipients) will be processed in local laboratories for clinical purposes as per normal protocols. For study purposes, the results of these investigations will be documented. These samples will be handled as per routine clinical practice.

9.9.2. Samples for central analyses

Optional perfusate samples and liver biopsy for central analyses will be collected in accordance with national regulations and requirements including standard operating procedures for logistics and infrastructure. Samples will be taken in appropriately licensed premises, stored and transported in accordance with the HTA guidelines and local trust policies. Samples for central analysis will be kept in the Oxford Radcliffe bioresource. The stored tissues will be held under an extension of the University of Oxford's HTA license (12217). Additional (optional) consent will be sought for long-term storage of these samples beyond the end of the study for use in future studies. Where consent is obtained, these samples will be stored in the Oxford Radcliffe bioresource under an extension of the University of Oxford's HTA license (12217). Where no consent for long-term storage is obtained, the samples will be destroyed at the end of the study.

9.10. Early Discontinuation/Withdrawal of Participants

All patients completing the 12-month follow-up assessment will be regarded as having completed the study. All patients will be encouraged to complete study follow-up, with letters sent to encourage completion of quality-of-life questionnaires. All reasonable efforts will be made to ensure completeness of follow-up, with central monitoring of registry returns and reminders to centres for timely submission.

Measures include ensuring that assessments are made at routine hospital visits, 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:

- Ineligibility either arising during the trial or retrospectively having been unknown/overlooked at screening
- Withdrawal of consent
- Loss to follow-up

In the event of a patient withdrawing from the trial, the reason for withdrawal must 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. If they do not consent to use of data collected, this will not be used in analysis.

9.11. Definition of End of Trial

Data will be collected from participants for 12 months post-transplant. Once all data from all participants has been collated, entered and cleaned, the database will be locked and the study will end. Longer-term (5-year) follow-up data (beyond the end of the study) will be collected from the UKTR.

10. DATA COLLECTION FOR RETROSPECTIVE CONTROL COHORT

10.1. Description of data

Outcome data for the retrospective control cohort will all be collected from the National Transplant Registry in an anonymised fashion. Outcomes will mirror those collected for the prospective study cohort, where possible, to allow direct comparison between cohorts. The data fields of the National Transplant Registry that are accessed for the purpose of this study are detailed in the PLUS Data Sources document. Registry data are collected at the time of registration, transplant, month 3 and month 12 post-transplant. Donor data are also available from the registry. These data will be used as the basis for the following assessments.

10.2. Baseline Assessments

10.2.1. Donor and Recipient Demographics

Donor and recipient demographics will be identical to those collected for the study cohort, as described in section 9.6.1. and 9.6.2.

10.3. Operative data

Donor timings

These are all routinely collected at the time of retrieval and will be obtained from the NHSBT database.

The parameters to be recorded include the same subset of parameters recorded for the prospective cohort described in 9.7.2 :

- Timings:
    - Withdrawal of support (DCD donors only)
    - Onset of functional warm ischaemia (DCD donors only)
    - Cessation of donor circulation (asystole in DCD donors)
    - Start of cold perfusion
    - Liver removal from the donor (hepatectomy)
    - Liver packed for transport time
- Perfusion solution used for aortic perfusion
- Perfusion solution used for storage and transport
- Degree of steatosis (graded mild, moderate, severe) – surgeon's assessment
- Quality of *in-situ* perfusion (graded poor, moderate, good)

Operative parameters

These will include the same subset of parameters collected for the prospective cohort and described in 9.7.2 :

- Cold ischaemic time
- Veno-venous bypass time
- Operative reperfusion time
- Intraoperative transfusion of blood products measured in units.

10.4. Subsequent Follow-up

10.4.1. Month 3 data

Immediate post-transplant data that are also collected in the same way for the prospective cohort:

- Length of ITU stay (days)
- Number of days ventilated
- Total length of hospital stay (days)
- Requirement for renal replacement therapy during transplant admission
    - Transient filtration
    - Short-term dialysis
    - Long-term dialysis
- Graft and patient survival at day 7 post-transplant
- Primary non-function: graft failure during the first 10 days after liver transplantation, documented by the site team as PNF with no initial function, as documented in the registry.

Inpatient Safety Outcomes as recorded in the registry:

- Recipient infection (CMV infection, fungal infection, post-operative sepsis)
- Biliary complications
    - Biliary tract strictures requiring intervention
    - Bile tract leaks requiring intervention.
- Vascular complications
    - Haemorrhage. Defined as bleeding requiring reoperation
    - Hepatic artery thrombosis
    - Portal vein thrombosis.
    - IVC/Hepatic vein occlusion.

Month 3 outcomes:

- Graft and patient survival at month 3 post-transplant
- Lifestyle activity score (see *Appendix 6*)

Immunosuppression

Details of maintenance immunosuppression at 3 months post-transplant will be included as recorded in the registry.

10.4.2. Month 12 data

Outcomes to be collected include:

- Graft and patient survival at month 12 post-transplant
- Number of readmissions in past 12 months (and reasons for readmission)
- Presence of transplant-related renal dysfunction
- Healthcare resource use in past 12 months (including inpatient days).
- Lifestyle activity score (see *Appendix 6*)

The following biochemical outcomes will be recorded at month 12 post-transplant:

- Serum bilirubin (measured in µmol/l)
- Serum alanine transaminase (ALT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L)
- Serum alkaline phosphatase (ALP; measured in IU/L)
- Blood urea (mmol/L)
- Serum creatinine (µmol/l)

Immunosuppression

Details of maintenance immunosuppression at 12 months post-transplant will be recorded.

11. THE ORGANOX METRA DEVICE

11.1. Device description

11.1.1. OrganOx Limited

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

11.1.2. The OrganOx metra

The OrganOx *metra* is a normothermic preservation device for use in human liver transplantation. 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.

11.1.3. The OrganOx metra Base Unit

The OrganOx *metra* normothermic perfusion device 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 plastic circuit
- a set of perfusion solutions suitable for 24 hours perfusion

11.1.4. Disposable Set

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

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

11.1.5. Perfusion Solutions

For the present study all the additives necessary to perfuse and maintain the organ during the storage process, with the exception of sodium taurocholate, are not included in the disposable set provided by OrganOx and will be sourced locally (OrganOx will provide a list of recommended suppliers in the

Instructions for Use (IFU) document). These solutions include bolus injections (given at the start of perfusion) and the maintenance infusions (given throughout perfusion).

The primary perfusion fluid for the liver comprises packed red blood cells, supplemented by colloid solution to normalise the haematocrit and osmolarity– these two components are not included and will be sourced locally.

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

- Antibiotic and antifungal agents as per current local protocols. Heparin (anticoagulant) to prevent thrombosis in the circuit. In clinical use, a half-life of ~90 minutes is assumed; on this basis heparin is also given as a maintenance infusion.
- Sodium bicarbonate (buffer) for adjusting the pH of the perfusate.
- Calcium gluconate/calcium chloride to correct the binding of citrate to calcium.

During the perfusion the following are infused at a constant rate:

- Parenteral nutrition solution - a source of amino acids and glucose for liver maintenance.
- Insulin to control the perfusate glucose level
- Heparin to maintain anticoagulation.
- A 2% solution of sodium taurocholate in isotonic saline to compensate for loss of bile salts.
- Prostacyclin to optimise micro-perfusion.

The primary fluid for perfusing the organ is packed red cells supplied from blood transfusion centres and supplemented by a commercially-available colloid solution (human albumin solution or Gelofusine as per local protocol) to normalise the haematocrit and osmolarity. Further additions are made to the perfusate to support the liver. All solutions required will be attached to the circuit during set-up and before the liver is attached. The recipient centre will provide the solutions necessary for perfusion with the *metra* including the packed red blood cells. All solutions are prepared immediately before the organ is attached to the device and contain sufficient solution for 24 hours operation, the intended maximum perfusion time for a liver on the device.

11.2. Device Safety

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

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

The OrganOx perfusion system is based on the principle that all the perfusion solutions, additives and packed red cells must be removed from the organ prior to transplant. Therefore following the completion of the perfusion, the perfusion solution is flushed out of the organ with HTK solution. OrganOx has deliberately designed the operation of the device such that it will require minimal changes to current transplant clinical practice.

11.3. Regulatory Aspects

The OrganOx metra has been used in over 800 clinical liver perfusions worldwide. It has been tested in a multicentre Phase III clinical trial demonstrating both safety and efficacy.

The Organox device is CE marked and will be used within its approved indication. Therefore this is not a regulatory device trial. There are no current plans for the manufacturer to use clinical data from the study to support a change to CE marking of the product.

11.4. Device and Disposables Accountability

All participating centres have access to an OrganOx metra device that is also available for general clinical use. Disposable sets will be provided for the study by OrganOx and should only be used for the preservation of livers recruited into this study. Any livers being perfused outside of the current study should use the hospitals own supply of disposable sets.

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

At the end of each procedure the OrganOx *metra*, and any unused disposable and perfusion solutions will be disposed of on site. Details of total numbers of disposable sets provided for trial use will be recorded.

11.5. Device Maintenance

Device cleaning and routine maintenance will be the responsibility of the local transplant centre storing the device. Full details for cleaning and routine maintenance required will be provided in the instructions for use (IFU), and appropriate training will be provided as part of the device training described in section 15.3.

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.

12. SAFETY REPORTING

The below sections describe the required reporting for adverse events within the clinical trial. This is in addition to the standard incident reporting to the device manufacturer and to Clinical Governance at NHSBT. It is a statutory condition of a licence for procurement or transplantation activity to rapidly report to NHSBT (acting on behalf of the HTA), relevant and necessary information concerning adverse events which may influence the quality and safety of organs. All study sites will therefore follow their usual procedures for highlighting concerns – by completing an NHSBT incident submission form:

https://safe.nhsbt.nhs.uk/IncidentSubmission/Pages/IncidentSubmissionForm.aspx

These reports will be reviewed periodically by the Data Monitoring Committee (DMC).

Untoward incidents related to the process of organ retrieval and transplantation are routinely collected by NHSBT. Further detail may be found here:

https://www.odt.nhs.uk/odt-structures-and-standards/governance-and-quality/tell-us-about-an-incident/

12.1. Adverse Event Definitions

Adverse Event (AE)	Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) whether or not related to the study intervention.

Serious Adverse Event (SAE)	An adverse event that:
	• Led to death
	• Resulted in serious deterioration in the health of the subject that:
	  ○ resulted in a life-threatening illness or injury
	  ○ resulted in persistent or significant disability or incapacity
	  ○ required in-patient care or prolongation of hospitalisation
	  ○ resulted in medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
	  ○ resulted in congenital anomaly or birth defect
	
	These are handled under the SAE reporting system.
	
	Planned hospitalisation for a pre-existing condition, or a procedure required by the trial protocol, without serious deterioration in health, is not considered a serious adverse event.

Severity definitions

The following definitions will be used to determine the severity rating for all adverse events:

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

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

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

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

12.2. Anticipated Serious Adverse Events

Liver transplantation is a life-saving procedure but is associated with serious life-threatening complications which would be expected during the study and which we do not propose to report as SAEs, except those reported as outcomes:

- Primary non function: 0.8% in DBD, 4.0% in DCD livers
- Early allograft dysfunction (Olthoff definition): 25% DBD, 40% DCD
- Hepatic artery thrombosis: 4-15%, depending whether anomalous arterial supply required reconstruction or not
- Post reperfusion syndrome: 30%, more common in DCD than DBD
- Biliary anastomotic breakdown in 10%, more common in DCD
- Post operative haemorrhage requiring reoperation: 10%
- Acute rejection: 20%
- Acute kidney injury: 25% in DBD, 50% in DCD
- Chest complications: sepsis; effusion; paralysed right hemidiaphragm: common
- Ascites: universal if pre-existing ascites, common if not pre-existing
- Line sepsis
- Biliary anastomotic stricture: 10%
- Ischaemic cholangiopathy: 4% DBD, 20-25% DCD
- Retransplantation in first year: around 11% for DBD, rising to 17% for DCD
- Graft failure or sepsis
- Hospitalisation for pre-existing condition that has not deteriorated.

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

12.3. Assessment of Causality

The relationship of each adverse event to the trial procedures, conduct or intervention must be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from the trial procedures. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

12.4. Procedures for Reporting Serious Adverse Events

It is the responsibility of the local investigator to ensure that all adverse events which fall in to the category of Serious Adverse Events (SAEs) are reported to NHSBT Clinical Trials Unit, Chief Investigator, central investigators and, if required, to their local R&D department as soon as possible after becoming aware of the event but no later than 24 hours. AE not meeting the definition for serious will not be collected.

Data will include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the trial procedures, conduct or intervention
- How the event qualifies as serious
- Expectedness

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

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

Serious adverse events will be collected from transplant until 12 months following the transplant, via a purposely designed MACRO database (access via www.ctu.nhsbt.nhs.uk/macro). SAEs will be automatically notified to NHSBT CTU. If the eCRF is unavailable for any reason, a paper version of the form should be completed, scanned and emailed to serious_adverse_events@nhsbt.nhs.uk. Within the following 5 working days, the local investigator may be required to provide additional information on the SAE in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic or relevant information that will assist the understanding of the event.

Additional and further requested information (follow-up or corrections to the original case) should also be added to eCRF using a new SAE Report Form. NHSBT CTU will ensure that all SAEs are reported to the Sponsor .

The clinical reviewers will review the SAEs and, if they feel they pose an immediate risk to patient health or safety, then they will report them to the DMC immediately, and the REC within 15 calendar days of the Chief Investigator becoming aware of the event. This includes any SAEs that the local investigator assesses to be unexpected and related to the study procedure.

All SAEs will be followed up to resolution. The DMC will review the accumulating data at regular intervals.
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12.5. Study Suspension or Early Termination

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

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

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

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

If suspension or premature termination occurs,

a) the research team remain responsible for providing resources to fulfil the obligations from the study protocol and existing agreements for following up the subjects enrolled in the study, and
b) the chief investigator or authorized designee shall promptly inform the enrolled subjects at their study site, if appropriate.

Device deficiencies and use errors should be reported directly to Organox for investigation by the manufacturer.

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

13. STATISTICS

13.1. Statistical Analysis Plan (SAP)

The statistical aspects of the study are summarised here with details fully described in a separate statistical analysis plan. The SAP will be finalised before any analysis takes place.

13.2. Description of Statistical Methods

Primary outcome reporting will occur after collection and analysis of data at 12 months following the enrolment of the last patient to the trial. The outcome measures are described in detail in section 6.

The study arm (NMP) will be compared against RWC cohort (SCS) defined in Section 8.1 at this point for all primary and secondary outcomes, except 5 year outcomes. 95% confidence intervals will be provided for all assessments of treatment effect. Baseline characteristics of the donors enrolled and transplanted will be presented separately, together with characteristics of the liver transplant recipients. A CONSORT diagram will show the flow of donor livers and recipients through the study, in particular the reasons for any post-enrolment exclusions which are necessary because study entry occurs before some exclusion criteria can be assessed.

The primary outcome is a binary outcome of functional utilisation at 12 months. As the 12-month visit may occur at a routine hospital appointment prior to 12 months, livers will be assumed to be functioning at 12 months if documented as functioning >10 months post-transplant. The primary outcome will be analysed using a logistic regression model to assess the effect of treatment on outcome, after adjustment for the important prognostic factors of donor type and use of normothermic regional perfusion (NRP). The odds ratio, 95% confidence interval and p-value for the treatment term in this logistic regression model will be the primary analysis for the trial. NMP will be concluded superior if the lower bound of the confidence interval exceeds the efficacy threshold which has been pre-specified at 40 extra livers transplanted with one-year function per year (an OR of 1.20).

Secondary outcomes will be presented using descriptive statistics and analysed using a similar approach, with adjustment for donor type and use of NRP. However, when performing analyses on the subset of transplanted livers, adjustment will also be made for the transplanting centre where numbers allow. General Linear and Generalised Linear Models or non-parametric methods will be used as appropriate for the different outcomes. Length of HDU/ITU and hospital stay post-transplant will be analysed using a competing risks approach where death is the competing risk. Measures of biochemical liver function at 12 months will be compared between the two treatment arms using linear regression, with appropriate transformation if required. Patient and graft survival (censored and non-censored) will be estimated using the Kaplan-Meier method and analysed using Cox Proportional Hazards regression. Safety outcomes will be summarised for each arm according to classification and severity. Some outcomes are only measured in the study arm and these will be presented using descriptive statistics. Measures of biochemical liver function over the first seven days will be presented graphically and area under the curve summarised for the study arm, along with a summary of the MEAF score.

During the trial, data will be collected regarding perfusion parameters (including machine logs and perfusate characteristics). These data will be analysed separately to the main clinical trial outcomes to explore relationships between perfusion parameters, utilisation and transplant outcomes.

After the final patient has reached five years post-transplant, patient and graft survival will be obtained from the transplant registry. The unadjusted survival rates at five years will be estimated using the Kaplan-Meier method. A Cox proportional hazards model will also be used to assess the treatment effect after risk-adjustment for any imbalance in baseline characteristics that is known to affect recipient outcome.

13.3. Sample Size Determination

There are 2465 livers in the retrospective control cohort. One-year functional utilisation in this group was calculated using complete organ utilisation data and a Kaplan-Meier estimate of transplant survival at one year, due to a small proportion of censored data prior to that point. One year functional utilisation in the control cohort was 21.8%. A 50% improvement in functional utilisation (to 32.7%) would represent an increase of around 135 extra transplants functioning at 1 year each year in the UK. This would transform waiting list mortality (50 patients in 2019-20) and average waiting times.

There will be some circumstances where, typically due to logistics (e.g. temporary NMP unavailability), centres will preserve a liver in the NMP study cohort using SCS. In the European Phase-3 trial, only one liver randomised to NMP crossed-over to the SCS arm (14). This figure is likely to be higher in the current study due to earlier study entry and logistical constraints, so we have allowed for a 15% crossover rate (25% due to logistical reasons, 5% eligibility message not relayed and 5% general cross-over). We will analyse the data on a modified ITT basis, whereby livers unable to be perfused for logistic reasons are excluded, along with those who were found to be ineligible after study entry.

Allowing for these exclusions and cross-over rate, the sample size was calculated based on an anticipated increase in the primary endpoint from 21.8% in the RWC cohort to 31.4% in the NMP mITT analysis. A ‘superiority by a margin’ design was used, such that with a 2.5% one-sided significance level the sample size will have 90% power to detect whether NMP is superior to SCS by the prespecified threshold (margin) of 40 extra livers transplanted with one year function per year. As there are 2465 livers in the control cohort, an allocation ratio of 3.5:1 was used to achieve the necessary power and this requires a sample size of 700 liver offers in the NMP study arm. As the primary endpoint uses mandatory data collected by NHSBT, loss to follow-up should be low. Allowing a 25% loss due to exclusions due to being unable to use NMP for logistical reasons and another 10% loss (due to withdrawal of consent, loss to follow-up and those meeting exclusion criteria after study entry), the number required increases to 1035 offers.

Currently, around 1242 eligible livers (i.e. DUI>0.27 and Hep C/HIV negative) are offered each year on average in the UK. Recruitment would therefore require 56% of all eligible livers offered during the 18-month recruitment period to be enrolled in the trial.

13.4. Analysis Populations

A modified intention-to-treat (mITT) analysis will be performed for the primary outcome. Liver offers will be analysed according to whether part of the study arm or RWC cohort, irrespective of whether NMP was actually used in the study arm. The exceptions to this relate to eligibility factors that are unknown at the time of study entry (recipients <18 years, split liver, multi-organ or non-UK transplants, recipient declined consent to NMP or data collection) and livers in the NMP study arm that are unable to undergo perfusion

due to logistical constraints (e.g. lack of trained staff or device availability) – these livers will be excluded from the mITT population. These exclusions are considered appropriate because enrolment prior to organ offer and establishing availability of NMP is for pragmatic reasons only, and the recipient and transplant exclusions are unaffected by participation in the different cohorts.

Secondary (recipient) outcomes will be analysed using a second modified intention-to-treat analysis. As well as excluding livers described above, this analysis will exclude livers offered but not transplanted for any reason.

In addition, secondary per-protocol analyses will be performed for the primary endpoint.

A propensity-score matched analysis will also be undertaken as a secondary analysis to allow for adjustment for any baseline differences between the two cohorts resulting from the non-randomised design. Due to anticipated cross-over in the trial, this will also be used to provide an unbiased estimate of the treatment effect if all livers retrieved in the study arm had adhered to NMP treatment. Exploratory analyses will be undertaken to assess the effect of a 'device to donor' vs 'back to base' approach to NMP on the primary outcome, along with any effect of the duration of NMP and use of NRP. Sensitivity analyses will also be conducted to investigate the impact of any other donor interventions that form part of other research studies within NHSBT OTDT if such interventions are imbalanced between the two cohorts.

We will also undertake the following pre-specified subgroup analyses (if sufficient data are available):

- DBD vs DCD donors, anticipating NMP to have a greater impact in DCD donors
- Impact of donor utilisation index (DUI), analysed according to the quartiles observed and anticipating NMP to have a greater impact in the lower DUI quartiles.
- Impact of donor risk (DLI), analysed according to the quartiles observed and anticipating NMP to have a greater impact in the lower DLI quartiles.

13.5. Embedded Feasibility Study

An embedded feasibility study will assess recruitment and the use of NMP in recruited livers, to ensure that NMP is used where feasible. Due to logistical constraints (absence of trained staff, device already in use etc.), NMP may not be available for use in all cases, but lack of use may reduce the ability of the study to demonstrate an improvement in utilisation.

Go/no-go points at the end of 6-months are as follows:

- Recruitment of a minimum of 200 liver offers
- NMP utilisation in transplanted livers of > 50%, as a measure of protocol compliance. This will be assessed at a centre level, and poorly performing centres may be withdrawn from the study.

If recruitment or compliance with the intervention are outside these targets, recruitment strategies will be reviewed and a decision made as to whether to proceed.

No further interim analyses are planned.

13.6. The Level of Statistical Significance

The level of statistical significance will be set at a one-sided level of 2.5% for the primary outcome as superiority of NMP is required. For all other hypothesis tests a two-sided significance level of 5% will be used.

13.7. Procedure for Accounting for Missing, Unused, and Spurious Data.

Withdrawals from the trial after implantation will be documented, and a narrative analysis of withdrawals will be performed. Recipients withdrawing from the trial after implantation will be included in analysis using imputation methods for missing data, as described in the SAP.

The primary outcome will be available for all unused livers, and the majority of transplanted livers where the recipient attends a follow-up visit >10 months post-transplant or the liver fails at any point. It is anticipated very few patients will be lost to follow-up, but it is likely that not all measurements at all time points will be recorded for every recipient. Where data are missing from the registry for the study cohort at 3 and 12 months, attempts will be made to chase missing data.

Assuming that registry data are missing at random, with no effect of cohort on the probability of missing data, multiple imputation methods will be used to estimate missing variables. Sensitivity analyses will assess the effect of these assumptions.

13.8. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

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

13.9. Health Economics Analysis

The health economics aspects of the study are summarised here with details fully described in a separate health economics analysis plan (HEAP). The HEAP will be finalised prior to any analysis taking place.

The unit cost of NMP is greater than that of SCS, but it is hypothesised that the increased cost of the NMP technology will be (at least partly) offset by reduced costs elsewhere (e.g., shorter time on the transplant waiting list, reduced length of hospital and intensive care stay, fewer livers needed on average per transplanted patient); it is also hypothesised there may be health benefits (measured in terms of quality-adjusted survival) that may be balanced against any cost differences. The health economic analysis will incorporate these potential impacts and express the cost-effectiveness of NMP in terms of the incremental cost per quality-adjusted life year gained (the cost-effectiveness measure preferred by NICE) of NMP versus SCS.

A key feature of the health economic analysis, which differentiates it from the statistical analysis described above, is that for the health economic analysis the unit of analysis is the patient, whereas for the statistical analysis it is the liver.

We will generate evidence evaluating the cost, value for money and budget impact of NMP versus SCS from an NHS perspective that will be useful for NHS managers and commissioners when deciding whether or not to use the new technology. The analysis will include the following elements:

(1) The cost of using NMP and SCS per liver. We will undertake a detailed micro-costing of both storage approaches, including staff and non-staff costs (both capital and consumables), valued using market prices. We will model the number of transplants anticipated per year for both the NMP and SCS approach, and multiply the respective costs of using NMP and SCS per liver by these numbers.

(2) Costs associated with use of NHS services during the one-year following transplant per patient. These will be based on a combination of previously published data regarding the costs of care for liver transplant recipients in the UK (where available), as well as resource use data collected prospectively during the study using routine NHSBT data collection forms. These record data on length of initial ITU and hospital stay, use of renal replacement therapy, and total number of inpatient days and number of stays up to one year post-transplant. These data will be collected irrespective of whether the patient had NMP or SCS – see below. Values will be monetised using national unit cost data from published sources.

(3) Health outcomes in transplant recipients, measured in terms of quality-adjusted life years during the one-year follow-up period per patient. Health related quality of life, survival and QALYs per patient will be estimated from published data (where available), as well as being collected prospectively from patients recruited to the study (irrespective of whether or not they had NMP or SCS – see below). EQ-5D-3L data (www.euroqol.com) will be collected pre-transplant, and at 3 and 12 months post-transplant. If the patient dies during the 12-month follow-up period they will be assigned a utility score of zero from that point onwards. Patient-specific utility profiles will be constructed assuming a linear relation between each of the patient's EQ-5D-5L scores at each follow-up point. The QALYs experienced by each patient from baseline to 12 months will be calculated as the area underneath this profile.

(4) We will estimate the impact of NMP on the number of days and associated costs a patient spends on the transplant waiting list and assign a mean unit cost per day to this time. These costs may be derived from previously published data, or if unavailable from observing a cohort of patients on the waiting list during the course of the study.

The total costs of NMP and SCS will comprise the sum of (1), (2) and (4).

For items (2) and (3) we will group participating patients into four outcome categories (uncomplicated transplant with good function, complicated post-transplant course requiring total hospital stay >2 months in the first year, graft failure requiring re-transplantation, death) and we will calculate mean resource use and costs (item (2)) and mean QALYs (item (3)) over the one year period for all patients in each category, irrespective of whether or not they had NMP or SCS. The probability of ending up in each of the four outcome categories will be taken from study data, based on the primary and secondary outcomes of the trial and will be allowed to vary by whether or not the patent had NMP or SCS. Hence, this approach assumes that the mean costs and mean QALYs associated with each outcome category is the same irrespective of whether or not the patient had NMP or SCS; it also allows that the probability a patient ended up in each of the four categories may vary by whether or not they had NMP or SCS.

Cost-effectiveness will be calculated as the mean cost difference between groups divided by the mean difference in outcomes (QALYs) to give the incremental cost-effectiveness ratio (ICER) and incremental net monetary benefits (NMBs). We will subject the results to extensive deterministic (one-, two- and multi-way) and probabilistic sensitivity analysis. The aim of the former is to identify the most important drivers of cost and cost-effectiveness; in addition we will undertake threshold analyses to identify the range of costs of NMP at which it is likely to be cost neutral and cost-effective. For the latter, we will apply recommended probability distributions around the parameter values and calculate confidence intervals

around the ICERs and incremental NMBs. These will also be used to construct cost-effectiveness acceptability curves, which will show the probability that each strategy is cost-effective for different values of the NHS' willingness to pay for an additional unit of outcome.

We will combine data on incremental costs with epidemiological data on projected numbers and undertake a budget impact analysis to evaluate what the total cost impact would be of rolling out NMP using a number of assumptions concerning adoption at individual NHS transplant centres and national levels.

14. DATA MANAGEMENT

A detailed Data Management Plan will be developed to outline the data management processing, data cleaning and QC procedures for the trial. The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

14.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, correspondence and UK transplant registry data.

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

14.2. Access to Data

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

14.3. Data Recording and Record Keeping

Study liver and participant data will be entered onto the trial database designed and administered by the NHSBT CTU data management team using MACRO™, a commercially available FDA 21 Code of Federal Regulations (CFR) Part 11 compliant clinical trial database system. Following completion of analysis, the trial database will be archived in accordance with University policy.

The study team must keep the signed Informed Consent forms, all trial documentation and source documents collected during the trial in a secure location (e.g. locked filing cabinets in a room with restricted access). Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

The participants will be identified by a unique patient trial ID in any database. Participant identifiers (e.g. NHS number) will only be stored where required for linkage to external data sources (e.g. NHS Digital, NHSBT). Individual participants will not be identified in the resulting publications and presentations from the trial.

All trial documentation will be retained for at least 5 years after trial completion or termination. In addition, the Investigator must not discard or destroy any trial specific materials unless otherwise instructed by NHSBT.

14.4. Use of registry data

The UK Transplant Registry will be the primary source of data about the donor and the organ retrieval. The primary source for the initial inpatient stay, 3- and 12-month and 5-year outcome data will be the UK Transplant Registry. Where primary or secondary outcome data are missing, we will use the electronic case report forms (eCRFs) to obtain missing data where recorded. For some outcomes data are only captured on the eCRFs for the prospective cohort, so the eCRFs will be the primary source for these outcomes. Linkage between trial and registry data will only be undertaken by statisticians working on the trial and registry identifiers will be removed from datasets after linkage has been undertaken. NHS Blood and Transplant Information Governance have conducted a Data Protection Impact Assessment, are satisfied that confidentiality and data protection measures are in place and approved the use of UK Transplant Registry Data for this study.

We will also request consent for linkage to other national data sources (e.g. NHS Digital, Office for National statistics) for the purposes of verifying resource use and clinical outcomes.

15. QUALITY ASSURANCE PROCEDURES

15.1. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

15.2. Monitoring

Regular monitoring will be performed according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy as defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol and GCP.

15.3. Local Investigator and Site Personnel Training

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

All personnel involved in recruitment and data entry will also be trained in the use of the online recruitment and data collection tool by members of the clinical trials unit, and records of such training will be maintained.

15.4. Study Documentation

It is the responsibility of the local investigator to maintain complete, accurate and current study records. Each investigator will be provided with an investigator site file, online access to the case reporting system and other associated study specific documentation by the co-ordinating centre. Such records will be maintained during the course of the study and for up to 5 years following the date on which the study is terminated or completed.

15.5. Trial committees

There are a number of committees involved with the oversight of the trial. These committees are detailed below.

15.5.1. Trial Management group (TMG)

A TMG comprising the CI, other lead investigators, local principal investigators and members of the CTU. The TMG will be responsible for the day to day running and management of the trial. It will meet at least

four times a year, more often during set up and close down phases of the trial. At least one face to face meeting will be held each year.

15.5.2. Trial Steering Committee (TSC)

The role of the TSC is to:

- provide expert oversight of the trial
- maintain confidentiality of all trial information not already in the public domain
- make decisions as to the continuation of the trial
- monitor recruitment rates and advise the TMG on recruitment issues
- review and approve V1.0 of the protocol, and any substantial amendments
- review regular progress reports of the trial from the Trial team
- receive feedback from the DMC and consider their recommendations, including any ethical implications arising from their advice
- assess the impact and relevance of any accumulating external evidence
- monitor completion of Case Report Forms (CRFs) and comment on strategies from TMG to deal with problems
- monitor protocol deviations and advise the TMG on remedial action
- monitor any quality issues e.g. serious breaches and advise TMG on remedial action approve additional sub-studies
- oversee the timely reporting of trial results
- approve the statistical analysis plan
- approve the publication policy
- approve the main trial manuscript
- approve abstracts and presentations of results during the trial and on completion
- approve any requests for release of data or samples including clinical data and stored biological samples

The ultimate decision on continuation of the trial lies with the TSC.

15.5.3. Data Monitoring Committee

The trial has a data monitoring committee (DMC) which consists of at least three independent members, including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DMC will periodically review accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. As a result of the reviews the DMC may make recommendations to the TSC, including premature termination of the trial, should they feel it is indicated.

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

16. PROTOCOL DEVIATIONS

16.1. Definitions

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

A "protocol deviation" is a failure to adhere to the requirements specified in this study protocol without adequate justification. Examples may include the enrolment of a liver or recipient not meeting all of the inclusion/exclusion criteria specified in section 8 or missed study procedures without documentation. Livers excluded after recruitment due to factors not known at the time of recruitment (see section 13.4) will not be deemed protocol deviations.

16.2. Reporting of protocol deviations

All protocol deviations must be recorded and reported to the data monitoring committee. The DMC will review all deviations and assess their impact on patient safety. Serious breaches must be reported as per section 16.3.

16.3. Reporting of serious breaches

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

a) The safety or physical or mental integrity of the subjects of the trial; or
b) The scientific value of the trial

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day, with a copy sent to the clinical trials unit (CTU) by e-mail (plus@nhsbt.nhs.uk). In collaboration with the CI and CTU, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

17. ETHICAL AND REGULATORY CONSIDERATIONS

17.1. Declaration of Helsinki

The investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki (2008).

17.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in in compliance with the approved protocol, Good Clinical Practice (GCP), UK General Data Protection Regulation and the UK Policy Framework for health and social care research.

17.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4. Other Ethical Considerations

Participation in this trial or a decision to withdraw will not affect a patient's position on the liver transplant waiting list or their likelihood or receiving a liver transplant.

17.5. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and final report will be submitted to the REC, host organisation(s) and Sponsor.

17.6. Donor and Recipient Confidentiality

The study will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so. The processing of the personal data of both donors and recipients will be minimised by making use of unique liver and patient trial IDs only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

17.7. Expenses and Benefits

Study assessments and investigations will be conducted during routine hospital attendances. Where study assessments cannot be made at routine clinic visits, collection of data and delivery of the patient questionnaire will be made remotely via telephone or internet and it is not expected that additional travel expenses will be incurred.

18. FINANCE AND INSURANCE

18.1. Funding

This study is funded by an NIHR Invention for Innovation (i4i) Challenge Award (NIHR201003). Funding will be managed through the Nuffield Department of Surgical Sciences (NDS) finance office.

An in kind contribution from OrganOx Ltd will provide device support and training, and disposable sets at reduced cost for the purposes of the trial.

18.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

18.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

19. DISSEMINATION POLICY

19.1. Data analysis and release of results

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

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

No data from the study will be presented in oral or written form without permission of the TSC. Approval to submit papers for publication will include all authors of the paper.

19.2. Primary outcome publications

All publications, abstracts and other outputs will be reviewed by the Trial Steering Committee (TSC) prior to publication. Publications will reflect the input of all participating centres in authorship, which will be agreed by the TSC.

Reports relating to primary outcomes will be published in peer-reviewed journals of appropriate relevance. Individual centres will undertake not to report any trial data independently. A final report on the primary outcomes of the study will be compiled by the chief investigator and NHSBT CTU and approved and signed off by each local investigator.

19.3. Other study papers, abstracts and presentations

Study investigators wishing to publish secondary data analyses will submit a proposal to the TSC for approval. If the committee accepts the proposal, then the author of the proposal may decide on the lead in each publication resulting from such a proposal.

Following primary study publication, anonymised data will be made available to researchers outside of the core study team on application. All data sharing requests will be reviewed and approved by the trial steering committee.

19.4. Identification

The ISCRTN trial identifier will be included on all presentations and publications.

19.5. Timing

No data may be made public before publication and never without agreement from the CI.

19.6. Acknowledgements

For the main report of this study submitted for publication, together with associated methodology and health economic papers or posters/presentations, we will use the International Committee of Medical Journal Editors definitions of Authorship and Contributorship (http://www.icmje.org/ethical_1author.html). The members of the TSC and DMC should be listed with their affiliations in the Acknowledgements/Appendix of the main publication and the support of the NHSBT CTU, and funder acknowledged in all publications/presentations. The NIHR must be acknowledged in all research publications and carry a disclaimer:

"The research was funded by the National Institute for Health Research (NIHR). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care"

20. MANAGEMENT OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University's technology transfer office, Oxford University Innovations.

21. ARCHIVING

Archiving will be authorised by the Sponsor following submission of the end of study report. The TMF including all essential documents will be retained for 5 years after the completion of the study. Consent forms will be retained in medical records in accordance with local requirements.

22. REFERENCES

1. NHS Blood and Transplant. Organ Donation and Transplant Activity report 2018/19 [Internet]. 2018; [cited 2020 Jan 14] Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/16537/organ-donation-and-transplantation-activity-report-2018-2019.pdf

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12. NHS Blood and Transplant. POLICY POL196/7: Deceased Donor Liver Distribution and Allocation [Internet]. 2019; [cited 2020 Jan 15] Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/17449/liver-allocation-policy-pol196.pdf

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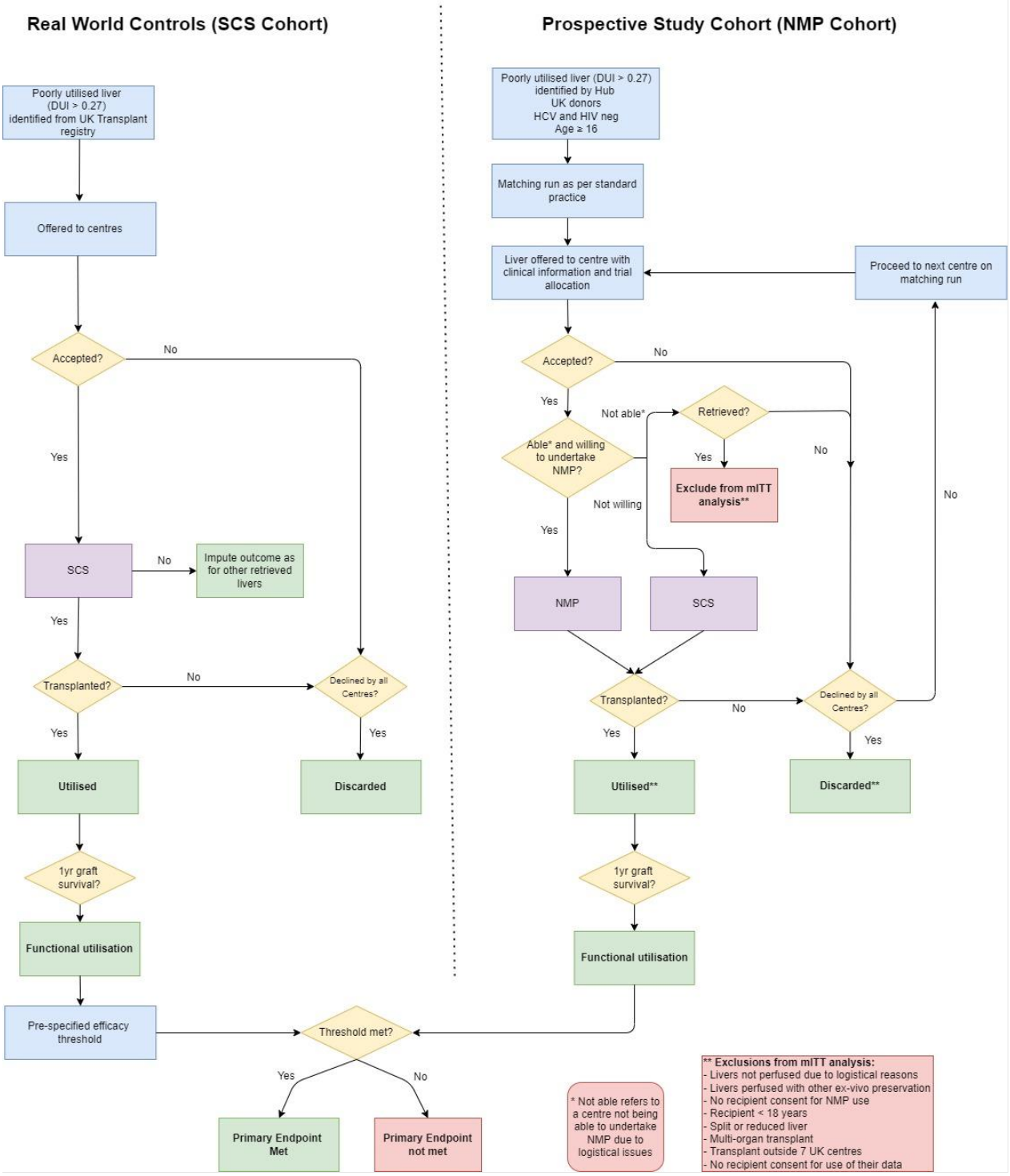
23. APPENDIX 1: FLOW OF LIVERS THROUGH THE STUDY



DUI - Donor Utilisation Index; NMP - Normothermic Machine Preservation; SCS – Static Cold Storage

24. APPENDIX 2: SCHEDULE OF PROCEDURES FOR STUDY COHORT

[1]

Activity	Pre-study Screening	Pre-study Baseline	Pre-reperfusion	Post-reperfusion	Postoperative							Follow-up		
					D1	D2	D3	D4	D5	D6	D7	M3	M12	Y5
Informed consent	X													
Meets inclusion/ exclusion criteria	X													
Recruitment		X												
Donor & recipient demographics		X												
Perfusion parameters/samples			X											
Reperfusion biopsy				X										
Surgical variables				X										
Serum ALT/AST [1]					X	X	X	X	X	X	X		X	
Serum Bilirubin [1]					X	X	X	X	X	X	X		X	
Serum GGT [1]					X	X	X	X	X	X	X			
INR [1]					X	X	X	X	X	X	X			
Serum lactate [2]					X	X	X	X	X	X	X			
Primary non-function														
Graft survival					X	X	X	X	X	X	X	X	X	X
Patient survival					X	X	X	X	X	X	X	X	X	X
Quality of life (EQ-5D-5L)		X										X	X	
Resource use					X	X	X	X	X	X	X	X	X	
Safety outcomes				X	X	X	X	X	X	X	X	X	X	

Biochemistry will be recorded as part of routine postoperative lab tests; [2] Serum lactate will be recorded daily during ITU/HDU stay

25. APPENDIX 3: DONOR UTILISATION INDEX (DUI)

The Donor Utilisation Index (DUI) is a clinical risk score that estimates the risk of non-utilisation of an offered liver. It was derived from analysis of a cohort UK liver offers between 1 February 2016 and 31 January 2020. The overall sample was randomly split into model development and validation cohorts, and separate models were derived for DBD (development n=2,590, validation n=1,067) and DCD (development n=1,746, validation n=758) donors. 26 candidate variables were considered, and those statistically significant at the 10% level were included in the final model.

Both models showed good performance, with a C-statistic of 0.78 for the DBD model, and 0.796 for the DCD model.

25.1. DBD Donor Utilisation Index

$$DUI_{DBD} = \frac{\exp(\eta_{DBD})}{1 + \exp(\eta_{DBD})}$$

$$\begin{aligned}\eta_{DBD} = -4.1731 + {} & (0.8005\ if\ HepB\ positive) + (0.7601\ if\ past\ history\ of\ diabetes) \\ & + (0.4792\ if\ past\ history\ of\ tumour) + 0.030043\ age + 0.042627\ bilirubin \\ & + 0.005034\ alk\ phos + (0.3155\ if\ moderate\ alcohol\ consumption) \\ & + (1.1254\ if\ heavy/very\ heavy\ alcohol\ consuption) \\ & - (0.3342\ if\ stopped\ consuming\ alcohol) + (0.2501\ if\ blood\ group\ A) \\ & + (0.2428\ if\ blood\ group\ B) + (1.3751\ if\ blood\ group\ AB) \\ & + (0.4115\ if\ cause\ of\ death\ CVA) - (0.0484\ if\ cause\ of\ death\ Anoxia) \\ & + \big(0.7446\ if\ cause\ of\ death\ Other\ (excluding\ Trauma)\big) + BMIspline \\ & + ALTspline\end{aligned}$$

$$BMIspline = -0.095502\ BMI\ +\ 0.034105\ F1\_BMI\ \ -0.062880\ F2\_BMI$$

$$F1\_BMI = \frac{(BMI - 19.7)_+^3 - (BMI - 36.5)_+^3}{36.5 - 19.7} - \frac{(BMI - 28.2)_+^3 - (BMI - 36.5)_+^3}{36.5 - 28.2}$$

$$F2\_BMI = \frac{(BMI - 24.5)_+^3 - (BMI - 36.5)_+^3}{36.5 - 24.5} - \frac{(BMI - 28.2)_+^3 - (BMI - 36.5)_+^3}{36.5 - 28.2}$$

$$ALTspline = 0.041311\ ALT\ - 0.007743\ F1\_ALT\ + 0.010706\ F2\_ALT$$

$$F1\_ALT = \frac{(ALT - 11)_+^3 - (ALT - 396)_+^3}{396 - 11} - \frac{(ALT - 57)_+^3 - (ALT - 396)_+^3}{396 - 57}$$

$$F2\_ALT = \frac{(ALT - 25)_+^3 - (ALT - 396)_+^3}{396 - 25} - \frac{(ALT - 57)_+^3 - (ALT - 396)_+^3}{396 - 57}$$

25.2. DCD Donor Utilisation Index

$$DUI_{DCD} = \frac{\exp(\eta_{DCD})}{1 + \exp(\eta_{DCD})}$$

$$\begin{aligned}\eta_{DCD} = {} & -5.2145 + (0.3026\ if\ past\ history\ of\ smoking) + (0.4152\ if\ past\ history\ of\ diabetes) \\ & + (0.3163\ if\ male) + (\ 0.5582\ if\ past\ history\ of\ tumour) \\ & + (0.4415\ if\ past\ history\ of\ hypertension) \\ & - (0.4145\ if\ past\ history\ of\ cardiac\ or\ respiratory\ arrest) + \ 0.000652\ ALT \\ & + \ 0.016575\ sodium + \ 0.004917\ alk\ phos + 0.002071\ creatinine \\ & + (0.2510\ if\ moderate\ alcohol\ consumption) \\ & + (1.0362\ if\ heavy/very\ heavy\ alcohol\ consuption) \\ & - (0.3321\ if\ stopped\ consuming\ alcohol) + (0.2293\ if\ blood\ group\ A) \\ & + (0.2847\ if\ blood\ group\ B) + (1.5143\ if\ blood\ group\ AB) \\ & + (0.1481\ if\ cause\ of\ death\ CVA) + (0.7689\ if\ cause\ of\ death\ Anoxia) \\ & + \big(0.9891\ if\ cause\ of\ death\ Other\ (excluding\ Trauma)\big) + \ BMIspline \\ & + agespline + ITUspline\end{aligned}$$

$$BMIspline = 0.007428\ BMI\ + \ 0.029538\ F1\_BMI\ - 0.058265\ F2\_BMI$$

$$F1\_BMI = \frac{(BMI - 19.9)_+^3 - (BMI - 39.7)_+^3}{39.7 - 19.9} - \frac{(BMI - 29.1)_+^3 - (BMI - 39.7)_+^3}{39.7 - 29.1}$$

$$F2\_BMI = \frac{(BMI - 24.8)_+^3 - (BMI - 39.7)_+^3}{39.7 - 24.8} - \frac{(BMI - 29.1)_+^3 - (BMI - 39.7)_+^3}{39.7 - 29.1}$$

$$agespline = 0.022407\ age\ + \ 0.000002\ F1\_age + 0.002921\ F2\_age$$

$$F1\_age = \frac{(age-26)_+^3 - (age-76)_+^3}{76-26} - \frac{(age-64)_+^3 - (age-76)_+^3}{76-64}$$

$$F2\_age = \frac{(age-52)_+^3 - (age-76)_+^3}{76-52} - \frac{(age-64)_+^3 - (age-76)_+^3}{76-64}$$

$$ITUspline = -0.014439\ ITU\_stay\ +\ 0.000626\ F1\_ITU - 0.000985\ F2\_ITU$$

$$F1\_ITU = \frac{(ITU\_stay-14)_+^3 - (ITU\_stay-324)_+^3}{324-14} - \frac{(ITU\_stay-103)_+^3 - (ITU\_stay-324)_+^3}{324-103}$$

$$F2\_ITU = \frac{(ITU\_stay-52)_+^3 - (ITU\_stay-324)_+^3}{324-52} - \frac{(ITU\_stay-103)_+^3 - (ITU\_stay-324)_+^3}{324-103}$$

26. APPENDIX 4: EQ-5D-5L QUALITY OF LIFE QUESTIONNAIRE

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

Mobility

I have no problems in walking about ❑

I have slight problems in walking about ❑

I have moderate problems in walking about ❑

I have severe problems in walking about ❑

I am unable to walk about ❑

Self-Care

I have no problems washing or dressing myself ❑

I have slight problems washing or dressing myself ❑

I have moderate problems washing or dressing myself ❑

I have severe problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

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

I have no problems doing my usual activities ❑

I have slight problems doing my usual activities ❑

I have moderate problems doing my usual activities ❑

I have severe problems doing my usual activities ❑

I am unable to do my usual activities ❑

Pain/Discomfort

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

Anxiety/Depression

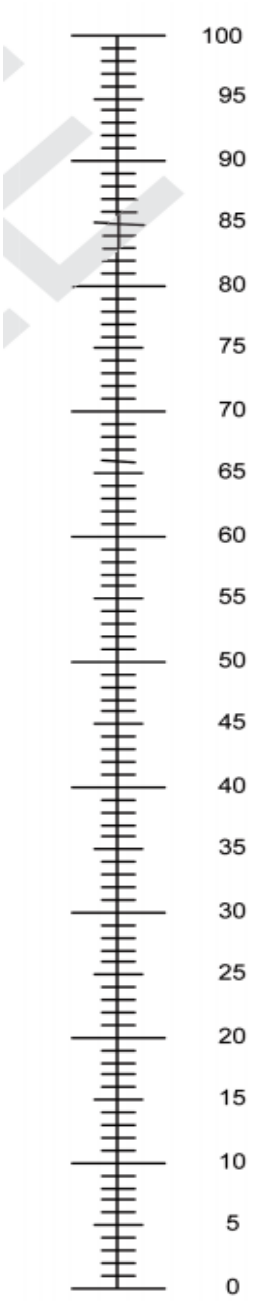
I am not anxious or depressed	❑
I am slightly anxious or depressed	❑
I am moderately anxious or depressed	❑
I am severely anxious or depressed	❑
I am extremely anxious or depressed	❑

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

- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
- 0 means the worst health you can imagine.

Mark an X on the scale to indicate how your health is TODAY.

Now, please write the number you marked on the scale in the box below.



YOUR HEALTH TODAY =

27. APPENDIX 5: CLAVIEN-DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS

Grade	Definition
I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.
II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
III	Requiring surgical, endoscopic or radiological intervention.
IIIa	Intervention not under general anaesthesia.
IIIb	Intervention under general anaesthesia.
IV	Life-threatening complications (including CNS complications) requiring HDU/ITU management.
IVa	Single organ dysfunction (including dialysis).
IVb	Multi-organ dysfunction.
V	Death of a patient.
Suffix 'd'	If the patient suffers from a complication at the time of discharge, the suffix 'd' (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

28. APPENDIX 6: LIFESTYLE ACTIVITY SCORE

1. Able to carry out normal activity without restriction
2. Only restricted in physically strenuous activity
3. Can move freely. Capable of self-care. Unable to do any form of work
4. Only capable of limited self-care. Confined mostly to bed or chair
5. Completely reliant on nursing/medical care

29. APPENDIX 7: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.1	2022/09/23	Charlie Brown	Updated investigators and trial management
2	1.2	26/10/2022	Charlie Brown	Updated investigators and fixed minor errors
3	1.3	10/01/2023	Charlie Brown	Increased sample size
4	2.0	22/02/2023	Charlie Brown	Updated safety reporting, data collection, recruitment dates, list of abbreviations, trial management & eligibility criteria
5	2.1	16/05/2023	Aleema Iqbal	Protocol date change from v2.0 22.02.2023 to Protocol v2.1 16.05.2023. Also change of PI in Addenbrookes Hospital in Cambridge, from Professor Chris Watson to Mr Rohit Gaurav.
6	2.2	23/05/2023	Aleema Iqbal	Change of PI at St James Hospital in Leeds, from Mr Gabi Oniscu to Miss Barbara Fiore; Mr Gabi Oniscu replaced with Mr Ahmed Sherif in TMG; update of statistician roles & abbreviations.
7	2.3	22/08/2023	Charlie Brown	Change of PI at Queen Elizabeth Hospital, Birmingham
8	3.0	30/08/2023	Charlie Brown	Removal of technical complications and device incidents as a secondary outcome; addition of option to collect consent remotely; link with information to reporting organ transplant incidents updated
9	3.1	23/07/2024	Charlie Brown	Updated investigator details; change of trial statistician; clarification of primary non-function, adverse outcome data collection, ineligible livers, end of trial definition, 12 month and 5 year follow up data, primary and secondary data, use of UK transplant registry data; addition of date fields for signatures

List details of all protocol amendments here whenever a new version of the protocol is produced

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, and HRA (where required).