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Deceased Donor Kidney Storage at 10 Celsius versus Conventional Storage

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PROPOSED RESEARCH PLAN—Summary

Protocol Overview:

Study Description	Based on preliminary clinical safety studies, and animal findings of improved ex-vivo kidney function after 10°C storage compared to conventional storage on ice, the aim of this study is to translate static storage at 10°C to clinical practice in kidney transplantation. Primary Outcomes: 1. Store pre-procurement deceased donor kidneys in the X°Port Lung Preservation System from the time of procurement until transplantation at Vanderbilt University Medical Center (VUMC) and evaluate recipient kidney allograft function by measuring urinary neutrophil gelatinase-associated lipocalin (NGAL), which is a biomarker for acute kidney injury associated with DGF. 2. Compare recipient outcomes between kidneys which were stored using the X°Port Lung Preservation System vs those stored on ice. a. Primary outcome is urinary NGAL level. Secondary Outcomes: Recipient urine output measurements, serum creatinine levels, the presence of DGF, and the recipient's 30-day creatinine and estimated glomerular filtration rate. The goal of this study is to compare the outcomes of recipients who received kidneys preserved using the X°Port Lung Preservation System to control recipients who received kidneys preserved at static cold storage on ice. It is a prospective, double-arm, open-label, single-center study. Kidneys will either be preserved with the investigational device or with static cold storage on ice. This research aims to optimize static preservation of kidney allografts as an accessible strategy to improve outcomes.
Study Population:	All adult single organ kidney transplant candidates on the waiting list at the study center will be eligible for enrollment. Both donation after brain death and donation after circulatory death kidney donors will be included in this study. Recipients must be 18 years old or older, must have given consent for the study, and must be listed for a single organ kidney transplantation.
Planned Sample Size:	30 in the intervention arm
Participating Institutions (if a multi-center clinical trial)	n/a (single-center trial)

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Background and Rationale:

Kidney transplantation is a life-saving treatment for patients with end stage kidney disease, which affects nearly 800,000 patients in the United States. However, deceased donor kidney transplantation is often complicated by delayed graft function (DGF), conventionally defined as the recipient requiring hemodialysis within the first week after transplant. DGF is unfortunately common, occurring in 25% of all transplanted kidneys and portends worse long-term graft survival, higher rates of rejection, and increased recipient mortality. DGF commonly occurs because of ischemic reperfusion injury (IRI), a complex pattern of injury occurring at the time of organ procurement. IRI results from decreased cellular ATP, decreased function of the Na/K pump, mitochondrial destabilization, the generation of reactive oxygen species (ROS), apoptosis, complement and immune cell activation. Most commonly, kidneys and other deceased donor organs have been stored on ice to minimize cellular oxygen consumption and prevent IRI. However, preliminary data show that porcine kidneys are exposed to high levels of ROS when stored on ice due to decreased mitochondrial protective pathways associated with hypothermic conditions. Promising studies in other organ systems demonstrate reduced ROS when organs are stored at 10°C instead of standard ice. In lung transplant, porcine lungs stored at 10°C vs standard ice show better physiologic metrics, decreased mitochondrial DNA release, less cell death, less lactate production, and less glucose consumption. Higher levels of protective mitochondrial metabolites were observed in lungs stored at 10°C than standard ice storage. These metabolites promote the innate anti-oxidative system within mitochondria, protecting against ischemic injury. Clinically, human lung transplant shows favorable clinical outcomes with prolonged cold ischemia at 10°C vs ice storage. Within kidney transplant, there have been no similar investigations of 10°C storage compared to ice. **We hypothesize that deceased donor kidney storage at 10°C will be superior to standard ice storage.** This study has the potential to transform deceased donor organ storage and establish a new standard of care by storing kidneys at 10°C. Further, preserving kidney mitochondrial function and decreasing the rate of DGF, would allow transplant centers to accept more organs by lowering the clinical risk associated with organ acceptance, with an overall increase in organ utilization.

Aims:

1. Store pre-procurement deceased donor kidneys in the X°Port Lung Preservation System from the time of procurement until transplantation at Vanderbilt University Medical Center (VUMC) and evaluate recipient kidney allograft function by measuring urinary neutrophil gelatinase-associated lipocalin (NGAL), which is a biomarker for acute kidney injury associated with DGF.
2. Compare recipient outcomes between kidneys which were stored in either the X°Port Lung Preservation System vs static cold storage on ice.
    a. Primary outcome is urinary NGAL level.
    b. Secondary outcomes: Recipient urine output measurements, serum creatinine levels, the presence of DGF, and the recipient's 30-day creatinine level and estimated glomerular filtration rate

There are 800,000 patients with end stage kidney disease and 96,000 patients waiting for a kidney transplant[1]. Kidney transplant remains life saving for these patients, but the need for organs dramatically exceeds the supply: 14 patients die each day while waiting for a kidney transplant,[2] and 25% of all recovered deceased donor kidneys are discarded, a problem that has worsened over the past decade.[2] When patients receive a transplant, it is complicated by DGF in 25% of kidney transplants.[3] Research has shown that when patients experience DGF, they are at higher risk of hospital readmission, worse allograft function, rejection and mortality.[3–6]

DGF arises in part from IRI to the graft that occurs at the time of organ procurement. Ischemic reperfusion is a complex pattern of injury that ultimately results in decreased cellular ATP, decreased function of the Na/K pump, lysosomal degradation, mitochondrial destabilization, generation of reactive oxygen species (ROS), apoptosis, and complement and immune cell activation. Mitochondrial ROS generation seems to have

a crucial role in the pathogenesis of renal IRI.[7] It is believed that storing kidneys at 4°C on ice minimizes cellular oxygen consumption and minimizes IRI compared to normothermia; however, preliminary data show that **cooling porcine kidneys on standard ice may be detrimental due to decreased mitochondrial protective pathways including ROS scavenging.** [8] While there is decreased oxygen consumption with standard ice storage, there is a much less robust decrease in the generation of ROS.[8] With standard ice storage, the amount of generated mitochondrial ROS is relatively high compared to the decreased oxygen consumption.[8]

Storage at 10°C has been used for other organs. In lung transplant, porcine lungs stored at 10°C vs ice show better physiologic metrics, decreased levels of mitochondrial DNA release, less cell death, less lactate production and less glucose consumption.[9] Higher levels of mitochondrial protective metabolites including itaconate, glutamine, and N-acetylglutamine were observed in lungs stored at 10°C than at on standard ice[9]. These metabolites promote the innate antioxidant system within mitochondria, protecting against ischemic injury.[10.] Clinically, human lung transplant shows no worse outcomes with prolonged storage at 10°C vs on ice, even with extended cold ischemia time.[11] Porcine livers stored at 10°C vs on ice show better oxygen consumption when placed on subsequent normothermic perfusion system, with improved bile flow, less cell death and a better biochemical profile. [12] Preliminary porcine studies performed by Dr. Crannell show that deceased porcine kidneys stored at 10°C at 24 hours and reperfused on normothermic machine perfusion (Figure 1) have increased urine output, better oxygen consumption, improved vascular flow and lower hemodynamic resistance compared to porcine kidneys stored on standard ice (Figure 2, unpublished data).

Within human kidney transplant, Dr. Crannell published a case report describing the successful kidney transplant of a 10°C-stored kidney[13]. From this work, we hypothesize that deceased donor kidney storage at 10°C will be superior to conventional storage, with decreased levels of neutrophil gelatinase-associated lipocalin (NGAL), a urine biomarker for acute kidney injury that has been shown to be predictive of delayed kidney graft function.[14,15]

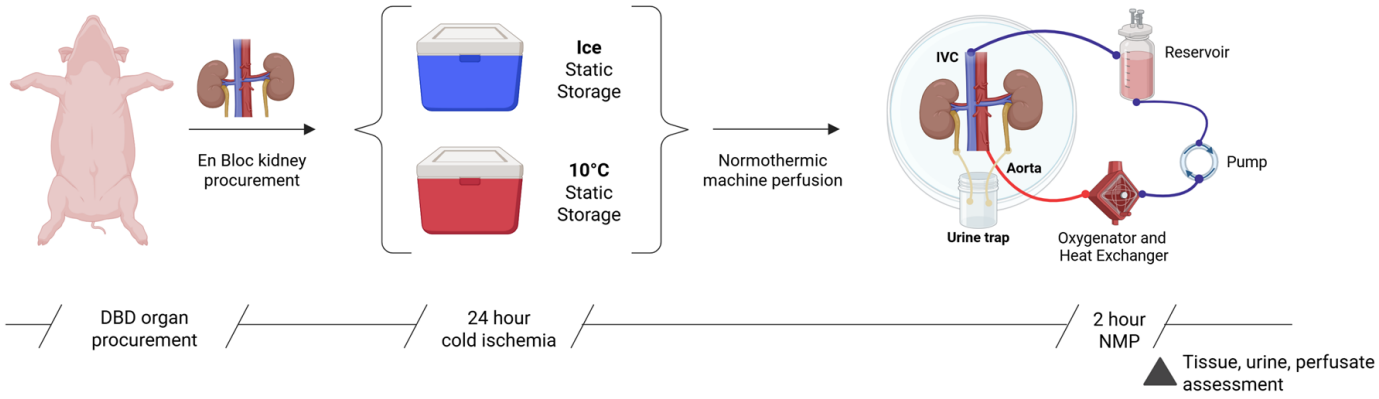

Figure 1: Experimental overview of normothermic machine perfusion after static storage at 10℃ or on ice for 24 hours.

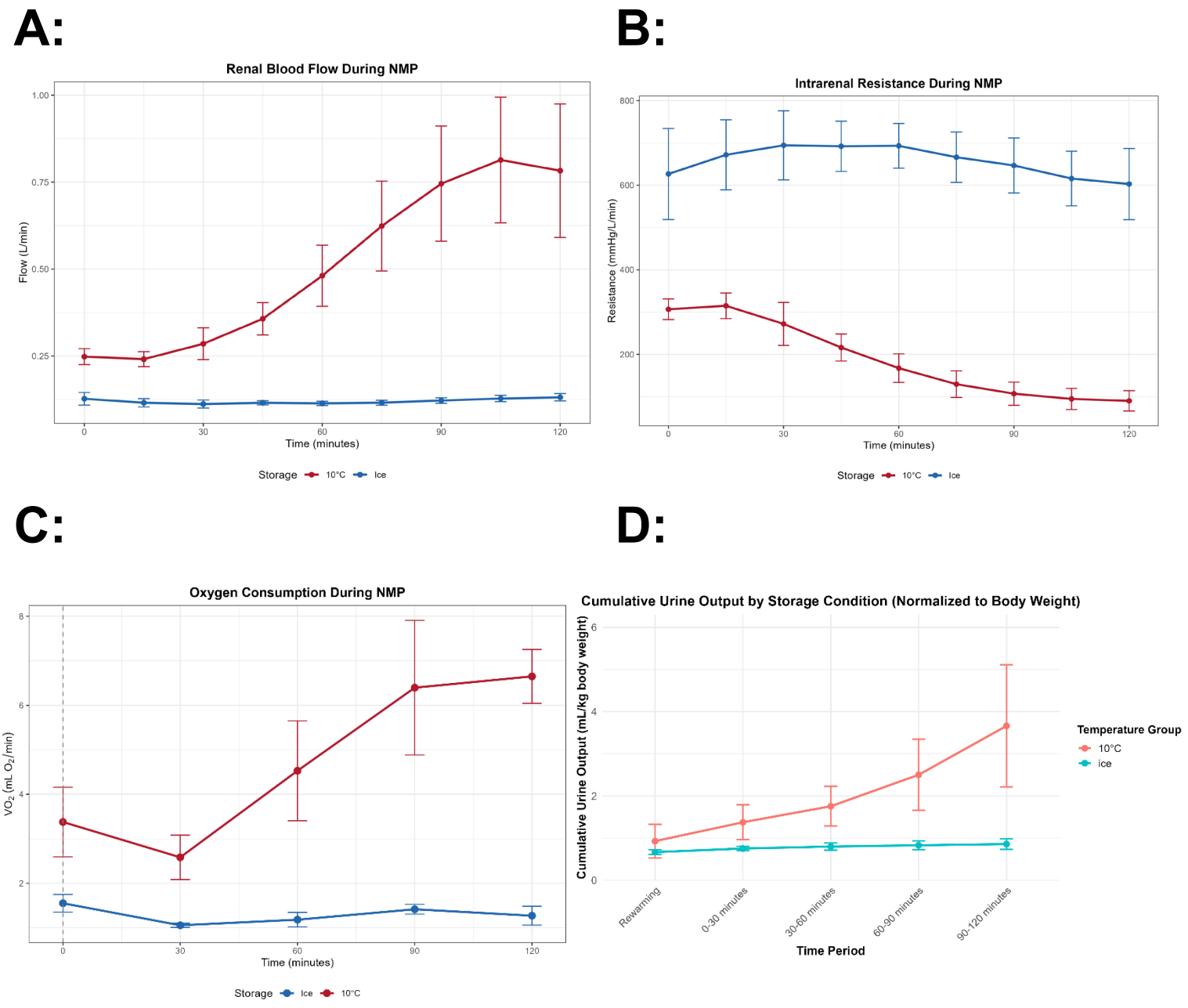

Figure 2: Porcine kidneys stored at 10°C for 24 hours and reperfused with normothermic machine perfusion (NMP) have better (**A**) total flow, (**B**) lower resistance, (**C**) have higher oxygen consumption and (**D**) make more urine compared to kidneys stored on ice. N=6 organs per study group

Inclusion / Exclusion Criteria

Deceased donor inclusion criteria:

1. Donation after brain death or donation after circulatory death kidney donors, whose health care proxy has consented for donation and the possibility of research
2. Deceased donor kidneys that have been allocated to Vanderbilt University Medical Center (VUMC) as a primary match offer prior to organ procurement will be eligible

Deceased donor exclusion criteria:

1. Donors whose health care proxy has declined consent for the possibility of research
2. Deceased donor kidneys that have been allocated to VUMC as a backup offer
3. Deceased donor kidneys that have been allocated to VUMC as a post procurement offer
4. Deceased donor kidneys that have already been placed on ice storage

Patient (recipient) inclusion criteria

1. All adult single organ kidney transplant candidates on the waiting list will be eligible for enrollment.

Patient (recipient) exclusion criteria

1. Kidney recipients less than 18 years old
2. Kidney transplant candidates that decline consent
3. Transplant candidates listed for multi-organ transplant

Methods

The proposed study is a prospective, double-armed, open-label, single-center trial. Once an organ offer is made to VUMC and reviewed for suitability, the kidney transplant recipient will be contacted by one of our research key study personnel (KSP), trained as kidney transplant organ coordinators to review the organ offer per standard operating procedure. Once the surgeon has accepted the organ offer, the coordinator will review the study with the patient using the phone script for informed consent.

Organ storage groups: X°Port Lung Preservation System vs. Static Cold Storage (Ice)

The study will test the difference between storage of donor kidneys in the X°Port Lung Preservation System (X°Port LPS) (experimental) and static cold storage on ice (standard of care). The storage method used will be determined by the location of the donor kidney because of study logistics (see below).

If the donor kidney is coming from an organ procurement organization(s) (OPO) participating in the trial, the study organ coordinator will alert the on-site procurement team that the kidney is to be placed in the X°Port LPS. The organ coordinators have regular and frequent contact with the OPOs as part of the procurement and transplant process. If the donor kidney is outside of a participating OPO, no alert is required. These organs are automatically stored on ice.

All donor kidneys will be obtained per standard clinical practice by the local procurement surgeon. After collection, the kidneys will either a) be placed in the X°Port LPS and stored at approximately 10°C or b) be stored on ice per standard of care procedures. Donor kidneys will be labeled with the anonymous United Network for Organ Sharing number and will be stored per UNOS policy in three layers of protection prior to being placed in the X°Port LPS.

Biospecimen Collection:

Urine will be collected from the transplant recipient's indwelling urinary catheter on the morning of postoperative day 1 (POD1), prior to initiation of the immunosuppressant tacrolimus. The samples will be processed, labeled with an anonymous study ID number and stored appropriately until further analysis.

Data collection:
Donor characteristics including age, body mass index (BMI), mechanism of death (anoxia, cerebrovascular accident, head trauma, unknown/other) type of donation (donation after brain death, donation after circulatory death, type of organ support (+/- normothermic regional perfusion), warm and cold ischemia times, kidney donor profile index, and terminal donor creatinine will be collected and recorded in REDCap. Recipient characteristics including age, sex, BMI, etiology of kidney disease, dialysis status prior to transplant (peritoneal dialysis, hemodialysis, or pre-emptive transplant) and major comorbidities will be collected in REDCap and analyzed. Post-transplant clinical measures including urine output, daily creatinine, estimated glomerular filtration rate (GFR), need for renal replacement therapy, creatinine at 30 days, eGFR at 30 days and death within 30 days will be recorded at the 1 month mark post-transplant.

Primary endpoint:
The primary study end point will be recipient urine NGAL level as measured in duplicate by ELISA and compared between recipients who received a kidney stored in the X°Port LPS versus those that were stored conventionally on ice.

Secondary endpoints:
Secondary outcomes will include recipient 24 h post op urine output measurements, daily serum creatinine and GFR, the presence of DGF, and the recipient's 30-day creatinine level and eGFR. These clinical outcomes will be compared between recipients who received a kidney stored in the X°Port LPS versus those that were stored conventionally on ice.

Consent

Consent and eConsent process:
For pre-procurement organ offers, eConsent will be performed as follows: One of our kidney transplant organ coordinators will ask if the patient would be interested in hearing about a research study for which they qualify. The coordinator will provide a brief summary about the study by phone. If the patient is interested in learning more, the coordinator will email him/her a printable copy of the consent to look over and set up a second phone call where they can review the document together. A link to the eConsent will be emailed to the patient at the start of the second phone call. The coordinator will review the entire document with the patient. He/She will be able to ask any questions, and sign the eConsent which will be returned through the REDCap system. Patients will be emailed a final signed copy of the consent document as a PDF for their records.

If a patient is unable to complete an eConsent, an in-person written consent will be performed. The consent form will be scanned into REDCap, and the patient will be provided with their signed consent form after it is scanned. No paper consent will be retained by study investigators.

Data and Safety Monitoring Plan

Because the true risk of kidney storage at 10°C is unknown, we will also collect data on several safety endpoints including:

1. Graft function
    a. Immediate kidney graft function: urine output with immediate improvement in recipient creatinine
    b. Slow graft function: urine output without immediate improvement in creatinine
    c. Delayed graft function: recipient requiring hemodialysis within the first 7 days post-transplant
2. Unanticipated transfer to the ICU
3. NSTEMI/STEMI/CVA

4. Mortality within 30 days.

The project will have a data safety monitor, Dr. Laura Hickman, a kidney transplant surgeon who routinely monitors kidney transplant clinical and quality outcomes. Data monitoring of the first 10 patients receiving the X°Port LPS stored kidney will be performed to determine if the trial meets all safety endpoints, and if the collection protocol meets all study needs.

After the first 10 patients, data will be monitored monthly examining the following safety outcomes:

- A statistically worse rate of delayed graft function (currently 24% at VUMC)
- Perioperative NSTEMI/STEMI/CVA
- 30-day perioperative mortality attributed to the transplant
- Primary non-function (defined as a return to dialysis within 3 months of kidney transplant)

The following adverse events (AEs) will initiate a pause in enrollment to further assess the study's efficacy and determine medical cause for the AE

- More than 2 investigational study patient deaths within 30 days among the first 10 cases
- A statistically worse rate of DGF

Dr. Hickman and Dr. Crannell will meet monthly to discuss the data and safety monitoring. If any of the safety endpoints are met, the IRB will be promptly notified according to IRB policies and procedures. Interim analyses at 6 and 12 months will be performed to evaluate the safety and potential benefits of 10°C kidney storage.

Statistical Methods

Differences between donors and recipients of ice-stored organs vs X°Port LPS-stored organs will be compared by Mann Whitney U for continuous variables and Chi squared for categorical variables. To examine the relationship between storage temperature and the primary outcome NGAL level, a univariate linear analysis examining donor and recipient covariates will be performed, with a subsequent multivariable linear regression analysis controlling for relevant clinical variables including type of donation (DBD vs DCD), kidney donor profile index and terminal donor creatinine. Logistic regression will be used to compare categorical outcomes. A median difference will be calculated between NGAL levels for kidneys stored in the X°Port LPS vs those stored on ice. To calculate the sample size needed, the concept of conditional power (CP) was implemented, defined as the chance the study will be statistically significant at its planned end. Using Welch's unequal-variance approach with a fixed 6:1 control-to-case ratio and an assumed between-group difference of 3,000 NGAL units (10C minus ice storage), it was determined that enrolling 11 cases and 66 controls provides an estimated 91 percent chance of statistical significance. This study will enroll 30 patients to ensure power for the primary outcome, sufficient evaluation of secondary outcomes and a comprehensive assessment of the safety and effectiveness of 10˚C storage.

Summary

Organ storage has typically been done on ice in the hopes of minimizing IRI, but there is very good clinical data that this paradigm may not yield optimal donor organ performance. Indeed, there has been a shift in recent years and livers are now being maintained under normothermic machine perfusion[16], lungs are being stored at 10°C with good clinical outcomes[9,11], and hearts have moved away from storage on ice[17,18].

Significantly, there has been no trial examining the effects of 10°C storage on the kidney. This study hopes to show the clinical benefit of 10°C storage for deceased donor kidney transplant, with lower biomarkers of acute kidney injury. Storage at 10°C is simple and more cost effective when compared to machine perfusion and has

obvious benefits for transportation simplicity and cost. This study has the potential to be very impactful and establish a new norm for deceased donor organ preservation temperature.

Christian Crannell, MD is a kidney transplant surgeon at Vanderbilt University Medical Center interested in optimizing organ grafts and organ utilization. Most recently he has published on a successful 10°C kidney transplant. Additionally, he has published on the role of heparin in deceased donor procurements and the labelling effect of high kidney donor profile index kidneys. He is a member of Dr. Bacchetta's Laboratory for Organ Regeneration, Recovery, and Replacement, which focuses on 10°C organ storage and perfusion systems. Dr. Crannell will be responsible for all aspects of the proposed project.

Rachel Forbes, MD, MBA is a kidney transplant surgeon at Vanderbilt University Medical Center with clinical and research interests in expanding access to transplantation through organ utilization and optimization. She has experience with clinical and translational research including evaluating the effect of pulsatile pump perfusion on Hepatitis C Virus Transmission and in collaboration with Dr. William Fissell works on efforts to create a bioartificial kidney.

Ciara Shaver, MD, PhD is a physician scientist and transplant pulmonologist. She has a basic and translational research laboratory focused on mechanisms of donor organ injury and post-transplant outcomes. She has extensive experience in translational studies of biomarkers and organ injuries during critical illness. She is codirector of the Laboratory for Science and Translational in Critical Illness and is funded by an R01. For this project, she will assist Dr. Crannell with sample processing and biomarker assessment and will provide guidance on statistical analysis.

Peter Reese, MD, Ph.D. Dr. Reese is the founding director of the Vanderbilt Center for Transplant Science. He is a board-certified nephrologist with subspecialty training in transplantation. Dr. Reese has a strong track record in conducting epidemiological studies and clinical trials to improve clinical outcomes of kidney transplant recipients. Dr. Reese will provide input on renal assessment at the cellular and organ levels.

Matthew Bacchetta, MD is Professor of Surgery and Biomedical Engineering, the Surgical Director of the Vanderbilt Lung Institute, and Director of the Laboratory for Organ Regeneration, Recovery, and Replacement (LOR3) at VUMC. He is the co-inventor of human-xenogeneic cross circulation for organ recovery and has been advancing development of this technology for more than a decade. Dr. Bacchetta has extensive experience with storing human lungs at 10°C for transplant. He has collaborative relationships with the entire project team. He has had continuous research funding from NIH, Department of Defense, foundations, and industry partners for more than 20 years and has expertise translating novel products to commercial use.

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