EXAMINE THE MECHANISMS INVOLVED IN THE POTENTIAL DOWN-REGULATION OF BRAIN-STEM DEATH INDUCED PROINFLAMMATORY RESPONSES BY SIMVASTATIN

RESEARCH PROTOCOL

FUNDER-NIHR EME, REFERENCE NIHR 134145

KEY PROJECT CONTACTS:

Principlal Investigator: Professor Simi Ali (Newcastle University)

Co-Investigators: Professor John Dark (Newcastle University)
Mrs Helen Thomas (NHS Blood and Transplant)
Professor Danny McAuley (The Queen's University of Belfast)
Dr Dan Harvey (Nottingham University Hospitals NHS Trust)
Professor Rutger Ploeg (University of Oxford)
Mr John Fuller (PPI based in England)

Study Coordination Centre: Newcastle Uniiverity

For general queries, please contact

Simi Ali:
Address:
Theme: Immunity and Inflammation
Translational and Clinical Research Institute Faculty of Medical Sciences
3rd Floor Leech Building, Room M3057
Newcastle University
NE2 4HH
Tel: 01912087158
E-mail address: Simi.Ali@newcastle.ac.uk

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1. SUMMARY OF RESEARCH:

Background: Despite a decade long increase in the number of deceased organ donors there is a considerable shortfall of organs available for transplantation. The pathophysiology of brain-stem death includes a catecholamine storm followed by a massive release of pro-inflammatory cytokines. Furthermore, the pro inflammatory state in the donor is reflected in adverse outcomes in the recipient.

Statins are 3-hydroxy-3-methyl coenzyme A reductase inhibitors, with many pleiotropic effects, which may modulate the inflammatory processes in brain-stem dead donors. We have recently been awarded a grant (NIHR 131124) for a 4 year study randomising organ donors to statin treatment or control. The hypothesis is that a statin within protocolised care after diagnosis of brain-stem death improves outcomes in patients undergoing transplantation.

Aims &objective
We aim to explore the underlying mechanistic pathways that confer statin induced organ protection.
Main objectives are

1) Examine whether statin administration reduces donor inflammation by cytokine modulation and if time of administration has an effect. Also determine whether pre-existing statin treatment affects donor baseline cytokine levels.
2) Determine whether inflammatory sub-phenotypes exist in the donor population and whether these sub-phenotypes are associated with distinct clinical outcomes (number of organs utilised per donor) and inflammatory cytokine levels
3) Examine gene expression profiles in heart tissue biopsies after donor statin administration.
4) Examine long-term effects of statin treatment in a selected cohort of transplant patients.

Experiments will be carried out with data already collected in the national transplant database and samples from the Quality in organ donation (QUOD) programme. No extra data or blood samples will be needed from recipients/donors.

Methods: Serum samples will be obtained before the drug is administered, but after brain death and at organ retrieval from donors in both arms of the study.These samples will be tested for cytokines, to detect whether there is a significant difference in cytokine expression between the treatment groups and to study the effect of time of administration on statin-induced cytokine changes. Secondly, these cytokine data along with baseline data will be used in a latent class analysis (LCA), without consideration to outcome, to identify donor sub-phenotypes. Post-LCA discovery, we will study whether sub-phenotypes correlate with distinct clinical outcomes and inflammatory cytokine levels in response to statin.

In order to identify genes which are involved in statin induced anti-inflammatory effects, the tissue samples from donors which show an anti-inflammatory response will be used to identify the differentially regulated genes in pairwise comparisons. Finally, in order to evaluate whether initial statin administration has long term anti-inflammatory effects in patients, analysis will be carried out in a smaller cohort of local transplant patients.

Timeline for delivery: Duration 2 years (SIGNET EME to start 12 months following the recruitment start of SIGNET HTA).

Impact & Dissemination: This will potentially allow us to identify organ donors based on cytokine and gene expression who are more likely to benefit from statin intervention. Thus leading to a larger organ pool and better function in the recipient.

We have a Patient Public Involvement (PPI) co-applicant who will liaise with the PPI panels of original trial (SIGNET) and NIHR Blood and Transplant Research Unit (BTRU). In addition, results will be disseminated by publications, conference presentations and press release.

2. LIST OF ABBREVIATIONS

BTRU Blood and Transplant Research Unit
CTU Clinical Trials Unit
DBD Donors after brain death
DGF delayed graft function
HARP Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction
HTA Heath Technology Assessment
IRI ischemia reperfusion injury
ITU intensive treatment unit
LCA Latent Class Analysis
PPI Patient public involvement
PPIE Patient and Public Involvement and Engagement
QUOD Quality in Organ Donation
RhoA Ras homolog family member A
SIGNET Statins for Improving orGaN outcomE in Transplantation

3. BACKGROUND AND RATIONALE:

A) HOW THIS RESEARCH FITS INTO THE EME REMIT:

We have recently been awarded a grant (NIHR 131124) for a 4 year study randomising organ donors to statin treatment or control. The hypothesis is that a statin within protocolised care after diagnosis of brain-stem death improves outcomes in patients undergoing transplantation. This proposal will explore the mechanistic pathways that confer statin induced donor organ protection. Experiments will be carried out with data already collected in the national transplant database and samples from the QUOD programme.

In the proposed study we will analyse cytokine and gene expression profiles in donor serum samples and cardiac tissue respectively after donor simvastatin treatment. This will help us to define the mechanism of statin mediated immunomodulatory and cardio protective effects. It will help determine if different sub-phenotypes in the donor population have divergent clinical outcomes in response to statin. Furthermore, it will allow us to identify organ donors (hyper-inflammatory) based on cytokine and gene expression who are more likely to have anti-inflammatory effects following statin intervention. Finally, it will allow correlation between laboratory observation and clinical outcome.

B) OVERVIEW OF THE PROTOCOL OF THE MAIN STUDY THAT THIS APPLICATION RELATES TO:

SIGNET (Statins for Improving orGaN outcomE in Transplantation), ISRCTN: 11440354

The aim of the SIGNET HTA ((NIHR 131124) is to

1) Determine if simvastatin confers an improvement in clinical outcomes in cardiac transplant recipients
2) Determine if simvastatin is safe in other organ transplant recipients (renal, lung, liver and pancreas)

Population: adult, brain-stem dead donors at major organ donation centres in the UK

Intervention: Administration of 80mg simvastatin enterally via nasogastric tube, as soon as consent for organ donation and research is given, in addition to standard donor management protocol.

Comparator: Standard donor management protocol, usual practice ensuring that treatment will be similar in all respects other than the intervention.

Primary Outcome: The primary outcome will be a composite outcome of mortality, cardiac mechanical support or renal replacement therapy within 30 days following heart transplant. A robust and clinically meaningful composite primary end point has been chosen, encapsulating all early adverse outcomes post-heart transplant, namely death, mechanical cardiac support and renal replacement therapy. A composite end point facilitates a study powered to detect our anticipated clinical effect with a mean 150 adult heart transplants per year in the UK. The three elements of the composite are similarly important, have the same anticipated direction of effect and no one element dominates the composite outcome numerically. Each component will also be reported separately as a secondary outcome.

Secondary outcomes: Important clinical and safety organ specific transplant outcomes are routinely collected by the UK Transplant Registry, and will be reported as secondary outcomes.

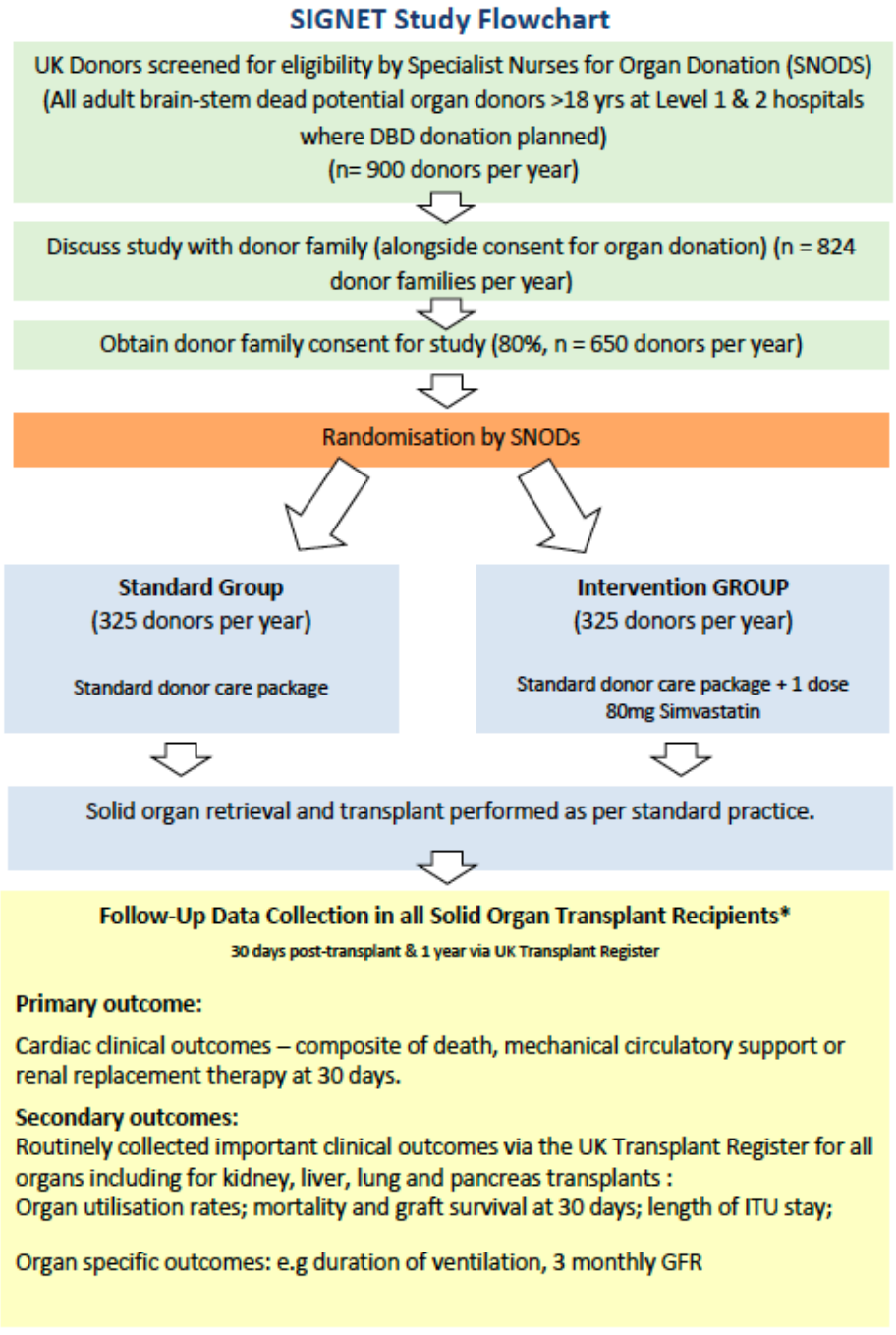
Project Plan Design This is a multi-centre, single-blind prospective, group sequential, randomised controlled trial. Randomisation will be in a 1:1 ratio, using permuted blocks of varying, undisclosed size, and will be stratified according to whether the donor was previously receiving statin therapy at ICU admission. Randomisation will not be stratified by transplanting centre (unknown at the time) or donation hospital (standard donor management applies at all hospitals and there is no evidence that donor hospital influences transplantation rates or recipient outcomes).

There is an established infrastructure for the collection and biobanking of samples of blood, serum and tissue from organ donors (QUOD, https://quod.org.uk/about-quod/). Mechanistic investigations are hugely aided by this infrastructure, with all the regulatory and ethical approvals already in place.

This study will be undertaken at up to 80 major donation hospitals (defined as > 6 organ donors per year). After approach for consent for organ donation, research consent from donor families will be requested by the Specialist Nurse in Organ Donation (SNOD), who will subsequently manage the randomization through an on-line system established by the NHSBT Clinical Trials Unit (CTU).

Recruitment Feasibility: At our selected sites we will screen 900 potential donors per year, and anticipate 824 actual adult donors after brain death (DBD) donors to donate at least one organ, per year. We anticipate a 20% refusal rate for research participation from donor families, therefore randomising 650 donors, and enrolling 325 donors to the intervention arm, per year. We aim to randomise 2600 donors in total over 4 years. Extrapolating from 2018-19 figures from the 80 leading donation hospitals, over 4 years we anticipate from these actual donors 488 cardiac recipients, 384 lung recipients, 3510 kidney recipients, 1988 liver recipients and 984 whole pancreas recipients.

Start date: 1.07.2021
End date: 30.06.2025
Status of study: Study began 1.10.2020, with recruitment planned to start 1.04.21. As a



result of the Pandemic effects on both donor hospitals and transplantation activity, start of recruitment was postponed to 1.07.2021. Although recruitment targets were not reached by the end date of the trial, recruitment was stopped on the grounds of futility in August 2025.

C) WHY IS THE RESEARCH NEEDED NOW:

i) Unmet health need:

Successful organ transplantation has substantial benefits for all recipients, with dramatically improved survival, improved quality of life and reduced costs. Median survival after cardiac transplantation is 12 years in the UK, with excellent quality of life (*1*). The health economic advantages are greatest for the kidney, with a saving of c£30k for every year free of dialysis, and an 85% 5 year graft survival (*2*). Similar benefits have been reported for cardiac and pulmonary transplantation.

Strategies to improve organ function in the donor might not only increase organ utilisation (number of transplants) but also have potentially the greatest impact in the recipient (organ transplant function). Post-transplant organ dysfunction in the recipient, be it the need for mechanical support for the heart, prolonged ventilation for the lung or delayed graft function and need for renal dialysis in the kidney, have huge short-term morbidity, mortality and cost, and for every organ reduces long term survival (*3, 4*).

PPI input into the development of the study emphasised the value of reducing early post-transplant morbidity. Two components of the primary end-point, mechanical support and renal support, epitomise the sort of prolonged and difficult post-operative stay the patients wish to avoid.

Early events during transplantation may determine the outcome in recipients. Brain death activates early immune responses in donor organs and subsequent transplant ischemia reperfusion injury (IRI) may further predispose to primary graft dysfunction and acute rejection in the recipient. Current immunosuppressive drugs do not prevent IRI. Therefore, treatment strategies that limit early injury and immune activation may have beneficial effects, especially when older and marginal donors are used.

In addition to cholesterol lowering, Statins also have many pleiotropic effects, which may modulate the inflammatory processes in brain-stem dead donors. In the SIGNET study (NIHR 131124) organ donors will be randomized to statin treatment or control. This EME will explore the mechanisms of action of this intervention.

ii. **Provide the size of the incident or prevalent patient population in the UK:**

Despite a decade long increase in the number of deceased organ donors, and the likely benefits of the recently-introduced deemed consent legislation, there is a considerable shortfall of organs available for transplantation. The COVID-19 pandemic has led to unprecedented challenges for UK transplantation. Many patients became suspended from the active transplant list. The total number of transplants fell in 2019/20 (from 3952 in 2018/19 to 3760 in 2019/20). In 2019/20, 372 patients died waiting and 746 were removed from the waiting list (ultimately leading to death). There was an 4% reduction in the number of kidney transplants, a fall of 5% in the number of heart transplants, and the number of liver transplants fell by 6%, however the number of pancreas transplants (including pancreas only, intestinal, kidney/pancreas and pancreas islets) increased by 1% (*5*).

Many offered organs, 75% for the heart and lung, are turned down for transplantation because of pre-existing disease or temporary brain-stem death related dysfunction. Despite this highly selective approach, more than 30% of recipients still required short-term mechanical cardiac support, reflecting donor heart dysfunction. Almost all the early deaths, 18% mortality in the first year, are in this group (*6*). The reported incidence of delayed graft function (DGF) following kidney transplantation from deceased donors has increased over time despite the progress in acute rejection treatment and translates to a 40% decrease in long-term graft survival (*7*). Similarly, Primary graft dysfunction following liver transplantation can be subdivided into early allograft dysfunction (EAD) and primary non-function (PNF), its more severe manifestation. Primary graft dysfunction occurs in 38.7% of liver transplants (*8*) with PNF incidence ranging from 0.9% to 8.5% (*9*).

iii. Provide context of your proposed research in terms of current practice.

A significant number of hearts and other organs offered for transplant by the donor family are not used; for the heart, this figure is about 75%. The reason for being so selective is that poor function of the donor heart in the recipient is by far the most common cause of death after a transplant. Any step in the donor which might improve the transplanted heart could have a major benefit to the recipient. The same principle applies to all the other organs transplanted.

This study capitalises on infrastructure unique to the UK to deliver cost effective research in organ donation. We have a national organ matching service and a national transplant registry, which collects detailed recipient outcomes (UK Transplant Registry). Additionally, there is an established infrastructure for the collection and biobanking of samples of blood, serum and tissue from organ donors (QUOD).

iv. If relevant, describe any time-limited opportunities.

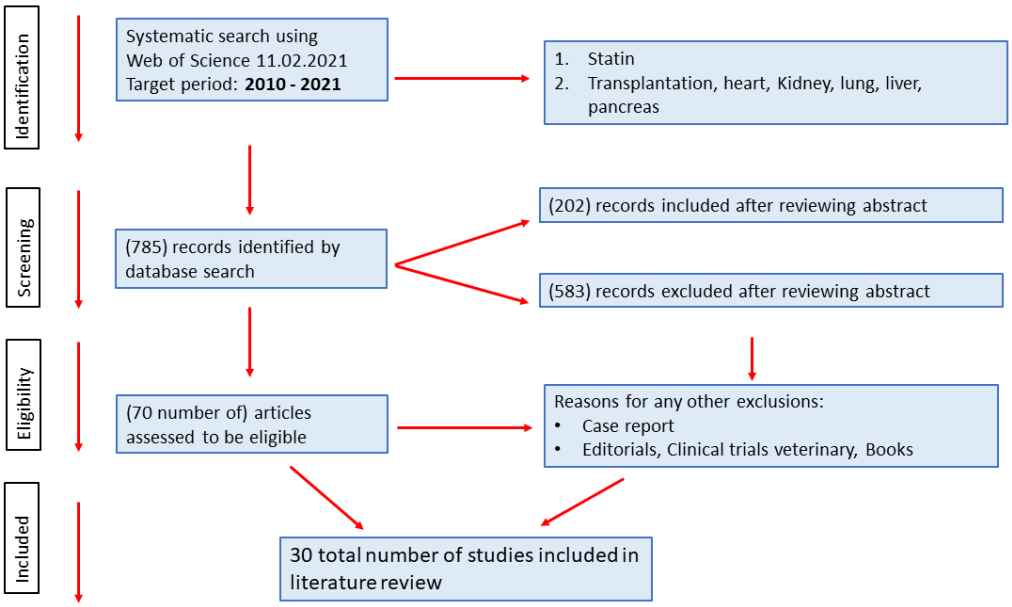
SIGNET HTA was funded under the 19/95 Solid organ replacement, transplant and donation cross-programme call. The proposed SIGNET EME which is to start 12 months after the start of SIGNET HTA recruitment will increase the impact of the trial. If the mechanistic study is able to identify organ donors based on cytokine and gene expression who are more likely to benefit from statin intervention these changes can be incorporated in donor management earlier. This will lead to larger organ donor pool and better function in recipient.

This will be the first study to examine the underlying mechanistic pathways that confer statin induced organ protection in a large donor population. Furthermore, it will also be first attempt to identify inflammatory sub-phenotypes in donor population and associate it with clinical outcome. This will be carried out with data already collected in the national transplant database.

D) KNOWLEDGE GAP THAT THIS RESEARCH WILL ADDRESS:

i. Methods used for reviewing the relevant literature:

A rigorous and systematic approach was taken to review the literature.



The pathophysiology of brain-stem death, with a catecholamine storm followed by a massive release of pro-inflammatory cytokines, has been well described in both animal models and the human setting (*10, 11*). The pro inflammatory state in the donor is reflected in events in the recipient. For instance, levels of IL6 and TNFα, in both serum and in terms of RNA expression in cardiac tissue, predicted which donor hearts were too dysfunctional for acceptability for transplantation (*12*). Levels of the pro-inflammatory cytokine IL8 in broncho-alveolar lavage of lung donors predicted early impaired gas exchange, longer duration of ventilation and survival in recipients (*13*). The same linkage between markers in the donor, in this case IL6 and TNFα, and outcome after liver grafting can be demonstrated (*14*).

Proof of concept for statin to improve donor cardiac function

Based on their studies of heart transplantation and kidney ischaemia in the rat (*15, 16*), where there was a clear benefit to animals pre-treated with statins, a group in Helsinki performed a randomised prospective study in brain-stem dead donors of a single dose of simvastatin 80mg via nasogastric tube early after consent for organ donation. They randomised 84 donors very likely to donate for cardiac transplantation, 42 patients received a heart from a statin treated donor (*17*). There was a striking reduction in early heart injury (measured by serial troponin levels), an improvement in early cardiac function (assessed by postoperative NT-proBNP), and a reduction in early rejection rates. There was no difference in early or one-year survival, however the study was not powered for clinical outcomes. The recipients had a reduced pro-inflammatory cytokine profile. In patients receiving other organs from the statin-treated donors, there was a significant reduction in Alanine Transferase (a marker of liver injury) at one week in liver recipients, and a non-significant improvement in gas exchange in the lung recipients. Importantly, there were no safety concerns in any organ recipient group.

Statins are 3-hydroxy-3-methyl coenzyme A reductase inhibitors, with many pleiotropic effects which may modulate the inflammatory processes in brain-stem dead donors. Elevated IL6 is associated with donor heart dysfunction in organ donors (3), and pre-admission treatment with statins reduces IL6 levels in patients with sepsis (*18*). Statins reduce pulmonary and systemic

inflammation in acute lung injury (ALI) (*19, 20*) a process which shares many features with lung injury and the related systemic inflammation in the brain-stem dead donor. The protective effect of statins against contrast-induced nephropathy, an analogous example of acute renal injury, has been reported (*21*).

Two sub-phenotypes of acute respiratory distress syndrome (ARDS) were identified in a multicentre, randomised control trials of simvastatin (HARP-2) with distinct clinical and biological features and disparate clinical outcomes (*22*). The hyperinflammatory sub-phenotype had improved survival with simvastatin compared with placebo. Statins reduce lung inflammation and injury in both animal models of ARDS (*23*) and preclinical human experimental studies and have endothelial stabilising properties (*24*). Thus, patients with more systemic inflammation, such as those with the hyperinflammatory sub-phenotype, could be most likely to respond to this therapy. A recent study has shown that brain death-induced inflammatory activity is similar to sepsis induced cytokine release (*25*). As previous sub-phenotypes were defined in ARDS, so we envisage that it is highly likely that we will be able to identify them in organ donor population as well. These findings support further pursuit of predictive enrichment strategies in clinical trials.

What is the mechanism(s) of the early mortality benefit seen in heart transplant recipients treated with statins?

It has been proposed that statins in the presence of calcineurin inhibitors may exert an immunomodulatory effect that leads to reduced natural killer cell activity, reduced T-cell response, reduced chemokine synthesis by mononuclear cells, and inhibition of the expression of MHC-II genes. However, many of these phenomena have been studied *in vitro* or in experimental models (*26*). To what extent these processes play a role in the clinical scenario of a transplant recipient on multiple immunosuppressive medications is unknown. Additional potential mechanisms may include the strong anti-inflammatory effects of statins (*27*) upregulation of endothelial nitric oxide synthase, and downregulation of growth factor genes responsible for smooth muscle cell proliferation (*28, 29*). Preclinical data, in which simvastatin is administered to heart transplant donors in a rat model resulted in inhibition of microvascular endothelial cell and pericyte RhoA/Rho-associated protein kinase activation and endothelial cell–endothelial cell gap formation, decreased intragraft mRNA levels of hypoxia-inducible factor-1α, nitric oxide synthase, and endothelin-1, and increased heme oxygenase-1 (*30*). In addition, statin treatment in the donor inhibited cardiac allograft inflammation, transforming growth factor β-1 signaling, and myocardial fibrosis.

This indicates that statin treatment of the donor results in mitigation of a number of noxious processes triggered by brain death and by ischemia and reperfusion of the allograft that take place in the transplant process. However, the mechanisms involved in statin mediated immunomodulatory and cardio protective effects in clinical setting are not defined.

ii. Recent or on-going relevant trials:

SIGNET HTA will commence recruitment on 01.07.2021. It was funded via 20/19 solid organ commissioned call and to our knowledge no other heart donor mechanistic trials were funded.

A group in Helsinki performed a randomised prospective study in brain-stem dead donors of a single dose of simvastatin 80mg via nasogastric tube early after consent for organ donation. They randomised 84 donors very likely to donate for cardiac transplantation, 42 patients

received a heart from a statin treated donor. There was a striking reduction in early heart injury (measured by serial troponin levels), an improvement in early cardiac function (assessed by postoperative NT-proBNP), and a reduction in early rejection rates. There was no difference in early or one year survival, however the study was not powered for clinical outcomes (*17*).

We have an extensive network of colleagues around the world, and are not aware of other planned Statin intervention studies in donor care. The group in Finland, with whom we have good relations, are not planning any clinical studies at present. We have also done a search on Clinicaltrials.gov, using the key terms “Organ donor” and “Statin” and have not uncovered any other studies

iii. Explain why your proposed research would lead to an improvement in the care of the patient population.

Recently, a randomized clinical trial showed that the simvastatin treatment of brain-dead donors conditions the heart transplant to withstand ischemia-reperfusion injury and to reduce the need for rejection treatments early after transplantation(*17*). In the proposed study we will examine whether statin reduces donor inflammation and will analyse cytokine and gene expression profiles in donor serum samples and cardiac biopsies respectively after donor simvastatin treatment. This will help us to define the mechanism of statin mediated immunomodulatory and cardio protective effects. It will help determine if different sub-phenotypes in the donor population have divergent clinical outcomes. This will potentially allow us to identify organ donors based on cytokine and gene expression who are more likely to benefit from statin intervention. Thus leading to larger organ pool and better function in recipient

E) DESCRIBE THE EVIDENCE THAT PROVIDES PROOF-OF-CONCEPT IN MAN FOR YOUR RESEARCH.
Results of a recent randomized clinical trial (RCT) in the cardiac donors provide safety and feasibility for a larger scale testing of this intervention in multiorgan donor. The investigators randomized 84 brain-dead organ donors to 80 mg of simvastatin or placebo. A single dose of the drug was administered through a nasogastric tube within 2 hours of declaration of brain death, and the heart was procured for transplant on average 12 hours later. Donor simvastatin treatment resulted in reduced heart recipient plasma levels of troponin and lower serum levels of NT-proBNP (N-terminal pro-B-type natriuretic peptide) within 1 week of transplant and in a reduction in the number of treated rejections with hemodynamic compromise. Meanwhile, biopsy-proven rejection and survival of the transplant recipients were similar in the 2 groups (*17*).

4. AIMS AND OBJECTIVES

Hypothesis:

- ***Donor simvastatin treatment has immunomodulatory and cardioprotective effects following heart transplantation. Furthermore, early administration of statin following brain death will have more beneficial effect then given later.***
- ***The heterogeneity of treatment response in donors is based on levels of inflammation. Distinct sub-phenotypes exist and they correlate with divergent clinical outcomes.***

We aim to explore the underlying mechanistic pathways that confer statin induced organ protection.

Main objectives are

1) Examine whether Simvastatin administration reduces donor inflammation by cytokine modulation and whether time of administration has an effect. Also determine whether pre-existing statin treatment affects donor baseline cytokine levels.
2) Determine whether inflammatory sub-phenotypes exist in the donor population and whether these sub-phenotypes are associated with distinct clinical outcomes (number of organs utilised per donor) and inflammatory cytokine levels.
3) Examine gene expression profiles in heart tissue biopsies after donor simvastatin administration.
4) Examine long term effects in a selected cohort of transplant patients

Experiments will be carried out with data already collected by QUOD programme. Mechanistic investigations will be hugely aided by this infrastructure, with all the regulatory and ethical approvals already in place

The QUOD scheme, supported by NHSBT but with a cost-recovery process is a standardised process for collecting serum, urine and bile specimens, together with kidney, liver and cardiac biopsies and bronchiolo-alveolar lavage specimens from some organ donors.

Regulatory aspects include oversight by the Human Tissue Authority and formal Research Ethics approvals

Consent for sampling is taken by the specialist nurses in organ donation. Plasma is taken from the archived hospital specimens taken after admission, and from samples taken after consent, at the end of donor management and at the time of organ retrieval. Tissue biopsies are taken after organ retrieval and stored in either formalin or RNALater. Samples are spun by the retrieval teams, using a portable centrifuge and taken on ice to a framework of recipient transplant centres linked to organ retrieval teams, where they are formally separated, aliquoted and frozen. Plasma and tissue samples are then stored in a the QUOD biobank in Oxford.

The QUOD biobank database contains detailed information about the timing of sampling and can be linked, through a robust data governance process, to NHSBT donor numbers and to anonymised transplant recipients using the UK Transplant Registry

Deliverables: Interventions to improve the organ function in the donor might increase organ utilisation and also impact organ function in the recipient. The main deliverables of this study will be better understanding of the mechanism of action of statin administration to the donors. This could possibly help identify biomarkers, which can stratify biologically distinct sub-types of donors with differential treatment response.

5. RESEARCH PLAN / METHODS:

1) **A) Examine whether Simvastatin administration affects the cytokine modulation**: We will use plasma samples from the QUOD programme. For donors in both arms of the SIGNET HTA, we have access to samples taken before the statin is given - a sample is taken for QUOD and stored by the ITU team as soon as research consent is obtained, and this will be very shortly before the statin is given in those donors randomised to receive it. Further QUOD samples are taken immediately before the organs are removed.

These samples will be tested for cytokines, to detect whether there is a significant difference in cytokine expression between the treatment groups and to study the effect of time of administration, relative to brain death, on statin-induced cytokine changes. We will also look at whether pre-existing statin treatment in the donor affects baseline inflammation and their response to statin.

To facilitate the measurement of multiple proteins of interest in one assay, electrochemiluminescent Mesoscale Discovery™ (MSD) Multiplex will be performed on serum samples to quantify cytokine expression. The 40-plex panel includes CRP, Eotaxin, Eotaxin-3, FGF (basic), GM-CSF, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PlGF, SAA, TARC, Tie-2, TNF-α, TNF-β, VCAM-1, VEGF-A, VEGF-C, VEGF-D, VEGFR-1/Flt-1. These assays will be performed according to manufacturer's instructions and provide higher sensitivity and dynamic range. We have successfully used them previously (*31, 32*).

Proposed sample size: Sample size assumptions were based on both previously published data (*17, 33-35*) and postulations made by the applicants of this funding proposal. This objective is powered to detect the effect of simvastatin versus control. The effect of time of statin administration has not been considered in our power calculation

In the proposed study, multiple cytokines will be simultaneously measured in the donor; we expect there to exist a certain level of correlation amongst cytokines. We considered powering the study on multiple cytokines simultaneously but, due to limitations in published data and prior knowledge, it was decided to power on C-reactive protein (CRP) only. (Note that other cytokines, in addition to CRP, will be studied at other stages in our study). CRP is the gold-standard marker of systemic inflammation in clinical care and has been shown to fall in statin-treated patients (*20*). Assuming a CRP mean value in the statin arm that is 25% lower than the control arm [standard deviation (SD) as per (*33*), at 80% of the largest mean], we estimate that a sample of 430 donors (half with and half without statin treatment) would need to be enrolled in order for the study to have 90% power, at a two-tailed significance level of 0.05. We do not allow for a dropout rate as only donors who have been enrolled in the SIGNET HTA (any-organ donor), and who have QUOD samples will be considered. Donors receiving statin therapy at ICU admission will be excluded from this primary outcome analysis and sample size calcualtion, but will be included in some of the other analyses.

We will recruit the first 430 donors from SIGNET HTA who meet this study's recruitment criteria. We estimate that 15% of adult DBD donors will already be on statin, which translates to needing 506 SIGNET HTA donors in total. SIGNET HTA aims to recruit 500 donors in the first year followed by 650 donors in each subsequent year, so the SIGNET EME target will be achieved within 15 months.

B) Inclusion of donors on pre-statins when admitted to ITU. We will also include an additional cohort of these donors (n=50) to investigate the effect on cytokine profiles of donors on pre-statins versus those without statins.
We estimate that donors on pre-statins at the time of ITU admission comprise 15-20% of donors. They are stratified within the randomisation for NIHR 131124, so the admission statin history is known and the samples collected through the Quality in Organ Donation (QUOD) programme are collected from the majority of study donor centres.
The best evidence available of the effects of statin treatment in the context of prior statin therapy (n=250 patients with severe sepsis, 123 randomised to atorvastatin and 127 to placebo, with 30.8% of the cohort on pre-statins) is found, in our opinion, in Kruger et al. 2013 (1). They found a statistically significant difference in IL-6 at baseline between prior

statin users and nonusers (lower mean IL-6 in the pre-statin group). Crucially, this difference disappears after around 48-72hrs. Evidence from the NHSBT Potential Donor Audit indicates that the mean (95% CI) time between DBD donor admission to family approach for organ donation is 60 hrs (53-63 hrs). We therefore hypothesise that prior statin use will not have a significant effect on cytokine expression at confirmation of donor death and this will be explored using the full cohort of 480 donors (including the n=50 donors on pre-statins), as described above.

Outcome: Identify whether statin administration reduces donor inflammation by cytokine modulation and whether time of administration has an effect. Also determine whether pre-existing statin treatment affects baseline inflammation levels in the donor.

2) **Determine whether sub-phenotypes exist in the donor population and whether these sub-phenotypes are associated with clinical outcomes (number of organs utilised per donor):** The baseline cytokine expression data above (TNF-α, IL-6 etc.) along with donor baseline characteristics will be used in a LCA analysis without consideration to outcome to identify sub-phenotypes. We will use up to 15 cytokines selected on the basis of their class-discriminatory power, data completeness and alignment to the research question (e.g. include cytokines closely implicated in the inflammation process). Donor age, sex, cause of death and requirement for vaso-constrictors will also be used as class-defining variables.

   Proposed sample size: There are no concrete guidelines for sample size for LCA analysis although published studies point to samples between 300 and 500 (*35*). We will perform LCA on the 430 donors (any organ) enrolled for objective 1A (donors receiving statin at ICU admission will be excluded).

   Once classes have been discovered, clinical outcome (number of organs utilised per donor) will be compared across treatment groups within sub-phenotypes. Inflammatory cytokine levels just prior to organ retrieval will also be compared.

Outcome: will identify if sub-phenotypes exist and whether they correlate with distinct clinical outcomes and donor inflammation.

3) **Examine gene expression profiles in heart tissue biopsies after donor simvastatin administration**:

A) The tissue samples from patients which show an anti-inflammatory response (based on cytokine data and clinical outcome, determined in objective 2) will be used for RNA-sequencing (RNASeq) to generate an unbiased profile of the transcriptome to identify the differentially regulated genes in pairwise comparisons. Heart biopsies from donors with hyper & hypo-inflammatory phenotype with and without statin treatment (n=40 in total, 10 from each of the 4 combinations) will be analysed and tissue samples will be used from QUOD programme. Two heart biopsies are taken when the heart is not transplanted and tissue is split into formalin and RNA later allowing pathology as well as multiomics.
To minimize variance in the analysed subgroup, the next generation sequencing analyses will be done from the 20 biopsies from each group with a male donor. The overall biological functions of these genes will be related to molecular pathways e.g. leukocyte migration, response to oxidative stress etc.

RNA extraction: RNA will be extracted from heart biopsies stored in RNA later. Samples will be lysed in Lysis buffer (Invitrogen) using a precellys homogenizer, RNA will be extracted using a Pure link RNA mini kit (Invitrogen) and contaminating DNA removed using Turbo DNase kit (Invitrogen). RNA will be quantified by measuring absorbance at 280nm (Nanodrop

spectrophotometer) and quality will be assessed using a 2100 bioanalyzer (Agilent) with a RNA nano chip (Agilent).

RNASeq: RNAseq Libraries will be made using TruSeq Stranded Total RNA library prep kit (Illumina) with 7 minutes fragmentation as per manufacturer's instructions. Final libraries will be amplified for 14 cycles by PCR and the final size assessed using a 2100 bioanalyzer (Agilent) with a HS DNA chip (Agilent). Pooling of finally libraries and sequencing will be carried out by Qiagen.

RNASeq analysis. Differential expression analysis will be carried out using DESeq2 after fitting an appropriate model within the R statistical environment In addition we will also assess changes in the expression of molecular pathways.

Assessment of RNA and protein correlates
We will further investigate whether the genes differentially expressed in donors with hyper and hypo-inflammatory phenotypes might be potentially useful biomarkers. Given proteins are easier to measure in a clinical setting we will search for potential candidates from our RNASeq data.

Outcome: Identify genes, which are involved in statin induced anti-inflammatory effects.

B) Differential miRNA analysis: The samples identified above in objective 2 will also be used for miRNA analysis to examine the changes in microRNA occurring during statin treatment. This will be assessed by nCounter microRNA analysis (Nanostring) which profiles the expression of 800 microRNAs. Global normalisation will be performed to the 100 highest expressed microRNAs using NSolver analysis software and microRNAs demonstrating a >2 fold increase or decrease in expression will be identified.

Four different computational tools (Diana, Target Scan, miRwalk, miRanda) will be used to identify the potential mRNA targets of differentially expressed microRNAs. MicroRNAs will be selected for further analysis if they interact with pathways likely to affect the pathways i.e inflammasome, cytokines & chemokines. We will identify 5 candidate microRNAs to take onto the next stage of analysis.

Differentially expressed microRNAs will be localised in heart tissue by *in situ hybridisation* using a double DIG labelled (5' and 3') miRCURY LNA microRNA detection probe (Exiqon, Denmark), using techniques established in our laboratory (*36*).
Any differences in these analyses related to statin treatment can be correlated with clinical outcomes already available to us, and have the potential to underpin the mechanism of statin action.
Outcome: Identify miRNA, which are involved in statin induced anti-inflammatory effects.

4) Examine long-term anti-inflammatory effects in a selected cohort of transplant patients (3 month & 1 year): Analysis will be carried out in a smaller cohort of local transplant patients. Samples will be collected under our existing ethics and available in local transplant biobank (REC reference: 17/NE/0022) from adult recipients of organs from SIGNET donors. For example, in lung transplant recipients blood samples will be collected peritransplant and 3 month and 1 year post transplantation.The cytokines and differentially expressed RNA and miRNA identified above will be analysed in this cohort to evaluate any long-term beneficial effects of statin administration. We will only examine the adult recipients from SIGNET donors at Newcastle, any paediatric recipients will be excluded.
Outcome: Evaluate whether initial statin administration has long-term anti-inflammatory effects.

CTU involvement: We have worked closely with NHSBT CTU in developing this proposal. They are fully registered with the UKCRC and will support both the design and delivery of this

research. They have expertise in the data on the UK Transplant Registry and experience in delivery of research within the clinical pathway. Statistical expertise will be provided by Helen Thomas, co-applicant, and Head of Clinical Trial Statistics for NHSBT CTU. Additionally, Helen Thomas is a coapplicant on SIGNET HTA as well, thus allowing better coordination.

6. **Study population: (Guidance on EDI) inclusion/exclusion criteria to ensure that certain groups are not excluded without justification**

Donors receiving statin therapy at ICU admission will be excluded from the primary outcome analysis and Objectives 2 and 3, but will be included in other analyses.

Participant Inclusion Criteria
Within a recruiting Intensive Care unit
Patients diagnosed dead using neurological criteria
Consent for organ donation in place, as defined by the Human Tissue Act and accompanying legislation and Codes of Practice.
Study specific consent from donor family

Participant Exclusion Criteria
Aged < 18
Planned donation after cessation of circulation (DCD)
Known donor allergic hypersensitivity to simvastatin (*note true allergic hypersensitivity is rare. For the purpose of SIGNET side effects such as myalgia and rhabdomyolysis would not be considered an exclusion as they are side effects from repeated dose, not an allergy. Only a single dose is administered for SIGNET.)*

Statistical analysis plan: Baseline characteristics of patients (donors and recipients) will be summarised, broken down by treatment arm and by organ where appropriate. Cytokine expression levels, for all measured cytokines, will also be summarised at each sample timepoint. The primary outcome will be analysed using a simple t-test to examine whether mean cytokine expression level (measured at organ retrieval) between treatment arms differs. The response variable DB4 CRP will be log-transformed if residual plots flag deviations from the Normal distribution. Then an unpaired parametric two-tailed t-test will be performed. T-tests will then be performed for each of the 14 subsequent cytokines; multiple comparison correction will use a Benjamini-Hochberg approach. For secondary analyses, multiple linear regression modelling will be performed with restricted cubic splines for continuous covariates and where appropriate, interaction terms between statin treatment and covariates. The outcome for these models will be rate of change of cytokine between DB2 (baseline) and DB4 or cytokine concentration at DB2 as appropriate. These data are likely to be skewed due to extreme values, and rate of change can be negative or positive. Therefore, a cube root transformation will be applied to handle extreme rate of change values, whilst preserving directionality. No random effect for donation hospital will be included as standard donor management applies at all hospitals but other confounders will be considered. For example traumatic brain injury, may predispose a transplanted lung and its recipient toward more severe early rejection episodes, suggesting more intense host inflammatory response (*37*).

To address objective 2, a LCA analysis will be performed. After data set up and exploration (inc. standardisation of continuous class-defining variables, elimination of highly-correlated variables to fulfil local independence and variable transformation) to select the final set of class-defining variables, a finite mixture model with k 'normal' classes will be fitted. Mixture models with different k's will be independently fitted; we envisage fitting k=1, 2 and 3 but larger k's could be examined, if appropriate. Fit statistics will be generated for each k, which will be used to select the optimal number of classes. As a general principle, we will select the model with the fewest

number of classes that best fits the data. Our definition of 'best' will be based on metrics such as the models' Bayesian information criteria and the size of the smallest class in each model (k's that result in a class with only a small number of observations could be considered unsuitable). Once the number of classes, k, and donors' membership into those classes are known, we will test for the effects of treatment (statin versus control) within class on the number of organs utilised per donor and also plasma inflammatory cytokine levels using regression models.Sensitivity analyses will be performed to determine the impact of eliminating candidate class-defining variables.

Additional organ-specific clinical outcomes for transplant recipients will also be available. Early survival, need for re-transplant and ITU stay will be collected for liver recipients, as will primary non-function rates for kidney recipients. This will be studied by summarising incidence/means in each of the treatment groups within each class. We will test for significant differences where appropriate.

Value for money:

The SIGNET HTA trial is funded to evaluate the benefits of a single dose of Simvastatin in potential organ donors. This proposed study will be carried out with data already collected in the national transplant database. No extra data or blood samples will be needed from recipients/donors.

1. Plasma samples will be obtained before the drug is administered, but after brain death and at organ retrieval from donors in both arms of the study (QUOD).
2. Heart and tissue biopsies available from QUOD will be used for RNA sequencing (QUOD).
3. Analysis on smaller local patient population following transplantation with the samples collected under our current ethics in the transplant Biobank (REC reference: 17/NE/0022).

Strategies to improve organ function in the donor might increase organ utilisation (number of transplants) but have potentially the greatest impact in the recipient (organ transplant function).

7. DISSEMINATION, OUTPUTS AND ANTICIPATED IMPACT

We will reach a diverse patient, clinical and research audience by disseminating our results with

- Open access publication in high-impact peer-reviewed journals
- Conference Presentations
- Co-ordinated press releases
- Use of social media outlets

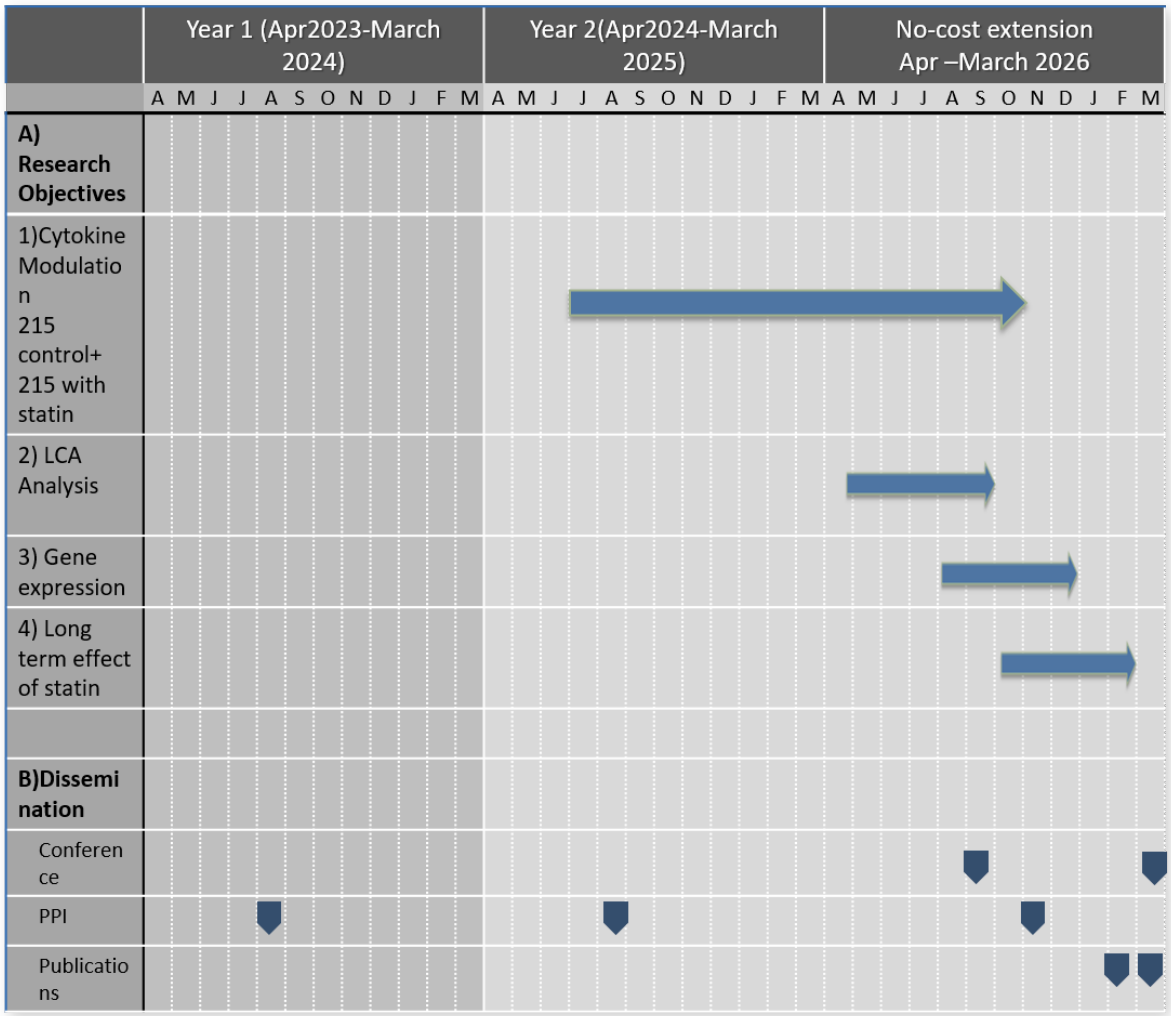
Our team includes national leaders in Organ Donation professional education, regulation and service delivery (Harvey) who can ensure rapid incorporation of novel interventions into standard UK protocols for organ donation.

Our lay co-applicant will play a key part in our public and patient involvement. This will ensure that the wider patient voice is addressed within the study and will also enable sharing of progress updates

8. PROJECT TIMETABLE

The original grant (NIHR 131124): Randomisation of donors at designated donation hospitals will begin July'2021. Analysis of consent and inclusion will be continuous, with review at the end of year 1. Recruitment will be monitored 3 monthly, with planned interim analysis at two and three years for harm, benefit and futility. Final analysis will be after close-down in June, 2025.

For the SIGNET EME, analysis will begin in June 2024 and continue until March 2026.



9. PROJECT MANAGEMENT:

The EME will be managed by the PI (SA) along with in-put from five scientific co-applicants and one lay co-applicant. All the applicants will be part of the Management group. We will have a monthly meeting on internet platform (Microsoft Teams. Topics of discussion will include, progress of research, any governance issue and financial management. It is envisaged that lay co-applicant will attend these meetings quarterly and as required to ensure that any items, which specifically need PPI, input will be tabled at the meetings they attend

Face-to-face meeting will be at planned conference along with SIGNET HTA at months 11 and 20 respectively. Lay co-applicant will work with research team to plan a half day project conference.

PI will provide training to staff (PDRA) on study processes and procedures. Statistical expertise will be provided by Helen Thomas, co-applicant, and Head of Clinical Trial Statistics for NHSBT CTU.

All research samples (serum/biopsies/etc.) will be stored in locked -80C freezer located within Newcastle university translational and clinical research institute.

10. ETHICS/REGULATORY APPROVALS

- QUOD samples are obtained in accordance with the relevant legislation, which includes the Human Tissue Act 2004, the Human Tissue (Scotland) Act 2006, the Mental Capacity Act 2005, the Adults with Incapacity (Scotland) Act 2000 and the Mental Capacity Act (NI) 2016. QUOD biobank holds generic ethical approval for research projects, subject to access being awarded by Steering committee. HTTPS://QUOD.ORG.UK/ABOUT-QUOD/

- For objective 4, serum samples, urine and tissue biopsies are already collected under our existing ethics and available in local transplant biobank (REC reference: 17/NE/0022).

11. PROJECT/RESEARCH EXPERTISE

Mechanistic studies will be done in a single laboratory with a long track-record of transplant immunobiology research (Ali). Prof Dark is the lead PI of SIGNET HTA and brings 35 years of clinical research experience in organ transplantation, including a 6 year spell as National Lead for Governance at NHSBT. There is expertise in organ donation and organ donor management (Harvey), and clinical trial experience of simvastatin in the critically ill (McAuley). CI, SIGNET HTA. Prof AcAuley has identified in ARDS, two sub-phenotypes with differential response to Simvastatin.

The NHSBT CTU has extensive experience with clinical transplant research and the associated national transplant database analysis (Thomas). The QUOD initiative is led by Professor Rutger, Professor of Transplant Biology, samples proposed to be used in this study will be accessed through this Biobank.

There are additional applicants from SIGNET HTA, who will contribute their expertise but are not included in costs, Professor Andrew Fisher (Lung Transplantation), Professor Neil Sheerin (Renal transplantation & renal injury) and Professor James Shaw (Pancreatic and islet transplants).

12.SUCCESS CRITERIA AND BARRIERS TO PROPOSED WORK

The key measure of success for this study is publication of the findings in a high impact factor journal. Further measures include the presentation of findings at international meetings, such as the American Transplant Congress, European society of organ transplantation etc.

The most likely risk is a failure to recruit the intended number of patients, resulting in a sample size that is underpowered or unsuitable for a full latent class analysis. This could occur due to a late start in recruiting, delays attributable to attaining ethical approval, or unforeseen recruitment difficulties in participating centres due to the COVID-19 pandemic. Such an event would not undermine the importance of the results of this study, as the data could be published as exploratory results and will inform future studies. However, in order to mitigate the risk of

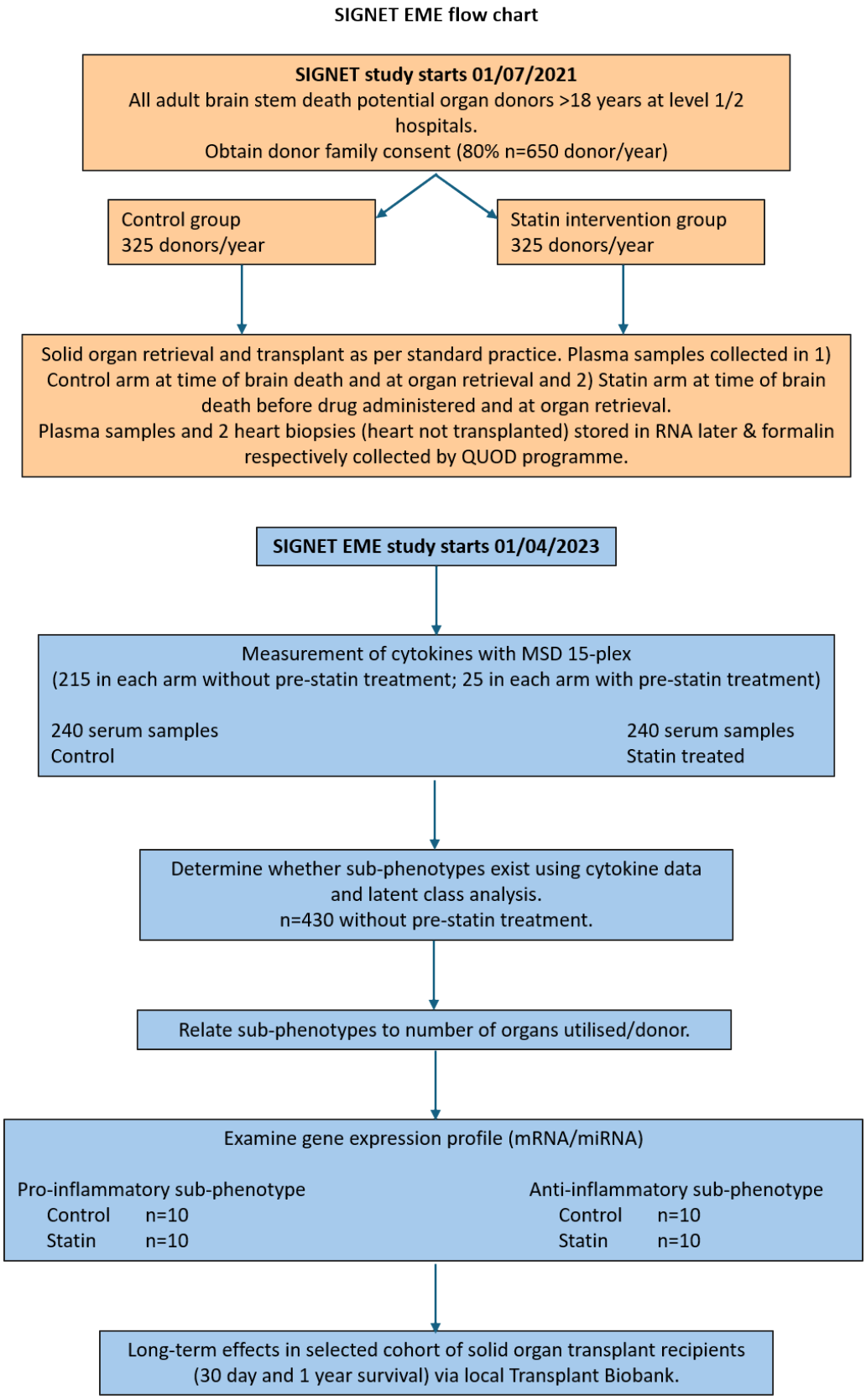
recruitment difficulties we have delayed the start of EME one year after the start of SIGNET HTA recruitment.

In the absence of a full latent class analysis, a non-parametric cluster analysis alternative will be pursued. Similar to LCA, in cluster analysis there is no prior information about the group or cluster membership of the individuals. Cluster analysis will allow us, as a contingency approach, to investigate whether distinct inflammatory sub-phenotypes are identifiable in the donor cohort.

A recent study has shown that brain death-induced inflammatory activity is similar to sepsis induced cytokine release(*25*). As previous sub-phenotypes were defined in ARDS,so we envisage that it is highly likely that we will be able to identify them in organ donor population as well.

However, a potential risk is that the previously defined subphenotypes (ARDS) are not identifiable in organ donor population by any or all methods. Again, this would not undermine the importance of the data; as such a result could conclusively determine that sub-phenotypes are disease-specific, informing future research.

13. Flow chart



14.REFERENCES

1. NHSBT. (2020), vol. 2019-20.
2. E. commission. (2013).
3. A. J. Fisher, J. Wardle, J. H. Dark, P. A. Corris, Non-immune acute graft injury after lung transplantation and the risk of subsequent bronchiolitis obliterans syndrome (BOS). J Heart Lung Transplant 21, 1206-1212 (2002).
4. E. Pareja, M. Cortes, D. Hervas, J. Mir, A. Valdivieso, J. V. Castell, A. Lahoz, A score model for the continuous grading of early allograft dysfunction severity. Liver Transpl 21, 38-46 (2015).
5. NHSBT. (2019-20), vol. 2021.
6. S. S. Avtaar Singh, N. R. Banner, S. Rushton, A. R. Simon, C. Berry, N. Al-Attar, ISHLT Primary Graft Dysfunction Incidence, Risk Factors, and Outcome: A UK National Study. Transplantation 103, 336-343 (2019).
7. S. G. Yarlagadda, S. G. Coca, R. N. Formica, Jr., E. D. Poggio, C. R. Parikh, Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. Nephrol Dial Transplant 24, 1039-1047 (2009).
8. D. P. Hoyer, A. Paul, A. Gallinat, E. P. Molmenti, R. Reinhardt, T. Minor, F. H. Saner, A. Canbay, J. W. Treckmann, G. C. Sotiropoulos, Z. Mathe, Donor information based prediction of early allograft dysfunction and outcome in liver transplantation. Liver Int 35, 156-163 (2015).
9. X. B. Chen, M. Q. Xu, Primary graft dysfunction after liver transplantation. Hepatobiliary Pancreat Dis Int 13, 125-137 (2014).
10. V. S. Avlonitis, A. J. Fisher, J. A. Kirby, J. H. Dark, Pulmonary transplantation: the role of brain death in donor lung injury. Transplantation 75, 1928-1933 (2003).
11. R. P. Watts, O. Thom, J. F. Fraser, Inflammatory signalling associated with brain dead organ donation: from brain injury to brain stem death and posttransplant ischaemia reperfusion injury. J Transplant 2013, 521369 (2013).
12. E. J. Birks, P. B. Burton, V. Owen, A. J. Mullen, D. Hunt, N. R. Banner, P. J. Barton, M. H. Yacoub, Elevated tumor necrosis factor-alpha and interleukin-6 in myocardium and serum of malfunctioning donor hearts. Circulation 102, III352-358 (2000).
13. A. J. Fisher, S. C. Donnelly, N. Hirani, C. Haslett, R. M. Strieter, J. H. Dark, P. A. Corris, Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation. Am J Respir Crit Care Med 163, 259-265 (2001).
14. M. S. Chae, K. U. Moon, H. S. Chung, C. S. Park, J. Lee, J. H. Choi, S. H. Hong, Serum interleukin-6 and tumor necrosis factor-alpha are associated with early graft regeneration after living donor liver transplantation. PLoS One 13, e0195262 (2018).
15. R. Tuuminen, S. Syrjala, R. Krebs, R. Arnaudova, E. Rouvinen, A. I. Nykanen, K. B. Lemstrom, Combined donor simvastatin and methylprednisolone treatment prevents ischemia-reperfusion injury in rat cardiac allografts through vasculoprotection and immunomodulation. Transplantation 95, 1084-1091 (2013).
NIHR 134145
16. R. Tuuminen, A. I. Nykanen, P. Saharinen, P. Gautam, M. A. Keranen, R. Arnaudova, E. Rouvinen, H. Helin, R. Tammi, K. Rilla, R. Krebs, K. B. Lemstrom, Donor simvastatin treatment prevents ischemia-reperfusion and acute kidney injury by preserving microvascular barrier function. Am J Transplant 13, 2019-2034 (2013).
17. A. I. Nykanen, E. J. Holmstrom, R. Tuuminen, R. Krebs, K. Dhaygude, M. Kankainen, J. J. Jokinen, J. Lommi, I. Helantera, A. Raisanen-Sokolowski, S. O. Syrjala, K. B. Lemstrom, Donor Simvastatin Treatment in Heart Transplantation. Circulation 140, 627-640 (2019).
18. P. Kruger, M. Bailey, R. Bellomo, D. J. Cooper, M. Harward, A. Higgins, B. Howe, D. Jones, C. Joyce, K. Kostner, J. McNeil, A. Nichol, M. S. Roberts, G. Syres, B. Venkatesh, A. N.-S. I.-A. C. T. Group,

A multicenter randomized trial of atorvastatin therapy in intensive care patients with severe sepsis. Am J Respir Crit Care Med 187, 743-750 (2013).
19. M. Shyamsundar, S. T. McKeown, C. M. O'Kane, T. R. Craig, V. Brown, D. R. Thickett, M. A. Matthay, C. C. Taggart, J. T. Backman, J. S. Elborn, D. F. McAuley, Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. Am J Respir Crit Care Med 179, 1107-1114 (2009).
20. T. R. Craig, M. J. Duffy, M. Shyamsundar, C. McDowell, C. M. O'Kane, J. S. Elborn, D. F. McAuley, A randomized clinical trial of hydroxymethylglutaryl- coenzyme a reductase inhibition for acute lung injury (The HARP Study). Am J Respir Crit Care Med 183, 620-626 (2011).
21. Y. Y. Sun, L. Y. Liu, T. Sun, M. Y. Wu, F. Z. Ma, Prophylactic atorvastatin prior to intra-arterial administration of iodinated contrast media for prevention of contrast-induced acute kidney injury: A meta-analysis of randomized trial data. Clin Nephrol 92, 123-130 (2019).
22. C. S. Calfee, K. L. Delucchi, P. Sinha, M. A. Matthay, J. Hackett, M. Shankar-Hari, C. McDowell, J. G. Laffey, C. M. O'Kane, D. F. McAuley, G. Irish Critical Care Trials, Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: secondary analysis of a randomised controlled trial. Lancet Respir Med 6, 691-698 (2018).
23. H. Shao, Y. Shen, H. Liu, G. Dong, J. Qiang, H. Jing, Simvastatin suppresses lung inflammatory response in a rat cardiopulmonary bypass model. Ann Thorac Surg 84, 2011-2018 (2007).
24. J. R. Jacobson, S. M. Dudek, K. G. Birukov, S. Q. Ye, D. N. Grigoryev, R. E. Girgis, J. G. Garcia, Cytoskeletal activation and altered gene expression in endothelial barrier regulation by simvastatin. Am J Respir Cell Mol Biol 30, 662-670 (2004).
25. P. Schwarz, G. Custodio, J. Rheinheimer, D. Crispim, C. B. Leitao, T. H. Rech, Brain Death-Induced Inflammatory Activity is Similar to Sepsis-Induced Cytokine Release. Cell Transplant 27, 1417-1424 (2018).
26. B. Kwak, F. Mulhaupt, S. Myit, F. Mach, Statins as a newly recognized type of immunomodulator. Nat Med 6, 1399-1402 (2000).
27. P. M. Ridker, E. Danielson, F. A. Fonseca, J. Genest, A. M. Gotto, Jr., J. J. Kastelein, W. Koenig, P. Libby, A. J. Lorenzatti, J. G. MacFadyen, B. G. Nordestgaard, J. Shepherd, J. T. Willerson, R. J. Glynn, J. S. Group, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 359, 2195-2207 (2008).
NIHR 134145
28. J. Alfon, J. F. Guasch, M. Berrozpe, L. Badimon, Nitric oxide synthase II (NOS II) gene expression correlates with atherosclerotic intimal thickening. Preventive effects of HMG-CoA reductase inhibitors. Atherosclerosis 145, 325-331 (1999).
29. J. A. Farmer, Pleiotropic effects of statins. Curr Atheroscler Rep 2, 208-217 (2000).
30. R. Tuuminen, S. Syrjala, R. Krebs, M. A. Keranen, K. Koli, U. Abo-Ramadan, P. J. Neuvonen, J. M. Tikkanen, A. I. Nykanen, K. B. Lemstrom, Donor simvastatin treatment abolishes rat cardiac allograft ischemia/reperfusion injury and chronic rejection through microvascular protection. Circulation 124, 1138-1150 (2011).
31. A. S. I. Andreasson, L. A. Borthwick, C. Gillespie, K. Jiwa, J. Scott, P. Henderson, J. Mayes, R. Romano, M. Roman, S. Ali, J. E. Fildes, N. Marczin, J. H. Dark, A. J. Fisher, D.-U. Investigators, The role of interleukin-1beta as a predictive biomarker and potential therapeutic target during clinical ex vivo lung perfusion. J Heart Lung Transplant 36, 985-995 (2017).
32. E. R. Thompson, L. Bates, I. K. Ibrahim, A. Sewpaul, B. Stenberg, A. McNeill, R. Figueiredo, T. Girdlestone, G. C. Wilkins, L. Wang, S. J. Tingle, W. E. Scott, 3rd, H. de Paula Lemos, A. L. Mellor, V. D. Roobrouck, A. E. Ting, S. A. Hosgood, M. L. Nicholson, A. J. Fisher, S. Ali, N. S. Sheerin, C. H. Wilson, Novel delivery of cellular therapy to reduce ischemia reperfusion injury in kidney transplantation. Am J Transplant, (2020).
33. D. F. McAuley, J. G. Laffey, C. M. O'Kane, G. D. Perkins, B. Mullan, T. J. Trinder, P. Johnston, P. A. Hopkins, A. J. Johnston, L. Murphy, C. McNally, A. M. Agus, C. McDowell, C. Jackson, in Simvastatin to reduce pulmonary dysfunction in patients with acute respiratory distress syndrome: the HARP-2

RCT. (Southampton (UK), 2018).
34. T. Lyngdoh, P. Vollenweider, G. Waeber, P. Marques-Vidal, Association of statins with inflammatory cytokines: a population-based Colaus study. Atherosclerosis 219, 253-258 (2011).
35. P. Sinha, C. S. Calfee, K. L. Delucchi, Practitioner's Guide to Latent Class Analysis: Methodological Considerations and Common Pitfalls. Crit Care Med 49, e63-e79 (2021).
36. S. S. Ladak, E. Roebuck, J. Powell, A. J. Fisher, C. Ward, S. Ali, The Role of miR-200b-3p in Modulating TGF-beta1-induced Injury in Human Bronchial Epithelial Cells. Transplantation 103, 2275-2286 (2019).
37. A. M. Ciccone, K. C. Stewart, B. F. Meyers, T. J. Guthrie, R. J. Battafarano, E. P. Trulock, J. D. Cooper, G. A. Patterson, Does donor cause of death affect the outcome of lung transplantation? J Thorac Cardiovasc Surg 123, 429-434; discussion 434-426 (2002).