Consortium for Organ Preservation in Europe

WP2

A multicentre randomised controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation

Statistical Analysis Plan
3.0 – 25Oct2016

Based on protocol version 3.0 (11st February 2016)

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GLOSSARY OF ABBREVIATIONS

ADE	Adverse Device Effect
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CET	Centre for Evidence in Transplantation
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COPE	Consortium for Organ Preservation in Europe
CRF	Case Report Form
CSM	Centre for Statistics in Medicine
DBD	Donation after brain death
DCD	Donation after circulatory death
DMC	Data monitoring committee
DPMP	Donors per Million Population per Year
DRI	Donor Risk Index
EAD	Early Allograft Dysfunction
EC	European Commission
ECD	Extended Criteria Donor
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ESOT	European Society for Organ Transplantation
ET-DRI	Eurotransplant Donor Risk Index
FP7	Seventh Framework Programme
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GST	Glutathione S-Transferase
HCC	Hepatocellular Carcinoma
HD	Haemodialysis
HDF	Haemodiafiltration
HDU	High Dependency Unit
HF	Haemofiltration
HMP	Hypothermic Machine Perfusion
IFU	Instructions for Use
IL6	Interleukin 6
INR	International Normalised Ratio
IQR	Interquartile range
IRB	Institutional Review Board
ITU	Intensive Care Unit
ITT	Intention-To-Treat analysis
IUD	Intrauterine Device
IVC	Inferior Vena Cava
MAP	Mean Arterial Pressure
MEAF	Model for Early Allograft Function

MELD	Model for End Stage Liver Disease
MHRA	Medicines and Healthcare Products Regulatory Authority
MRCP	Magnetic Resonance Cholangiopancreatography
NHSBT	National Health Service Blood and Transplant
NMP	Normothermic Machine Perfusion
OCTRU	Oxford Clinical Trials Research Unit
PGD	Primary Graft Dysfunction
PH	Proportional Hazards
PIL	Patient Information Leaflet
PNF	Primary Non-Function
PP	Per-Protocol analysis
QUOD	Quality in Organ Donation
R&D	Research and Development
REC	Research Ethics Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SC	Stratified Cox model
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SD	Standard Deviation
SITU	Surgical Intervention Trials Unit
SME	Small and Medium-sized Enterprises
TMC	Trial Management Committee
TNF	Tumor Necrosis Factors
UK-DRI	United Kingdom Donor Risk Index
USADE	Unanticipated Serious Adverse Device Event
UW	University of Wisconsin
vWF	Von Willebrand Factor
WP	Work Package

1 INTRODUCTION

This document details the proposed presentation and analysis for the main paper(s) reporting results from *the European Union Seventh Framework Programme (FP7) funded multicentre randomised controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation (WP2).* The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.

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[1] Chris Watson and Josep Grinyo are the Chair and the Vice-Chair, respectively, of the COPE DMC but Josep Grinyo will take the place of DMC Chair for this trial to ensure independence, due to Cambridge being a participating centre.

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2 CHANGES FROM PREVIOUS VERSION OF SAP

Issue Date	Details
04Feb2015	First version of SAP based on Protocol version 2.0 14th October 2014.
22Mar2016	Second version of SAP based on Protocol version 3.0 11th February 2016 including the following changes: – Patrizia Burra removed as DMC member (Section 1.1) – Primary analysis timepoint updated from 6 months to 30 days following new protocol version (Section 3.2) – Mention of Data Management Plan in Section 4 removed as none present for the study – MELD and DRI formulas updated; eGFR equation added (Section 6.2) – Baseline characteristics table updated (Section 6.2) – Patient groups for analysis (Section 7) updated with specific protocol deviations added – Subgroup analysis (section 8.5) updated – Secondary outcomes analysis (Section 9 and subsections) updated and clarified – Exploratory analyses (Section 10.1) added
25Oct2016	Third version including following changes: – New hepatologist added in DMC memberships (Section 1.1) – Flow diagram refined (Section 6.1) – Baseline characteristics table and section updated with further characteristics and p-value reporting for recipient characteristics (Section 6.2); UK-DRI formula updated and reference added – Summary of intervention details (Section 6.5) updated – Secondary analysis and subgroup analysis of primary outcome updated (Section 8) – Secondary outcomes analysis (Section 9 and subsections) updated and clarified – Exploratory analyses (Section 10.1) updated

3 BACKGROUND INFORMATION

The experimental precursor to the proposed normothermic perfusion system was developed 15 years ago and the method of perfusion of the isolated pig liver with autologous blood has since been extensively tested and refined, although the overall design of the perfusion circuit remains unchanged [1]. The circuit incorporates a centrifugal pump, membrane oxygenator, and heat exchanger. Arterial perfusion is directly pumped and the portal vein is perfused via a soft-shell reservoir using gravitational force. The addition of various substrates to the perfusion solution enables maintenance of metabolic function [2].

The initial preservation experiments were carried out to compare preservation by warm perfusion with conventional cold preservation [3]. Porcine livers were retrieved and stored for a period of 24 hours, either flushed with UW solution and placed in an icebox or attached immediately to the preservation circuit. Both groups of livers were then reperfused on the circuit for 24 hours (as a surrogate for transplantation) and markers of cellular injury and of synthetic and metabolic liver function were measured. These experiments demonstrated significant superiority of normothermic machine perfusion in terms of haemodynamic, biochemical and histological parameters

Subsequent experiments investigated the use of oxygenated, normothermic perfusion in an experimental setting that reflected the clinical situation of DCD donor organ retrieval [4]. Perfusion with normothermic blood was again compared with static cold storage after 60 min of warm ischaemia. Normothermic perfused livers demonstrated recovery of function by synthetic function, substrate utilisation and perfusion hemodynamics. Furthermore these livers displayed less cellular injury as shown by hepatocellular enzymes. In contrast, cold stored livers showed no evidence of viability during reperfusion and massive necrosis on histological examination.

It is recognised that the combination of warm ischaemia and conventional cold preservation leads to a poor outcome in DCD liver transplantation [5]. In the experimental setting, it is possible to institute warm perfusion with minimal exposure of the organ to cooling. However, in contrast, the logistics of clinical multi-organ retrieval in a distant donor hospital are complex and would be simplified by a period of cold preservation prior to normothermic preservation. This would enable the liver to be retrieved in the normal way, transported in an ice box and then attached to the perfusion machine once back at the base hospital. This scenario was simulated in the same experimental model by inserting a period of cold preservation prior to normothermic preservation [6]. Porcine livers were subjected to 60 minutes of warm ischaemia and then assigned to either normothermic preservation for 24 hours or cold preservation in University of Wisconsin solution for 4 hours followed by 20 hours normothermic preservation to achieve a total preservation time of 24 hours [6]. Livers that underwent normothermic preservation throughout had superior bile production, metabolic activity (base deficit and greater glucose use), and less hepatocellular damage (transaminase levels), and sinusoidal endothelial cell dysfunction (hyaluronic acid). The histology of livers that had been exposed to 4 hours of cold preservation before normothermia showed more necrosis and destruction of architecture. A similar study investigated 60 minutes of warm ischemia followed by 1 hour of cold preservation before 23 hours of normothermic perfusion [7]. This also showed evidence of increased hepatocellular injury, sinusoidal cell injury, but no detriment in terms of protein synthesis (factor V), bile production or histological features. These studies, therefore, demonstrated the need for the warm preservation device to be transportable so that normothermic preservation can be instituted with a minimal period of cooling at the time of organ retrieval.

In order to confirm these results in a preclinical model of organ transplantation, a series of liver transplants in a pig model was performed [8]. In these experiments pig livers were cold-preserved or warm-preserved (using the same machine perfusion methodology as before) for either 5 hours or 20 hours, followed by liver transplantation. As a model of DBD and DCD clinical scenarios, organs were cold-perfused *in situ* either at the time of cessation of circulation (as in a DBD organ donation) or after 40 and 60 minutes of warm ischaemia (simulating DCD organ donation). The two preservation

times were selected because 5 hours is comfortably within, and 20 hours substantially beyond, the limit of the conventional cold preservation technology in pigs (in which a generally accepted limit for survival is 12 hours). Similarly the 40 and 60 minute periods of warm ischaemia are considerably longer than would be acceptable in current clinical practice where warm ischaemia rarely exceeds 30 minutes. Indeed, success at 40 minutes would raise the realistic prospect of transplantation of donor livers from uncontrolled DCD donors.

There was no difference in outcome between the two groups at 5 hours of preservation. After 20 hours of preservation, there were significant advantages consistently in warm compared to cold preservation of both DBD and DCD organs. These advantages applied to postoperative enzyme release and animal survival. Notably, in the 20 hour warm-preserved groups, there was no difference in survival or postoperative transaminase levels in recipients of DBD compared to DCD (40 minute warm ischaemia) donor organs (86% versus 83%). At 60 minutes of warm ischaemia and 20 hours normothermic preservation, however, there were no survivors.

Analysis of haemodynamic, synthetic and metabolic parameters showed that those groups of livers that subsequently went on to successful transplant were predictable before transplantation on the basis of portal flow/pressure, acid-base homeostasis and several other biochemical parameters [8]. It may be concluded that normothermic perfusion, in this context, is not only a more effective means of organ preservation than conventional cold storage, but also that this method can be configured to provide an effective means of viability assessment [9].

The prototype version of the automated clinical investigation device has been tested and demonstrated to be effective during pre-clinical studies in which human livers, discarded as unsuitable for transplantation, were perfused for 24 hours. 13 such livers were perfused with human blood and the perfusion characteristics and control algorithms have been shown to be equally applicable to human as to pig livers (manuscript in preparation). More recently, the clinical trials device has been tested, using livers declined for clinical transplantation, and all key functional aspects of the device shown to be operational, including particularly transport to the donor hospital, automation and 24 hour perfusion.

Phase 1 clinical trial data

A phase 1 clinical trial was opened at King's College Hospital in 2012 and extended to the Queen Elizabeth Hospital, Birmingham in 2013. The first patient was transplanted with a normothermically-perfused liver in February 2013. As of December 27th 2013, the trial completed recruitment and transplanted the twentieth recipient with a liver preserved using the OrganOx *metra* device (in the configuration intended for the COPE study). In all these cases, perfusion parameters were stable with good acid-base maintenance. Postoperatively, all patients have made good recoveries.

3.1 Objectives

Hypothesis

Normothermic machine perfusion (NMP) is superior to static cold storage (SCS) of human liver allografts for reduction of preservation injury.

Primary objective

To compare the effect of NMP to SCS in the prevention of preservation injury and graft dysfunction, as measured by peak transaminase levels in the first week following transplantation.

Secondary objective

- To compare graft and patient survival between NMP and SCS livers
- To compare biochemical liver function between NMP and SCS livers

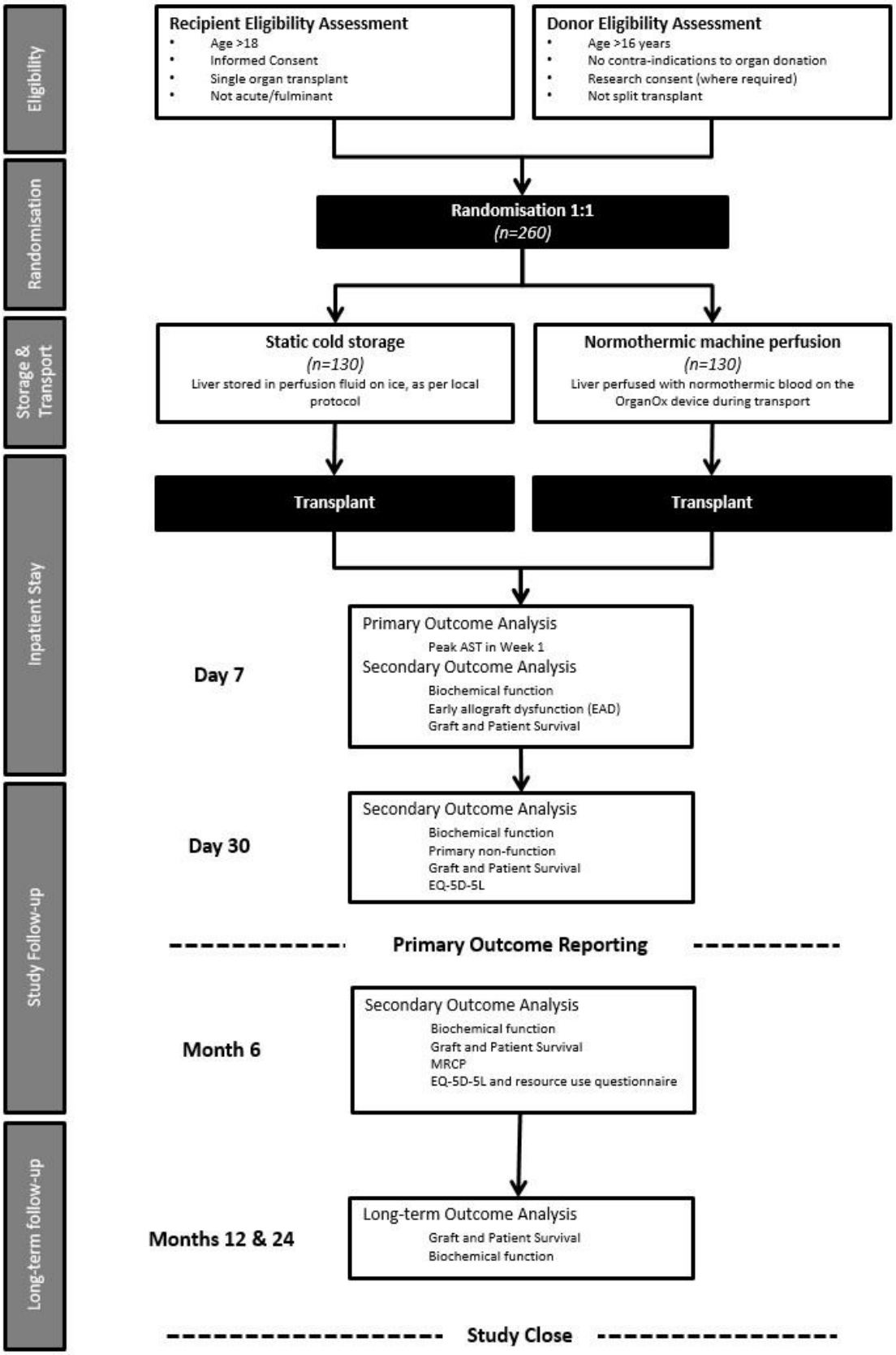
- To compare evidence of post reperfusion syndrome between NMP and SCS livers on implantation
- To compare evidence of ischaemia reperfusion injury between NMP and SCS livers
- To compare evidence of ischaemic cholangiopathy between NMP and SCS livers
- To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation
- To assess the feasibility and safety of NMP as a method of organ storage and transportation
- To assess the health economic implications of normothermic liver perfusion

3.2 Study Design

This is a randomised controlled, non-blinded, clinical trial comparing static cold storage (SCS) to normothermic machine perfusion (NMP) for organ preservation prior to liver transplantation. Following assessment of donor and recipient eligibility and confirmation of consent, the liver will be randomised to either NMP or SCS. At the end of preservation, the liver will be transplanted and the patient managed according to standard local practice and protocols.
Enrolled patients will participate in the study for 6 months, with outcomes assessed during the initial inpatient stay and at study visits at day 30 post-transplant and at month 6 post-transplant. Additional biochemical and survival data will be collected from routine clinical measurements taken in participating centres at 12 months and 24 months post-transplant.
Primary outcomes will be analysed and reported 30 days following enrolment of the last patient to the study. The study will close after the final patient has completed 24 months follow-up.
Anticipated flow of patients through the trial is depicted in Figure 1.

<u>Anticipated trial dates</u>

Date of start of recruitment:	May 2014
Date of expected end of recruitment:	June 2016
Date of expected primary analysis:	December 2016
Date of expected long-term follow-up analysis:	June 2018
Target number of subjects (livers):	220 transplanted livers (110 per arm) 260 randomised livers (130 per arm)
Participating Centres:	Addenbrooke's Hospital, Cambridge, UK King's College Hospital, London, UK Queen Elizabeth Hospital, Birmingham, UK Royal Free Hospital, London, UK Hospital Clinic Barcelona, Spain University Hospital, Essen, Germany University Hospitals, Leuven, Belgium



*Randomisation performed soon after organ retrieval by recipient centre

Figure 1. Flow of participants/livers through the trial

3.3 Eligibility

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

3.3.1 Donor criteria

Inclusion: Donors over the age of 16 years. Liver allografts from donation after brain death (DBD), standard and extended criteria donors (SCD, ECD) and donation after circulatory death (DCD) donors.
Exclusion: Living donors; liver intended for split transplant; donor age <16 years; liver in which investigator is unwilling to randomise to either arm.

3.3.2 Recipient criteria

Inclusion: Adult patients (18 years or more), active on the waiting list for liver transplantation; able to give informed consent.
Exclusion: Age less than 18 years; acute/fulminant liver failure; transplantation of more than one organ (e.g. liver and kidney); refusal of informed consent; unable to give informed consent.

3.3.3 Donor assessment

The transplant recipient co-ordinator is usually an advanced practice nurse, one of whose roles is to facilitate calls for organ offers and co-ordinate all aspects of the transplant process. On receiving an organ offer, the local recipient co-ordinator/surgeon will ascertain baseline demographic information from the offering organisation to assess eligibility of the liver for inclusion in the trial.

3.3.4 Recipient assessment

All patients on the transplant waiting list in participating centres will have been screened for suitability for transplantation; further screening assessment is not required as part of the present trial. On offer of a suitable donor organ consent will be confirmed by signing and dating the informed consent form for a second time; this process may also be done with a documented phone call. The online randomisation tool will require confirmation that the recipient meets the inclusion criteria for the trial and require the entry of baseline demographic information prior to randomisation and release of the randomisation code.
On admission to hospital, the recipient will be assessed for fitness to proceed to transplant according to local procedures. If a recipient is deemed unfit for transplant at the time of admission[2], they will no longer be active on the transplant waiting list and as such will be excluded from the trial. Any data collected from these individuals will not be included in the trial and will be deleted.

3.4 Treatment Interventions

On receipt the organ will be assessed and if not suitable for transplantation this will not go ahead.

3.4.1 NMP Group

If the liver is randomised to the NMP group, arrangements will be made to transport the OrganOx *metra* device to the donor hospital (see section 7.6). The recipient co-ordinator will also request that

[2] In UK, livers allocated to a recipient deemed unfit and so excluded from the trial are usually allocated to another recipient in the same hospital, so personnel will try to allocate them to the next patient who has consented.
In the other countries (participating centres) these livers will probably be lost from the trial.

However, patients unfit for transplantation are expected to be very few, probably less than 5%.

the donor co-ordinator arrange for 3 units of donor-type red blood cells to be cross-matched at the donor centre for use as the perfusate in the device. Following the routine retrieval procedure at the donor hospital 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 device instructions for use (IFU) document. The device is then transported to the recipient transplant centre. 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. 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.
If cannulation proves impossible, the liver will be transported using standard static cold storage as described below. Results will be analysed in the randomised group (intention-to-treat)[3].

3.4.2 SCS group

Following the routine retrieval procedure, the liver will be placed in ice-cold perfusion solution (according to local protocols) on the back-table, followed by storage in cold perfusion solution within an icebox. The organ will be transported to the recipient centre, and removed from storage prior to implantation for standard back-table preparation. The duration of cold storage will be dictated by logistics and local policy.

3.5 Sample Size

Data from 416 liver transplant recipients from University Hospital Essen demonstrate the geometric mean of peak AST to be 608.59 IU/L (the geometric mean is used as peak AST is non-normally distributed). 220 transplants (110 per arm) would have 90% power at 5% significance level to detect a 33% reduction (to 401.67 IU/L) in the geometric mean of peak AST.
2011/12 NHSBT data suggest that 12% of livers retrieved are not transplanted. Assuming losses of 15%, randomisation of 260 livers into the trial will be required to achieve adequate power.
Data from a study of hypothermic machine perfusion of human livers demonstrate an approximate 35% reduction in the peak AST with machine preservation when compared to historical controls undergoing static cold storage [10]. It is expected that normothermic machine perfusion will be at least as effective as hypothermic machine preservation in preventing reperfusion injury. In studies of a porcine model of DBD liver transplantation, normothermic machine perfusion led to a 52% reduction in peak post-transplant AST [8]. In a DCD model, the reduction was 73%.
Given the relationship between peak AST and primary non-function, graft and patient survival described above [11, 12], a 33% reduction in peak AST is likely to represent a clinically significant difference in outcome between the study arms.
It is recognized that a proportion of DCD donor livers randomized in the study will not proceed to donation due to the donor not arresting within the time defined by local protocols. These livers will be replaced in the study so that the numbers above reflect the number of livers actually retrieved.

The sample size calculation has been performed using R statistical software, whose output is shown below:

```
> exp(mean(log(peakast)))
```

[3] In phase I trial "aberrant arterial anatomy", which prevents from performing cannulation, happened in 10-15% of cases but the surgeon was able to reconstruct it, so no liver was actually moved to SCS.
In phase III this is less likely to happen, but an estimated percentage cannot be provided.

```
[1] 608.5898
> exp(mean(log(peakast)))*0.66
[1] 401.6693
> control <- exp(mean(log(peakast)))
> study <- exp(mean(log(peakast)))*0.66
> log(control)-log(study)
[1] 0.4155154
> sd(log(peakast))
[1] 0.9456289
> (log(control)-log(study))/sd(log(peakast))
[1] 0.4394065
> pwr.t.test(d=0.4394, sig.level=0.05, power=0.90, type="two.sample", alternative="two.sided")
Two-sample t test power calculation
n = 109.8137
d = 0.4394
sig.level = 0.05
power = 0.9
alternative = two.sided
```

The actual number of livers needed, assuming 15% of losses, was then calculated by hand based on the following formula:

$$N_A = \frac{N}{1-p} = \frac{220}{1-0.15} = 258.82 \sim 260$$

Where p is the proportion of losses, N is the total sample size and N_A is the total number needed to achieve N considering the loss rate.

3.6 Strategies for achieving adequate recruitment

The emergency nature of liver transplantation means that once a potential recruit is called in for a transplant there will only be a 3-4 hour window for the consent and screening process to occur. This does not allow sufficient time for the potential participant to consider the implications of participating in the study. For this reason, all patients who fulfil the entry criteria and who are on the waiting list for liver transplantation at the participating centres will be approached in advance of the study either during a routine clinic appointment, inpatient admission or in advance of discussion by letter. If the patient expresses interest in the study, a face-to-face meeting will be arranged during a routine admission or outpatient appointment. Detailed information will be given both verbally and in the form of a patient information sheet. The study coordinator and/or a medically qualified researcher will give information.

3.7 Randomisation

Full details will be stored in a separate Randomisation and Blinding Plan document to ensure adequate concealment of the schedule.

3.7.1 Sequence generation

Participants will be randomly assigned to NMP or SCS with 1:1 allocation as per a computer generated randomisation schedule using variable block randomisation using the following stratification factors: participating (recipient) centre and by donor type (DBD or DCD).

3.7.2 Allocation concealment mechanism

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

3.7.3 Implementation

Prior to study enrolment, the local investigator will confirm the availability of the NMP device. Once informed consent has been obtained from the potential recipient of an organ offer, the local investigators will login to an online data collection and randomisation tool. This will require confirmation of consent, as well as compliance with inclusion and exclusion criteria. Baseline recipient and donor characteristics will be recorded by the recipient co-ordinator and are required prior to release of the randomisation group. This will ensure that the patient is eligible for inclusion in the trial prior to allocation.

3.7.4 Blinding/Masking

Whilst it is not possible to blind the local investigators to the method of organ preservation, outcome assessors will be blinded where possible. This includes the histopathologist interpreting the biopsy specimens as well as the radiologist interpreting the 6-month MRCP images.
Note the primary outcome and many secondary outcomes are objective measures, so blinding is not a requirement.

Randomisation is expected to occur before retrieval of the liver, provided that both donor and recipient exclusion and inclusion criteria are met. However, note that it is expected that in some European participating centres this may occur at a slightly different time-point i.e. just after assessment of the organ upon retrieval. Therefore, in such cases there will be a smaller rate of liver withdrawn from the trial for reasons like DCD donor not proceeding and organ deemed not transplantable.

3.8 Primary and Secondary Outcomes

Primary outcome
The primary endpoint is defined as the difference in peak serum aspartate transaminase level (AST) within 7 days post-transplant between the two treatment arms. Serum AST will be measured daily during the first post-transplant week, and the peak level will be defined as the highest of these values (in IU/L). In order to ensure consistency, the first post-transplant measurement should be taken at 12 to 24 hours post-reperfusion.

A number of studies have demonstrated a relationship between peak AST in the early post-transplant period and patient survival, graft survival, early graft dysfunction and primary non-function following liver transplantation [11, 12]. Peak AST is also significantly elevated in liver allografts with histological evidence of moderate to severe perfusion injury [13, 14].

Secondary outcomes

Objective	Outcome Measures
To compare graft and patient survival between NMP and SCS livers.	1. Primary non-function: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes. 2. Graft survival at 30 days and 6, 12 and 24 months following transplantation. 3. Patient survival at 30 days and 6, 12 and 24 months following transplantation.
To compare biochemical liver function between NMP and SCS livers.	1. Serum bilirubin, GGT, AST, INR and creatinine daily at days 1-7 following transplantation then at day 30 and months 6, 12 and 24 following transplantation. 2. Daily serum lactate at days 1-7 whilst in high level (ITU/HDU) care 3. Early allograft dysfunction (EAD) [15]; defined by any one of: a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant b. INR >1.6 on day 7 post-transplant. c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant
To compare the physiological response to reperfusion between NMP and SCS livers	1. Post-reperfusion syndrome, defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than one minute during the first five minutes after reperfusion. This will be assessed in the context of vasopressor use [16, 17] 2. Length of stay in high level (HDU/ITU) care 3. Length of hospital stay 4. Need for renal replacement therapy (haemodialysis, haemofiltration, haemodiafiltration)
To compare evidence of reperfusion injury between NMP and SCS livers.	Histological evidence of reperfusion injury in post-reperfusion biopsies (taken immediately prior to abdominal closure). These will be compared to baseline pre-reperfusion biopsies (on removal of the liver from SCS/NMP) and graded using standard histological criteria [13, 18]
To compare evidence of ischaemic cholangiopathy between NMP and SCS livers.	Evidence of biliary stricturing on magnetic resonance cholangiography (MRCP) at 6 months post-transplant.
To assess the ability of perfusion parameters and biomarkers in perfusion fluids 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 mmHg) c. pO_2, pCO_2 and pH d. Blood temperature (°C), Glucose (mmol/L) and bile production (ml/h) 2. Perfusate ALT and AST at 15 minutes, 1 hour and the end of NMP 3. Perfusate IL6, TNF, vWF at 15 minutes, 1 hour and the end of NMP 4. In addition to these pre-specified outcomes, additional biological samples will be taken for the COPE WP7

	bioresource at specified timepoints as detailed in protocol appendix A1.
To assess the feasibility and safety of NMP as a method of organ storage and transportation.	1. Organ discard rate 2. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture (cold preservation or warm perfusate). 3. Adverse event rates and severity, graded according to the Clavien-Dindo classification [19] as described in Appendix 1: CLAVIEN DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS. a. Recipient infection b. Biopsy proven acute rejection c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks) d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis) e. Reoperation rate f. Technical complications/device failures
To assess the health economic implications of normothermic liver perfusion.	Full health economic analysis utilising: 1. Logistical costs, measured using national unit costs where available. 2. Healthcare resource use; measured by a combination of hospital episode records and a patient-completed resource use log. 3. Quality of life by delivery of the EQ-5D-5L questionnaire at baseline, day 30 and month 6 post-transplant.

3.9 Outcomes Assessment Schedule

Activity	**Pre-study Screening**	**Pre-study Baseline**	**Pre-storage**	**Pre-reperfusion**	**Post-reperfusion**	**Postoperative**									**Follow-up**		
						D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D30**	**M6**	**M12**	**M24**
Informed consent	X																
Meets inclusion/ exclusion criteria	X																
Randomisation		X															
Donor & recipient demographics		X															
Perfusion parameters/samples				X													
Surgical variables					X												
Graft biopsy			X	X	X												
CBD biopsy					X												

Serum AST						X	X	X	X	X	X	X		X	X	X	X
Serum Bilirubin						X	X	X	X	X	X	X		X	X	X	X
Serum GGT						X	X	X	X	X	X	X		X	X	X	X
Serum Creatinine						X	X	X	X	X	X	X		X	X	X	X
INR						X	X	X	X	X	X	X		X	X	X	X
Serum lactate*						X	X	X	X	X	X	X					
Primary non-function													X				
Graft survival						X	X	X	X	X	X	X	X	X	X	X	X
Patient survival						X	X	X	X	X	X	X	X	X	X	X	X
MRCP															X		
Quality of life (EQ-5D-5L)	X													X	X		
Resource use log														X	X		
Safety outcomes					X	X	X	X	X	X	X	X	X	X	X	X	

* Serum lactate will be recorded daily whilst the recipient is admitted to high level (ITU/HDU) care.

3.10 Data Management Responsibility

Data management responsibility is detailed in a separate data management plan prepared by the trial coordinator, the trial statistician and the database manager.

4 QUALITY CONTROL AND DATA VALIDATION

Source documents are where data are first recorded, and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records, clinical and office charts, laboratory reports, pharmacy records, subject diaries or logs, microfiches, radiographs, correspondence, device accountability records, recorded data from automated instruments and copies or transcriptions certified after verification as being accurate and complete.
eCRF entries will be considered source data if the eCRF is the site of original recording (e.g. there is no other written or electronic record of the data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name. Signed consent forms will be kept at the site and not sent to the Trial Office.
The central database will be monitored for discrepancies and missing data. The surgical interventional trials unit (SITU) will be responsible for managing the database, and if such discrepancies are identified the trial manager will be responsible for identifying the problem and contacting the local centre to ensure resolution. The trial manager will be responsible for the production of reports to each participating centre containing information and details of missing data or missed visits requiring completion.

5 DATA MONITORING COMMITTEE AND INTERIM ANALYSES

The trial has a data monitoring committee (DMC) which consists of five independent members, including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DMC will periodically review (approximately 6 monthly) accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. The DMC will advise the Trial Management Committee and WP8 if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms.

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

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

6 DESCRIPTIVE ANALYSES

6.1 Representativeness of Study Sample and Patient Throughput

Participants will be adult patients active on the waiting list for liver transplantation at any of the participating transplant centres. Patients screened for eligibility, reasons for exclusion and the flow of livers and recipients through the study will be summarised in the following adapted flowchart.

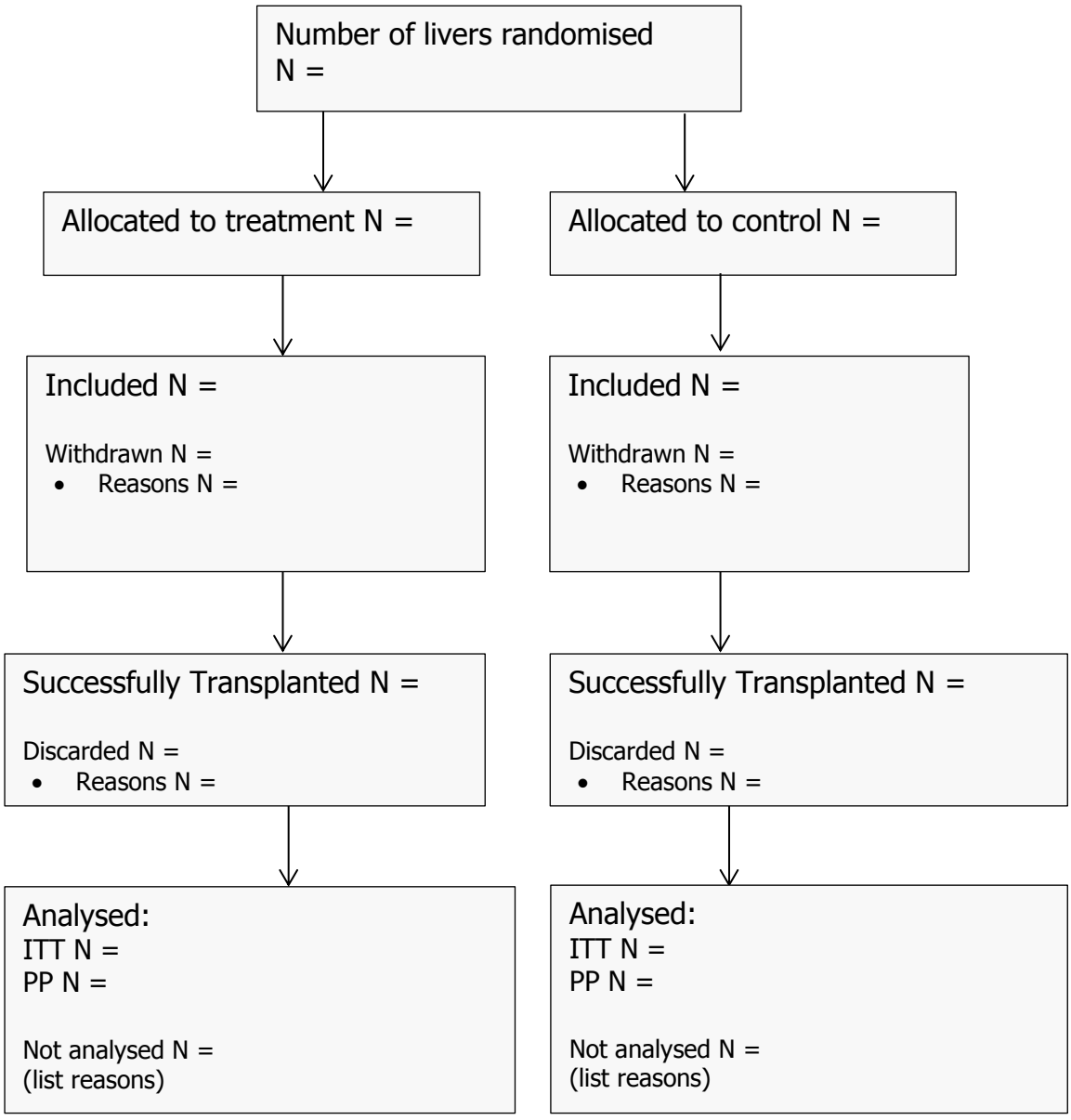


Figure 2. Adapted flowchart for WP2

6.2 Baseline Comparability of Randomised Groups

Participants will be described with respect to the stratification factors (participating centre and donor type) and to both donor and recipient demographics and other key prognostic factors at baseline, both overall and separately for the two randomised groups.

MELD Score will be described overall and separately by country due to variations in national donor rates causing likely differences in average MELD scores in different countries.
The formula to calculate MELD Score from serum creatinine, bilirubin and INR, developed and validated by Kamath and Kim [20] is the following:

$$MELD\ Score = 0.957 \times \ln\left[serum\ creatinine\left(\frac{mg}{dL}\right)\right] + 0.378 \times \ln\left[serum\ bilirubin\left(\frac{mg}{dL}\right)\right] + 1.120 \times \ln[INR] + 0.643$$

The resulting score will be multiplied by 10 and rounded to the nearest whole number.
UNOS has made the following modifications to the score [21]:

- If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0
- Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any positive value below 1 would yield a negative result).

DRI will be described overall and calculated using both the UK-DRI and the Eurotransplant DRI formulas. The UK-DRI formula has recently been developed and the relevant paper will soon be published in *Transplantation* (Collett D, Friend PJ and Watson CJE. *Factors associated with short and long term liver graft survival in the United Kingdom: development of a UK Donor Liver Index*.)

$$UK\text{-}DRI = exp\big[2.3159 + (0.9106\ if\ DCD) - 0.01434 \times \big(height(cm)\big) + (0.3058\ if\ history\ of\ cardiac\ disease) + (0.2545\ if\ steatosis\ present) + 0.01222 \times \big(bilirubin(\mu mol/L)\big) + (0.1736\ if\ positive\ smoking\ history) + (0.6453\ if\ black\ ethnicity)\big]$$

The ET-DRI formula has been adapted for the purposes of this study from the one developed by Braat et al [22].

$$ET\text{-}DRI = exp\big[0.960\big((0.154\ if\ 40 \le age < 50) + (0.274\ if\ 50 \le age < 60) + (0.424\ if\ 60 \le age < 70) + (0.501\ if\ 70 \le age) + (0.079\ if\ COD = hypoxia) + (0.145\ if\ COD = CVA) + (0.184\ if\ COD = other) + (0.411\ if\ DCD) + (0.105\ if\ different\ retrieval\ team)\big) + 0.06((latest\ lab\ GGt(U/L) - 50)\ /\ 100)\big]$$

The calculation of eGFR is based on the CKD-EPI Creatinine equation [23] as follows:

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

Scr is serum creatinine in mg/dL;
κ = 0.7 for females
κ = 0.9 for males;
α = –0.329 for females
α = –0.411 for males.

Serum creatinine can be recorded in mg/dL or in µmol/L. However, it will be reported and used in mg/dL for the purposes of analysis so all values will need to be converted $\left(\text{mg/dL} = {}^{\mu\text{mol/L}}/_{88.4}\right)$.

Numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with lower and upper quartiles) for continuous variables will be presented.
There will be no tests of statistical significance nor confidence intervals for differences between randomised groups for stratification factors and donor characteristics. However, due to the nature of the study (the randomisation unit is the donated liver and not the participant) and to a potential differential discard rate between the two groups, an appropriate statistical test will be applied for recipient characteristics and a p-value reported to demonstrate that the final recipients were well matched.

Stratification factors	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Centre*			
Addenbrooke's Hospital, Cambridge, UK			
King's College Hospital, London, UK			
Queen Elizabeth Hospital, Birmingham, UK			
Royal Free Hospital, London, UK			
University of Barcelona, Spain			
University Hospital, Essen, Germany			
University Hospitals, Leuven, Belgium			
Donor type*			
DBD			
DCD			
Donor demographics	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Gender*			
Male			
Female			
Age^			
Ethnicity*			
Caucasian			
African-Caribbean			
Other			
Cause of death			
CVA			
Hypoxia			
Trauma			
Other			
BMI^			
UK-Donor risk index^			
ET-Donor risk index^			
Recipient demographics at randomisation	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Transplant centre*			
Addenbrooke's Hospital, Cambridge, UK			
King's College Hospital, London, UK			
Queen Elizabeth Hospital, Birmingham, UK			

Royal Free Hospital, London, UK
University of Barcelona, Spain
University Hospital, Essen, Germany
University Hospitals, Leuven, Belgium

Gender*

Male
Female

Age^

Cause of Liver Failure*

Alcoholic
Auto-Immune Hepatitis
Drug Induced
Hepatitis B
Hepatitis C
Hepatocellular Carcinoma on background of Cirrhosis
Hepatocellular Carcinoma without Cirrhosis
Metabolic
Non-Alcoholic Fatty Liver Disease
Non-Alcoholic Steato-Hepatitis
Other Cancers
Primary Sclerosis Cholangitis
Primary Biliary Cirrhosis
Other

BMI^

MELD score^

UK
Essen, Germany
Leuven, Belgium
Barcelona, Spain

eGFR^

**Frequency and percentages are displayed*
^Median, IQR and range are displayed

6.3 Comparison of Losses to Follow-up

The numbers (with percentages) of losses to follow-up will be summarised at each stage. Those lost to follow-up will be reported and compared (where appropriate) between the NMP and SCS groups with absolute risk differences (95% confidence interval [CI]). Any deaths (and their causes) will be reported as described in the secondary outcomes analysis section.

6.4 Description of Available Data

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. Methods to handle missing data are described in section 8.4.

HRQL questionnaire, EQ-5D-5L, will be collected at baseline (pre-study screening) and at each early follow-up visit following liver transplantation (day 30 and month 6). Details of completed forms will be provided for each follow-up point, overall and separately for the two treatments and will include the numbers of forms expected, received and the compliance rate as in the following table.

Time Point	Overall		
EQ-5D-5L	Expected	Received	Compliance
Baseline			
Day 30			
6 months			

6.5 Description of Intervention

A summary of the treatment received will be provided, particularly the warm ischaemic time, cold ischaemic time, duration of machine perfusion, total preservation time, implantation time, perfusion parameters (logged automatically by the device) and any procedural complications. They will be reported as frequencies and percentages or median and ranges, as appropriate.
The duration of the total preservation will also be compared between the two groups using non-parametric tests. This is defined as time from "cross clamp time" to "time of arterial/portal reperfusion" (whichever occurs first) in the recipient.
Perfusion parameters collected will be:

1. Arterial and caval pressures (in mmHg)
2. Arterial, portal and caval flow rates (in mmHg)
3. pO2, pCO2 and pH
4. Blood temperature (°C), Glucose (mmol/L) and bile production (ml/h).

Perfusate ALT and AST and perfusate IL6, TNF, vWF will be collected at 15 minutes, 1 hour and the end of NMP.
Pre and post-reperfusion vasopressor and total duration of operation will also be reported as described above.
Protocol deviations (see Section 7) and any conversion from NMP to SCS will be reported with reasons for not receiving the assigned treatment.
Fisher exact test or Chi-squared test will be used to test the association between compliance and treatment group.

6.6 Unblinding of Randomised Treatments

As the endpoints are objective the study is open-label but, where feasible, outcome assessors will be blinded, e.g. histopathologist interpreting the biopsy specimens and radiologist interpreting MRCP imaging.

6.7 Reliability

Calculations performed by the computer will be checked by hand calculations for a minimum of 5% or 20 patients, randomly sampled, where appropriate.

Missing value codes will be checked for consistency and the proportion of missing values per variable will be presented. Patterns of missing data will be explored. Any missing value imputation used will be checked to ensure the missing values have been imputed within the limits of the data.

7 PATIENT GROUPS FOR ANALYSIS

All livers/patients who undergo preservation and transplant will be analysed.

Intention-to-treat analysis (ITT): liver analysed in the groups to which their liver is randomly assigned, irrespective of whether the assigned method of preservation is actually used.

Per protocol analysis (PP): Liver analysed as treated; therefore, in the groups of the intervention they actually received. This will be carried out as a sensitivity analysis and will also exclude patients who had the following protocol deviations:

- Machine preservation time <4 or >24 hours;
- Intervention/machine failure that resulted in the treatment not being carried out as per protocol.

Also, in case of change of transplant centre (stratification factor), in the PP analysis the liver will be analysed according to the centre where it was actually transplanted.

8 ANALYSES TO ADDRESS PRIMARY AIMS

It is anticipated that the analysis will be undertaken using STATA, SAS, R or other validated statistical software.
The trial is expected to complete recruitment within 24 months. Primary outcome analysis time point will be 30 days after the last liver has been transplanted.

8.1 Definition of Primary Outcome

Clinically significant response to treatment is defined as a 33% difference in peak serum aspartate transaminase level (AST) within 7 days post-transplant between the two treatment arms.

$$\frac{Peak\ Serum\ AST(SCS) - Peak\ Serum\ AST(NMP)}{Peak\ Serum\ AST(SCS)} \geq 0.33$$

For these analyses of the primary outcome a P-value of 0.05 (5% level) will be used to indicate statistical significance. Exact P values will be presented to three decimal places.

8.2 Statistical Methods Used for Analysis of Primary Outcome

The distribution of peak serum AST between the two arms will be assessed by a Normal plot for each treatment group. If this appears to be normally distributed the difference in peak serum AST between the two groups will be compared by using ANOVA with adjustment for stratification factors: participating (recipient) centre and donor type (DBD and DCD).
If peak serum AST has departure from Normality the first approach will be transformation. If the data cannot transform to Normal distribution, the difference in peak serum AST between arms will be analysed using Mann-Whitney U test. The difference will be presented along with 95% CI for the difference in medians. If a non-parametric analysis needs to be performed there will be no adjustment for stratification factors or other covariates.

8.2.1 Secondary Analysis of Primary Outcome

The analysis comparing the difference in peak serum AST between the two groups using ANOVA will be repeated with additional adjustment for important prognostic factors.

8.3 Adjustment of P values for Multiple Testing

There is no multiple testing as only a single primary outcome is considered. Therefore significance levels used will be 0.05 and 95% confidence intervals will be reported.

Interim analyses of primary and secondary endpoints will not be carried out unless requested by the DMC. In this case p-values of 0.001 will be used for significance.

8.4 Missing Data

All randomised patients completing the 30 days follow-up assessment will be regarded as having completed the primary study. All patients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments, and that patients do not incur extra financial costs (e.g. travelling costs) as a result of study participation.

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 unless the participant requests otherwise, as is stipulated in the trial consent form. In the event 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 data accrued before the date of withdrawal being included in the trial analysis. A narrative analysis of withdrawals will be performed.

As the primary outcome is the peak serum AST within 7 days post-transplant and there is a very strict protocol for post-operative procedure in place in every centre, it is expected that there will be no/few missing data. Even in case of missing daily values, the peak of available values will be taken, which is expected to occur within the first 36 hours post-transplant. Therefore, no imputation methods will be employed.
Strategies to handle missing data are, therefore, expected to be used only for secondary outcomes analyses or in long-term follow-up (12 and 24 months) analysis and only if the missing rate is 5% or more.

8.5 Pre-specified Subgroup Analysis

Subgroup analyses will be performed for donor type (DCD vs. DBD), donor risk index (ET-DRI) and MELD Score. Value intervals for ET-DRI will be 'low', 'medium' and 'high' based on the 33rd percentiles. Value intervals for MELD Score will be <9, 10-19, 20-29, 30-39, ≥40 based on the UNOS standard reference ranges[24].
The study is not powered to detect differences in the subgroups and should only be regarded as hypothesis-generating. Interaction methods will be used to look for consistency of treatment effect across the different subgroups and reported using forest plots.

8.6 Treatment by Centre Interaction

Consistency of effect will be assessed across the 7 centres by examination of the within centre effects. We are not expecting, however, differences as centre is a stratification factor. Any differences between centres will be explored graphically using forest plots as reported above and no formal test will be undertaken as there will be little power for a test of interaction.

8.7 Sensitivity Analysis

Sensitivity analyses will be carried out for the primary outcome using the per protocol population.

9 ANALYSIS TO ADDRESS SECONDARY AIMS

The secondary aims will determine the effect of NMP compared to SCS on graft and patient survival, biochemical liver function, physiological response to reperfusion, reperfusion injury and ischaemic cholangiopathy. They also will assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation and to assess the feasibility, safety and health economic implications of NMP as a method of organ storage and transportation.

Binary outcomes will be reported in terms of proportions along with 95% confidence intervals and will be assessed using chi-squared test. Logistic regression will be performed to adjust for potential confounders and the treatment difference will be reported using odds ratios with relevant 95% confidence intervals. Continuous outcomes will be reported as mean with standard deviation (SD) or as median and interquartile range (IQR), and compared using the T-test if normally distributed, or by the Mann-Whitney U. The treatment difference will be reported in terms of difference in means together with 95% confidence intervals. Time-to-event outcomes will be analysed using survival analysis methods, including Kaplan-Meier and Cox proportional hazards regression model with calculation of hazard ratios. The proportional hazards (PH) assumption will be tested and if non-proportionality is present appropriate methodology will be used such as stratified Cox (SC) model and extended Cox model containing time-dependent variables. Median survival times will be reported together with 95% confidence intervals.
Outcomes will be reported with 95% confidence intervals and p-values to 3 decimal places. A p-value of less than 0.05 will be regarded as statistically significant.

Primary timepoint for secondary outcomes analysis is at 6 months with long-term follow-up at 2 years.

9.1 Primary Non-Function

Primary Non-Function (PNF) is defined as irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes.

Presence of PNF will be compared across the two randomised groups by performing unadjusted and adjusted analysis as described above to take account of the stratification factors and important prognostic factors such as peak AST, DRI and MELD Score.

9.2 Graft and patient survival

Graft survival is defined as time (in days) from transplant to graft failure. Patients who die with a functioning graft will be censored at their date of death. Patients who are still alive with a functioning

graft at the end of the study will be censored at their last known date alive with functioning graft.

Patient overall survival is defined as time (in days) from transplant to patient death. Patients still alive will be censored at their last known alive date.[25]

Graft survival and patient overall survival will be compared across the two groups using the methods described above reporting at 6 months and at long-term follow-up time points. Cox proportional hazards regression model will be performed both in a univariate and multivariate framework adjusting for stratification factors and known or suspected prognostic factors such as donor and recipients demographics (BMI, DRI, indication for transplant, MELD Score).

9.3 Biochemical liver function

Liver function will be explored by assessing biochemical components of the blood serum. These are a number of serum biochemical tests: Biliburin, Gamma glutamyl transpeptidase (GGT), Aspartate transaminase (AST), International normalised ratio (INR), creatinine and lactate dehydrogenase.
All of these tests are interpreted using reference ranges.
The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the International Sensitivity Index (ISI) value for the analytical system used.[26]

$$INR = \left(\frac{PT_{test}}{PTnormal}\right)^{ISI}$$

There are circumstances where the INR is medically corrected. Although this is expected to be very uncommon, cases of INR being medically corrected will be recorded and this value used instead of the ordinary one. Proportion of INR medically corrected will also be reported overall and by treatment arm.
As Bilirubin, GGT, AST, INR and creatinine are measured daily at days 1-7 following transplantation and serum lactate daily at days 1-7 whilst in ITU/HDU care, they will be assessed using a number of different methods: their average value as well as area under the curve (AUC) will be calculated and compared across the two treatment arms.
Bilirubin, GGT, AST, INR and creatinine will also be measured at day 30 and month 6, 12 and 24 following transplantation. A mixed model for repeated measurement will be used at 24 months to compare each of these measures across the two groups.

9.3.1 Early Allograft Dysfunction

Early allograft dysfunction (EAD) has been defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function [15]:

a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant
b. INR >1.6 on day 7 post-transplant.
c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant

In case of missing data in any of the three variables required to define the EAD, this will be considered as present if any of the available values satisfies the specified criteria. In case of Bilirubin and/or INR missing due to the patient being discharged before Day 7 post-transplant, this will be considered as absence of EAD as the recipient would not be discharged earlier if the graft was not functioning. Subjects with missing data due to patient death or graft failure will be excluded from this analysis as expected to be few in numbers in the first 7 days post-transplant.
Presence of EAD will be compared across the two groups using the methods described above to adjust for potential confounders such as DRI and MELD Score.

9.4 Physiological response to reperfusion

Post-reperfusion syndrome is defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than a minute during the first five minutes after reperfusion.

$$\frac{MAP_{baseline} - MAP_{5mins}}{MAP_{baseline}} > 0.30 \text{ mmHg } for > 1min$$

Need for renal replacement therapy indicates the requirement of treatments for renal failure such as haemodialysis, haemofiltration and haemodiafiltration.
Differences in proportions of both post-reperfusion syndrome, need for renal replacement therapy abd post-reperfusion lactate levels between groups will be assessed by performing unadjusted analysis as described above.
Duration of renal replacement therapy at Day 7 will also be compared between the two groups using appropriate tests for continuous outcomes as described above.

Length of hospital stay and length of stay in HDU/ITU care will be summarised as median together with interquartile range and will be compared between the two groups using Kruskal-Wallis test. This will then be used as a resource for the health economic analysis (see Section 9.9).

9.5 Reperfusion injury

Graft biopsies will be taken immediately prior to abdominal closure and examined for evidence of reperfusion injury. These biopsies will be compared to the baseline biopsies prior to organ reperfusion (on removal of the liver from SCS/NMP) and graded according to standard histological criteria [13, 18]. The trial histopathologist, who will be blinded to the method of perfusion, will assess all biopsies.

Evidence of reperfusion injury will be compared across the two treatment groups by performing unadjusted analysis as described above.

9.6 Ischaemic cholangiopathy

All study participants will undergo magnetic resonance cholangiopancreatography (MRCP) with T2-weighted turbo-spin echo sequences at 6 months post-transplant unless contraindicated. The trial radiologist, who will be blinded to the preservation method, will assess all MRCPs. Evidence of ischaemic cholangiopathy will be taken as the presence of extra-anastomotic biliary structuring in the absence of hepatic artery thrombosis [27, 28].

Proportions of MRCP evidence of ischaemic cholangiopathy will be compared across treatment arms by performing unadjusted analysis as described above.

9.7 Perfusion parameters and biomarkers

Perfusion parameters will be collected and used to give a summary of the intervention, as described in section 6.5.
Biomarkers will also be collected to be used with perfusion parameters as described in the Exploratory Analyses section (10.1).

9.8 Feasibility and safety of NMP

Organ discard rate and adverse events rates and severity will be assessed as described in the Safety Analysis section (11).

9.9 Resource Use and Cost Data – only include if written by Economists

These will not be analysed by the statistician. All the planned health economic analyses will be detailed in a separate document held by the trial health economist.

Summary of cost-effectiveness analysis:
The trial health economist will perform an economic analysis with the objective of estimating average costs and effectiveness in each arm of the study. This will inform a cost-effectiveness analysis using a health service perspective and incremental cost effectiveness ratios (ICER's) will be reported. Quality adjusted survival will be obtained by administration of the EuroQol EQ-5D-5L questionnaire. Quality of life data will be collected at baseline (pre-transplant, at time of consent) and at each study follow-up visit following liver transplantation (day 7, day 30 and month 6).
Costs will be estimated based upon measured resource use and national unit costs. Resources will include machine and disposables costs, immunosuppression and other drugs, inpatient hospital stays (including intensive care days), radiological investigations, biopsies and other procedures, outpatient visit and visits to the family doctor. Resource use will be identified from case report forms, hospital episode statistics/insurer claims and from patient self-reporting using a simple log/questionnaire (to assess out-of hospital resource use). These questionnaires will be kept by the patient during the study and collected at the final study visit. Resource use will be transferred to an eCRF, and the original document kept at the participating centre as source material.

10 ADDITIONAL ANALYSES

10.1 Exploratory analyses

Pre-specified exploratory analyses

Histological and molecular markers collected whilst the liver is perfused on the device will be combined with perfusion parameters to develop a composite liver grading scoring system and to assess their ability to predict clinical outcomes i.e. viability assessment.

A model for the assessment of early allograft function was recently developed [29] and also showed to be associated with patient and graft survival. MEAF stands for Model of Early Allograft Function and is calculated by applying the formula below using the peak ALT and INR value from the first 3 post-operative days and the bilirubin value from the third postoperative day. The final MEAF score is the sum of the 3 values obtained rounded to the nearest integer.

$$Score\ ALT_{\max 3POD} = 3.29 \Big/ \left(1 + exp\left(-1.9132 \times \ln\left(\max_{3POD} ALT\right) - 6.1723\right)\right)$$

$$Score\ INR_{\max 3POD} = 3.29 \Big/ \left(1 + exp\left(-6.8204 \times \ln\left(\max_{3POD} INR\right) - 0.6658\right)\right)$$

$$Score\ Bilirubin_{3POD} = 3.4/\left(1 + exp(-1.8005 \times \ln(Bilirubin\ 3POD) - 1.0607)\right)$$

$$MEAF = Score\ ALT_{\max 3POD} + Score\ INR_{\max 3POD} + Score\ Bilirubin_{3POD}$$

This will be compared by treatment arms (NMP vs SCS) using ANOVA adjusting for Donor Type (DBD, DCD) and centre if the MEAF Score is normally distributed. In case MEAF Score has departure from Normality the first approach will be transformation. If the data cannot transform to Normal distribution, the difference in MEAF Score between arms will be analysed using Mann-Whitney U test. If a non-parametric analysis needs to be performed there will be no adjustment for other factors.

Another recent publication reported that high AST levels on day 3 correlates with patient and graft survival [30]. The relationship of patient and graft survival with the AST on day 3 will be explored using survival analysis techniques as described in Section 9.

Other exploratory analyses will be performed to explore the reasons for any differential discard rate between the two arms.

Additional Exploratory Analysis Not Specified Prior to Receiving Data

Any analyses not specified in this statistical analysis plan will be exploratory in nature and a significance level of 0.01 will be used to declare statistical significance. 99% confidence intervals will be presented.

10.2 Blinded analysis

This is an open label trial. Blinded review of the data will not be carried out.

11 SAFETY ANALYSIS

Safety outcomes to be reported will be:

- Organ discard rate (number of livers not suitable for transplantation)
- Adverse event:
  a. Recipient infection (defined as a positive microbiological culture result)
  b. Biopsy-proven acute rejection episodes
  c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)
  d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
  e. Reoperation rate
  f. Technical complications/device failures

Rates of adverse events and organ discards will be compared between NMP and SCS groups by examination of 95% confidence intervals for the difference in incidence. Their severity will be graded according to the Clavien-Dindo classification (Appendix 1: CLAVIEN DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS). Discarded organs will also be distinguished between declined (if accepted in a different hospital) and actually discarded.
The number of patients with any serious adverse events will be compared by assessment of the difference in incidence with 95% confidence interval. The number of serious adverse events per patient will be reported.

12 APPENDIX 1: 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.

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