
CLINICAL STUDY PROTOCOL

Protocol Number: 8

Study Title: Renal protection against ischaemia-reperfusion in transplantation

Investigational Product: N/A

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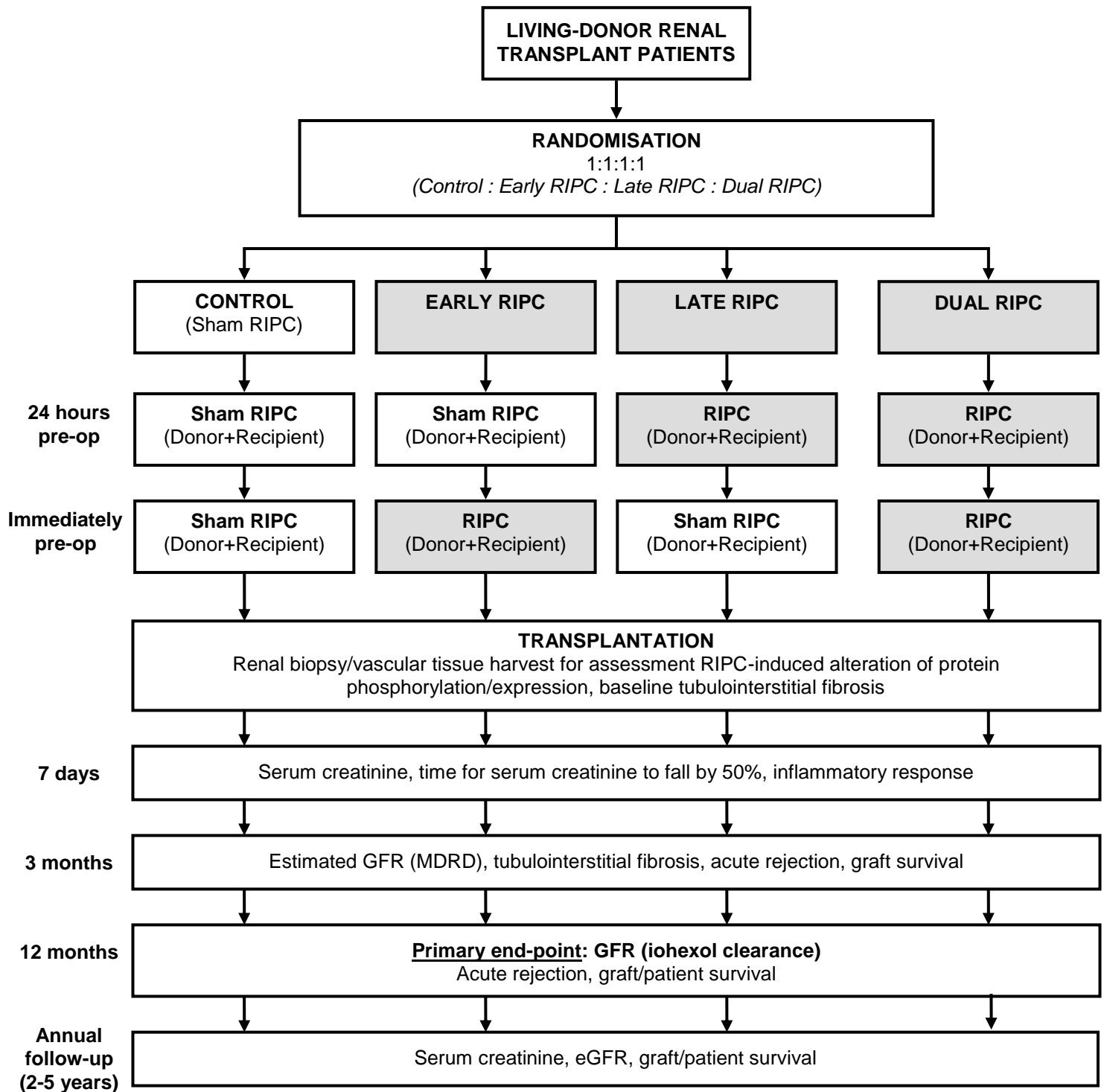
1 STUDY SYNOPSIS

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| Title of clinical trial | Renal protection against ischaemia-reperfusion in transplantation |
| Sponsor name | University College London |
| EudraCT number for proposed trial | N/A |
| Medical condition or disease under investigation | Renal Transplantation |
| Purpose of clinical trial | To determine if remote ischaemic preconditioning (RIPC) improves renal function after transplantation. |
| Primary objective | To determine the effect of RIPC on glomerular filtration rate (GFR) 12 months after transplantation |
| Secondary objective(s) | To determine the effects of RIPC on <ol style="list-style-type: none"> 1. Rate of fall in creatinine in the first 72 hours after transplantation 2. Inflammatory response to surgery in the first 5 days after transplantation 3. Protein expression in kidney parenchyma samples using histochemistry 4. Protein activation and expression in renal vasculature using immunoblotting 5. Kidney fibrosis 6 months after transplantation 6. Alloreactivity of T cells in the first 18 months after transplantation 7. Patient outcomes 2-5 years after transplantation using renal registry data |
| Study Design | Randomised double blind placebo-controlled trial |
| Study End-points | <ol style="list-style-type: none"> 1. GFR at 12 months after transplantation using iohexol clearance 2. estimated GFR (eGFR) at 3 months 3. Time for serum creatinine to fall by 50% 4. White cell count, CRP and plasma IL6, interferon gamma and TNF alpha before, and 1-5 days after surgery (donors and recipients) 5. Urinary IL6, interferon gamma and TNF alpha 1-5 days after surgery (recipients) 6. Kidney graft cortical tubulointerstitial fibrosis at 6 months (digital analysis of Sirius red staining in biopsy material) 7. RIPC-induced protein expressional changes in vascular and kidney tissue 8. Incidence of delayed graft function (need for dialysis in the first 7 days after |

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| | <p>transplantation or serum creatinine levels increase, remain unchanged, or decrease less than 10% per day in three consecutive days in the first week after transplantation)</p> <p>9. Incidence of acute rejection during the first 12 months after transplantation</p> <p>10. T cell activation, cytokine synthesis and proliferation in response to donor cells</p> <p>11. Long-term outcomes using renal registry data 2-5 years after transplantation (serum creatinine/eGFR, graft survival, patient survival)</p> |
| Sample Size | 400 living-donor renal transplant patients |
| Summary of eligibility criteria | <ol style="list-style-type: none"> 1. Patients undergoing living-donor transplantation 2. Patients aged 18 years and above 3. First transplant |
| Investigational medicinal product and dosage | N/A; this is not a trial of an investigational medical product |
| Active comparator product(s) | N/A |
| Route(s) of administration | N/A |
| Maximum duration of treatment of a subject | N/A |
| Procedures: Screening & enrolment | All patients undergoing living-donor transplantation will be considered for enrolment |
| Baseline | Baseline donor and recipient data will be recorded. |
| Treatment period | <p>The intervention is non-pharmacological. Patients (donors and recipients) will be randomised to one of four groups: control (sham RIPC), late RIPC, early RIPC, or dual RIPC (refer to flow diagram). Active treatment will consist of four 5-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg above systolic blood pressure. The inflations will be separated by 5-minute periods when the blood pressure cuff will be deflated. To activate late phase RIPC, the inflations will occur 24 hours before surgery. Placebo treatment (sham RIPC) will consist of four 5-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg.</p> <p>The intervention will be applied once or twice over a 24 hour period pre-operatively. Most visits and tests (including GFR and biopsies)</p> |

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| | in the first year are part of the transplantation protocol in the centres. Data for years 2-5 will be obtained from central databases in the UK (Transplant UK) and Holland (Eurotransplant). |
| End of Study | Patients will be followed-up for 5 years |
| Procedures for safety monitoring during trial | Serious unexpected adverse event reports will be forwarded to the Trials Co-ordination Group, London School of Hygiene and Tropical Medicine. Reports will be made to the Sponsor and the Data Monitoring Committee (DMC). Non-serious unexpected adverse events will be collated and summarised by the Trials Co-ordination Group, London School of Hygiene and Tropical Medicine and reported to the DMC. |
| Criteria for withdrawal of patients on safety grounds | Non-tolerability of 4 cycles of arm ischaemia |
| Regulatory submissions on safety grounds | Not required as not a CTIMP |

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3 INTRODUCTION

3.1 Background

3.1.1 Introduction

Chronic kidney disease requiring renal replacement therapy affects 44,000 adult patients in the UK, a population that is growing at 6% per annum (1). Kidney transplantation remains the best form of renal replacement therapy for many of these patients. In the UK the prevalent kidney transplant population is approximately 20,000; the remainder of those needing renal replacement therapy are treated by dialysis (2). There is an increasing demand of recipients for kidney transplants, due not only to the increase in incident cases, but also from those patients whose transplanted kidney has failed; this occurs in 3% of the prevalent transplant population, resulting in approximately 600 patients per year returning to the transplant list (2). However, the supply of kidneys for transplantation is more-or-less static, so that between 1997 and 2007, the number of patients in the UK waiting for a transplant increased by 43% (1). By 2007, this amounted to 6,480 patients, of which only 17% (1,440) received a kidney in 2007 (1). The picture is similar in other European countries (3). The consequences for a patient deemed best treated by transplantation of remaining on or returning to the transplant list are substantial; not only is there significant morbidity of dialysis but appreciable annual mortality (approximately 3%) among these patients. This is in addition to the cost of dialysis (£31,000 per patient per year in 2007), consuming approximately 1% of the NHS budget (1). Therefore, approaches that maximise the lifespan of each transplanted kidney will benefit patients directly, contribute to a reduction in the transplant list and moderate the costs of renal replacement therapy. This research protocol will evaluate the efficacy of an intervention that we hypothesise will improve the chances of early and late success of renal transplantation.

3.1.2 Renal injury is caused by ischaemia and reperfusion during transplantation

In many renal transplants, the kidney is exposed to warm ischaemia prior to harvest, cold ischaemia caused by the delay in transplanting the harvested organ, and a further period of warm ischaemia during the transplantation procedure (4). Cell death follows interruption of the blood supply to the kidney and successful reperfusion is mandatory for tissue salvage. Whilst this may be an integral part of the healing process, it may also contribute to tissue injury (5). Therefore tissue damage is a composite of injury that occurs during ischaemia and reperfusion; so-called ischaemia-reperfusion (IR) injury. The degree of IR injury determines the speed of recovery of organ function in the short term (6), and is most significant following non-beating-heart transplantation. In addition, it may modulate organ rejection in the longer term (4;6-8) by priming the immune response early after transplantation (9;10). Reduction in IR injury has potential to improve the outcome of kidney (and other organ) transplantation, in the short- and long-term (11;12).

3.1.3 Ischaemic preconditioning reduces IR injury

Ischaemic preconditioning (IPC) utilises sub-lethal ischaemia (preconditioning stimulus) to induce a state of protection against subsequent prolonged ischaemia (13). There are 2 phases of protection. There is a phase of IPC occurring within minutes of the preconditioning stimulus and lasts for up to 4 hours (14). The mechanism of early IPC has been extensively studied in animals and involves mediators that are generated during hypoxia (e.g. adenosine), a cascade of second messengers (e.g. phospholipases and kinases), and end effectors, including ATP-sensitive potassium channels (15) and the

mitochondrial permeability transition pore (16). A late phase of IPC occurs 24 hours after the preconditioning stimulus, which lasts for up to 72 hours and is termed the “second window of protection”, distinguishing it from early IPC (17;18). The prolonged (24-hour) interval between the preconditioning event and its renewed protection one day later is consistent with new protein synthesis (including heat shock and other cytoprotective proteins) (19). Although the majority of studies to date have demonstrated protection by IPC against IR injury to the myocardium of animals and humans (20), a smaller number of studies have investigated the potential of IPC to protect other organs, including the kidney (21). In animal models IPC attenuates injury and preserves function following renal IR (22-24) and after renal transplantation (25).

3.1.4 Remote ischaemic preconditioning

Despite the 20 years that have elapsed since the first description of IPC, its therapeutic value in the clinical setting remains untested. This is largely due to the logistical difficulties of applying ischaemic stimuli to induce preconditioning in vital organs in humans. Nor has it yet been possible to induce IPC pharmacologically, a reflection of the incomplete understanding of the mechanisms and the likelihood that multiple biological targets need to be activated. Demonstrating that there is clinically relevant tissue protection would stimulate renewed interest in pharmacological approaches to modulate ischaemic preconditioning.

However, the realisation that IPC protects tissues that are distant from those undergoing preconditioning has led to recent interest in direct clinical application of IPC (26). This facet of preconditioning (termed remote ischaemic preconditioning; RIPC) has been shown to be protective against IR injury of the myocardium (27-29), skeletal muscle (30), small intestine (31) and kidney (32). RIPC is mechanistically similar to IPC and causes a similar degree of tissue protection, as does IPC (33). The preconditioning signal is spread systemically by a mechanism that includes activation of the autonomic nervous system (34;35), and as yet unidentified humoral mediators (36;37).

We were the first group to show that RIPC could be triggered by limb ischaemia, and reduce experimental myocardial infarct size in pigs (38). Subsequent studies by our group and by others have determined that short periods of limb ischaemia also induce RIPC in humans. Therefore it may be possible to induce ischaemic preconditioning in the kidney using a remote stimulus such as limb ischaemia. This clinical study will test this possibility, and determine whether transient periods of limb ischaemia can induce a protective state to reduce renal IR injury that occurs following living-donor renal transplantation.

3.2 Data from pre-clinical studies in humans

This trial does not involve a pharmacological intervention, merely repeated 5-minute cycles of limb ischaemia. The human limb can tolerate ischaemia for up to 120 minutes without sustaining direct tissue damage. No safety concerns have arisen from the small number of pre-clinical studies performed to date, and this reflects the benign nature of the intervention. In healthy humans, we have shown that RIPC reduces IR injury to skeletal muscle and the vascular endothelium (38;39). Limb ischaemia was achieved by inflation of a blood pressure cuff to suprasystolic pressure on the upper arm for 5 minutes, after which the cuff was deflated for 5 minutes. The inflation/deflation cycle was performed 3 times. We confirmed that there were 2 phases of protection; an early and late phase, both of which are dependent on the function of autonomic nervous system (39). Activation of ATP-sensitive potassium channels is also a critical component of the mechanism (40).

3.3 Clinical Data

3.3.1 Efficacy

Limb ischaemia has been investigated as a preconditioning stimulus in paediatric cardiac surgery where there is a component of myocardial damage due to IR injury. Cheung et al used 4 cycles of limb ischaemia to induce early phase RIPC, and demonstrated that RIPC reduces myocardial injury following cardiac surgery in children (41). Moreover, a randomised controlled trial from our group has shown that early phase RIPC (3 cycles of arm ischaemia) is protective against myocardial injury in adults undergoing coronary artery bypass graft (CABG) surgery (*figure 1*) (42). More recently, RIPC has been demonstrated to reduce troponin release after angioplasty in patients with coronary artery disease (43).

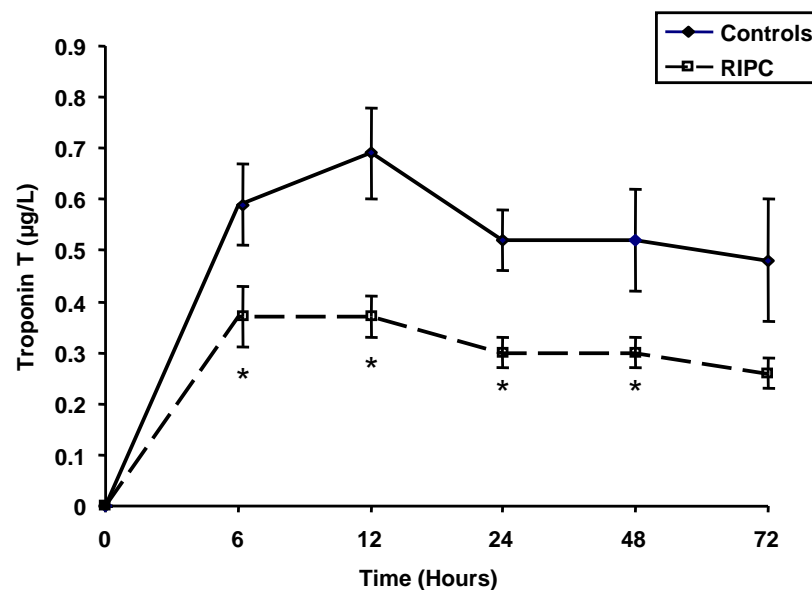


Figure 1 Serum troponin-T levels over the 72 hour perioperative period in adult patients undergoing elective coronary artery bypass graft surgery, demonstrating that compared to control, RIPC reduces serum troponin-T at all time-points. [$*P < 0.05$ control ($n=27$) vs. RIPC ($n=30$)].

In a clinical trial of paediatric living-donor renal transplantation, protective effects of late (“second window”) RIPC were evident. A blood pressure cuff was used to cause 5-minute periods of limb ischaemia (3 cycles, applied to the donor and recipient) 24 hours in advance of surgery. The outcome markers of renal IR injury we used were urinary retinol binding protein (RBPu) and the fall in plasma creatinine post surgery. RBPu is a low molecular weight protein that is freely filtered by the glomerulus and is normally 99.9% reabsorbed by the proximal tubule (44). The proximal tubule (distal segment) is the primary site of injury in renal ischaemia and reperfusion due to its marginal oxygenation under normal physiologic conditions coupled with high basal metabolic demand (45-47). Consequently, we predicted that IR-induced proximal tubule injury would increase urinary concentration of RBP post-transplantation, and confirmed this in initial pilot studies. Following this, a prospective cohort of patients ($n=20$) were randomised in a blinded fashion to sham RIPC or RIPC ($n=10$ in each group). RIPC reduced urinary RBP

(figure 2) consistent with an effect to reduce IR injury to the proximal tubules, and accelerated the post-transplantation decline in serum creatinine (figure 3). In addition, there was a beneficial effect of RIPC on long-term renal function, which was maintained for up to two years following transplantation (figure 4).

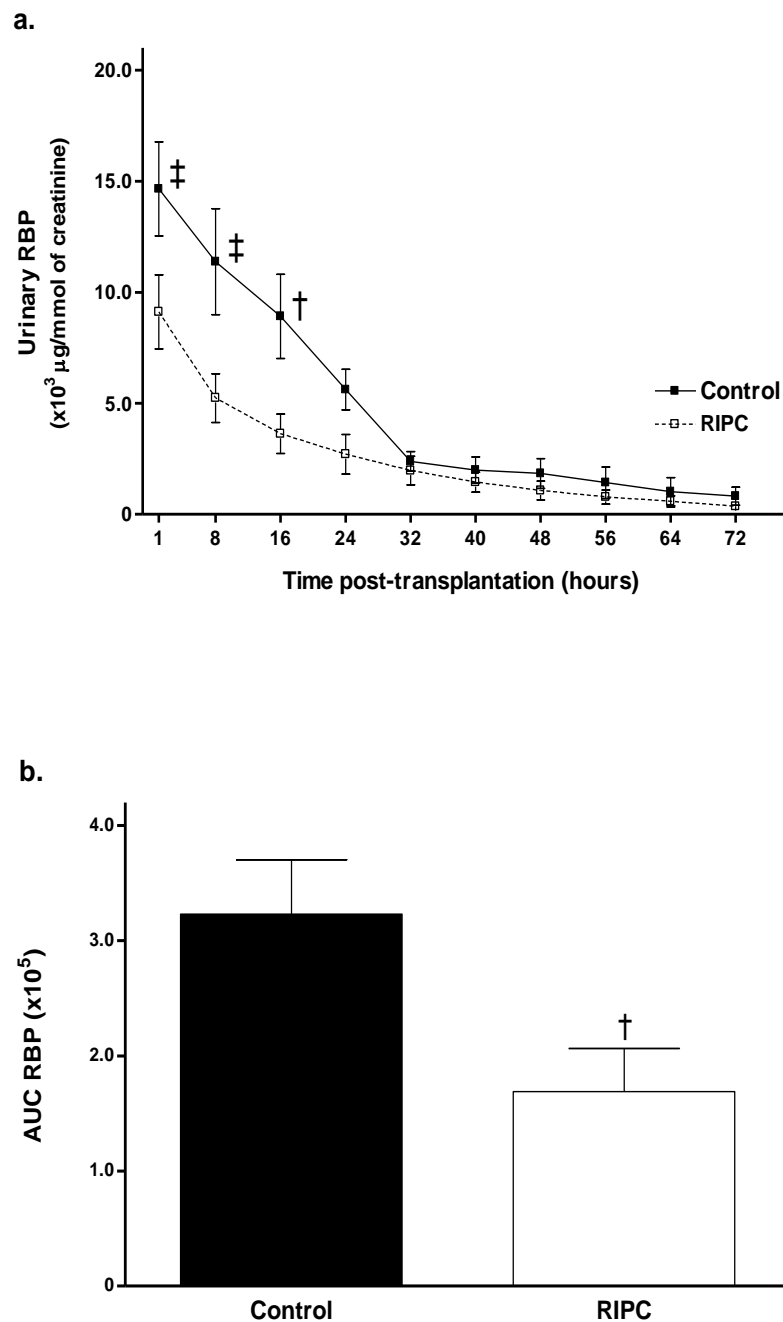


Figure 2 Effect of RIPC on urinary RBP following transplantation. (a) Urinary RBP concentration (expressed relative to urinary creatinine concentration) against time (for the first 72 hours post-transplantation) in control and RIPC groups [$p < 0.0001$ control vs. RIPC; ANOVA] (b). Area under the time curve for RBP (AUC RBP) in control and RIPC groups ($\dagger p < 0.05$ control vs. RIPC).

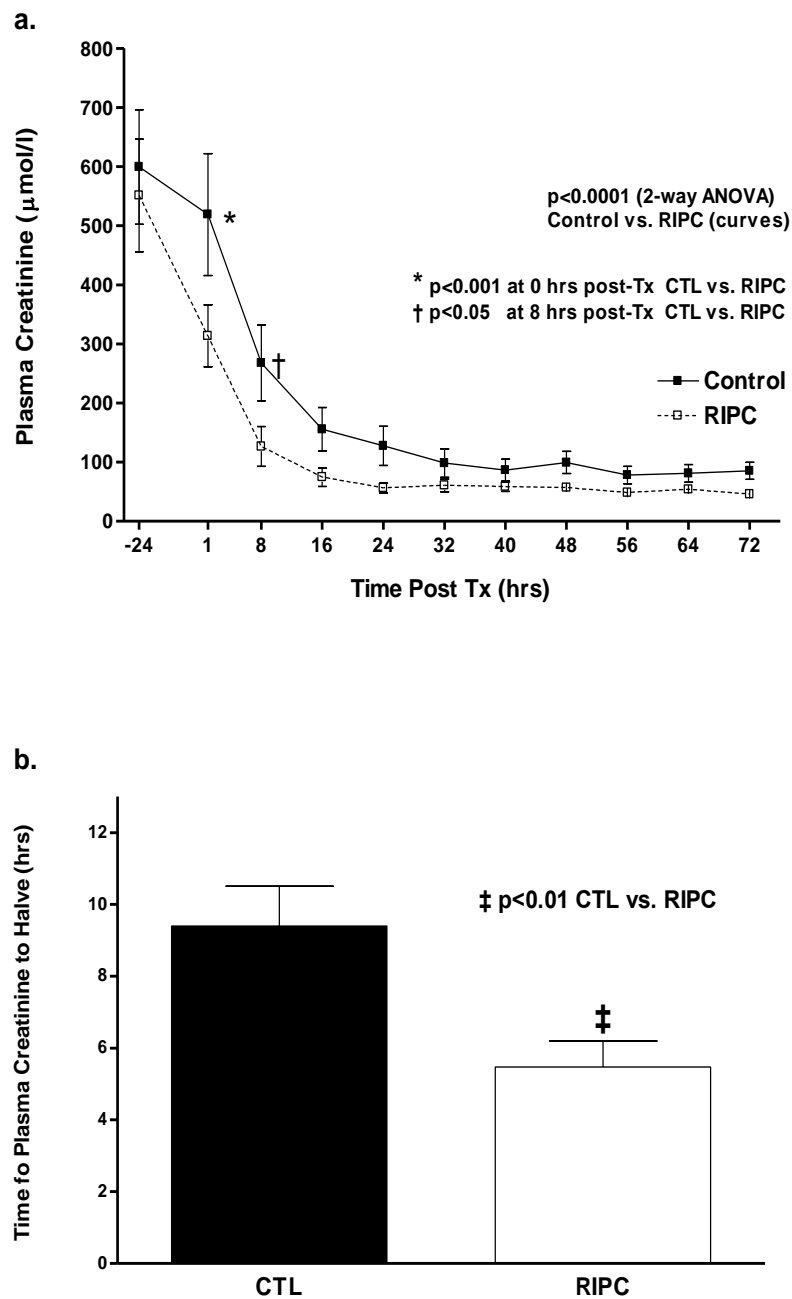


Figure 3 Effect of RIPC on plasma creatinine following transplantation. (a) Plasma creatinine concentration against time (for the first 72 hours post-transplantation in control and RIPC groups [$p < 0.0001$ control vs. RIPC; ANOVA]). (b) Time for plasma creatinine to halve in control and RIPC groups (‡ $p < 0.01$ control vs. RIPC).

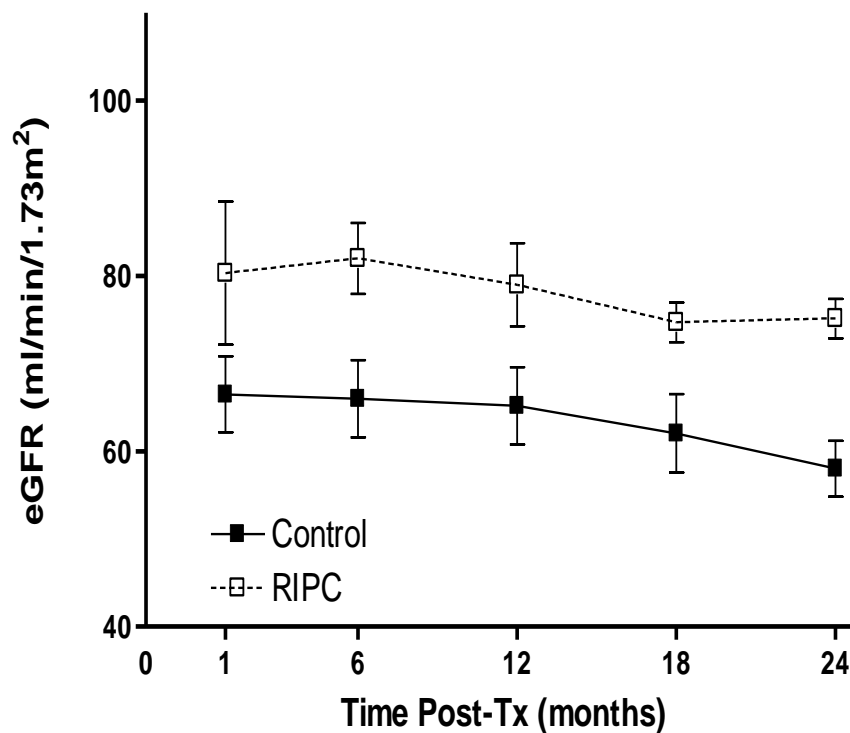


Figure 4 Effect of RIPC on long-term graft function following transplantation. Estimated GFR (eGFR) against time (1 to 12 months post-transplantation) in control and RIPC groups [$p < 0.01$ control vs. RIPC (ANOVA), $p < 0.05$ (6, 12 and 24 months post-transplantation)].

Renoprotection by RIPC has also been demonstrated in patients undergoing surgery for abdominal aortic aneurysm; in this study, surgery caused an approximate 50% increase in serum creatinine that was prevented by first window RIPC (48).

3.3.2 Safety and tolerability

There have been no unexpected adverse events utilising 3 cycles of 5 minutes ischaemia of the arm, in pre-clinical and clinical studies we have performed to date. The inflation of the arm cuff caused a sensation of upper arm pressure and towards the end of the 5 minute inflation the subject sometimes experiences a tingling sensation in the arm, but this has never been so severe as to warrant abandoning the preconditioning protocol. The procedure has no effect on platelet function as assessed by bleeding time.

4 RATIONALE FOR THE STUDY

We have confirmed that there are 2 phases of protection of RIPC in humans, each with measurable biological effects in patients undergoing IR injury in the clinical setting. A preconditioning stimulus causes an initial short-lived (up to 4 hours) period of protection from IR injury followed by a late phase 24 hours later, lasting for up to 72 hours. This trial will measure the effects of early (RIPC stimulus applied immediately before transplantation), late (RIPC stimulus applied 24 hours before transplantation) and combined (dual) RIPC in living-donor kidney transplantation.

4.1 Establishing the effects of late RIPC

The data presented above suggest a biological effect of RIPC to preserve kidney function post-transplantation. The small sample size precludes a definitive statement about the clinical usefulness of RIPC in this setting. A larger study is needed to determine robustly the effect of RIPC on clinical outcomes, including long term graft function. Given the small number of paediatric transplants, we propose to extend the work to consider the effect of RIPC in adult patients undergoing living-donor transplantation. Late RIPC had demonstrable benefits in our pilot study of renal transplantation, which we hypothesise are secondary to its prolonged and sustained phenotype. This profile might reduce IR injury and blunt the secondary inflammatory response to tissue injury. Applying the RIPC stimulus 24 hours before surgery will enable the late and sustained effects of RIPC on renal function (primary end-point) to be assessed, and establish aspects of the mechanism in humans.

4.2 Establishing the effects of early RIPC

Early RIPC activates an immediate but non-sustained protective effect, though this is followed 24 hours later by the late phase of protection. Whether this profile will result in reduction in renal IR injury will also be determined by this trial, as will aspects of the mechanism in tissues harvested peri-operatively. Identifying a protective effect of the early phase of RIPC has implications for future studies in deceased-donor transplantation, where the unpredictable availability of organs precludes the scheduling of a preconditioning protocol 24 hours in advance of surgery. In this clinical setting (and currently the majority of transplants) the only feasible preconditioning stimulus will be early RIPC.

4.3 Establishing the effects of dual RIPC

It is rational to hypothesise that a combination of early and late RIPC will augment protection beyond that seen with either alone. As detailed in section 3.1.3, the early phase of protection appears to recruit different mechanisms (at least in animal studies) from those activated in late preconditioning. This new scientific principle will be examined in this trial by randomising patients to early, late and dual (early and late) preconditioning limbs of the trial. By examining the effects of dual RIPC on clinical end-points (early fall in creatinine and late GFR) we should establish if there is an augmented biological effect and assess its clinical value in this study.

4.4 Investigations of the mechanism of RIPC

A detailed understanding of the mechanism of ischaemic preconditioning in humans will facilitate pharmacological approaches with potential to activate preconditioning pathways to supra-physiological levels. The first step will be to validate mechanisms that have been established in animal studies. Tissue samples will be harvested peri-operatively from donor and recipient. These will be small sections of renal graft artery and vein that are trimmed from vessels to facilitate anastomosis. In three of the centres participating in the study (Leiden, Cambridge, Royal Free Hospital), tissue biopsies are taken routinely from all kidney grafts during cold ischaemia. Where a section is deemed surplus to clinical need, this will be harvested for immunohistochemistry.

4.4.1 Mediators of RIPC

Samples of donor vascular tissue will be used for immunoblotting, to probe for proteins induced by late RIPC. These will be manganese superoxide dismutase (MnSOD) (49), cyclo-oxygenase 2 (COX-2) (50;51), inducible nitric oxide synthase (iNOS) (52), and heat shock proteins (HSP) 27/72 (53). Antibodies against the human isoforms of these proteins will be used and comparisons made with control. Immunohistochemistry will also be used to identify expression of these proteins in renal biopsy samples. For putative mechanisms of early RIPC, tissues will be examined for activation of protein kinase C epsilon, comparing cytosolic (inactive) with membrane bound (active) fractions (54), and downstream mitogen activated protein (MAP) kinases, including activated (phosphorylated) Akt and ERK (55). Again comparisons will be made with tissues from controls. Similar assessment will be undertaken in tissues from subjects who have undergone dual RIPC and these analyses will give insight into potential interactions between the two phases of RIPC. Co-morbidities might interfere with RIPC mechanisms in patients with kidney failure and account for a null effect in this trial. Additional comparisons between tissue from donors with normal renal function and the recipients will assess this possibility.

4.4.2 Biological effects of RIPC

The anti-inflammatory effects of RIPC will be assessed in donors and recipients. Down-regulation of TNF alpha pathways has been described in leucocytes from healthy volunteers undergoing RIPC (56). Kidney harvest and transplantation will induce a systemic acute phase response in recipients and donors, including increases in plasma and urine IL6, TNF alpha and interferon gamma (57;58).

Blood and urine samples will be taken from the 4 groups and analysed for these cytokines. The degree of kidney graft tubulointerstitial fibrosis at 6 months following living-donor kidney transplantation has been shown to correlate with long-term graft survival and function (59;60). This analysis will be done in Leiden, the transplant centre with unique experience of this analysis.

Alloreactive T cells will be analysed with regard to their detailed surface phenotype (to give insight into their probable effector functions and tissue homing capacities). Monocyte-derived dendritic cells, lymphoblastoid cell lines and “inflammatory cytokine stimulated lymphocytes” from donor peripheral blood samples will be used as in vitro stimulator cells to test recipient T cell activation, cytokine synthesis and proliferation (61). This analysis will be done at the Royal Free Hospital.

Genomic and proteomic approaches will be used to identify putative gene and protein signals in response to RIPC and subsequent IR injury. Plasma, DNA and RNA samples will be taken before and after RIPC protocols from donors and recipients in each of the 4 groups, and after transplantation surgery from the recipients. Samples will be biobanked for subsequent secondary analyses in the future.

5 TRIAL OBJECTIVES

The trial has 3 main objectives

1. To determine the effect of RIPC on renal ischaemia-reperfusion (IR) injury in adult living-donor renal transplantation

2. To establish whether combining early and late phase RIPC provides extra protection from IR injury after renal transplantation
3. To identify mechanisms of RIPC in humans

6 TRIAL DESIGN

6.1 Statement of design

A double-blind randomised trial of the effect of RIPC to reduce renal IR injury in adults undergoing renal transplantation.

6.2 Number of Centres

Patients will be recruited from 10 centres:

1. Guy's and St. Thomas' NHS Trust
2. Leiden University Medical Centre, Leiden, The Netherlands
3. North Bristol NHS Trust
4. Cambridge University Hospitals, NHS Trust
5. Royal Free Hospital NHS Trust, London, UK
6. University Hospitals of Leicester NHS Trust
7. Queen Elizabeth Hospital, Birmingham
8. VU University Medical Centre Amsterdam, The Netherlands
9. St George's Hospital, London
10. The Royal London Hospital, London

The trial will be co-ordinated by the Trials Co-ordination Group, London School of Hygiene and Tropical Medicine, London.

6.3 Number of Subjects

We estimate a sample size of 400 subjects will need to be recruited.

6.4 Sample size determination

6.4.1 *Primary analysis*

There will be two main analyses to reflect the factorial design of the trial using mean GFR in the first year after transplantation. These will be (i) the two arms receiving late RIPC compared to the two arms not receiving late RIPC (ii) the two arms receiving early RIPC compared to the two arms not receiving early RIPC.

Mean GFR in the first year after transplantation is estimated to be 47.3 ml/min/1.73m² in those not receiving RIPC with a SD of 13.9 (data from the Cambridge transplantation programme). The following calculations are based on either intervention increasing GFR by 10% (or 4.73 ml/min/1.73m²). A trial of 80 patients in each of the four arms (160 for each comparison group: 320 in total) gives 80% power (with a 5% type 1 error) to detect this difference in GFR at 12 months for either comparison allowing for a 15% dropout rate. The primary analysis will be performed when all patients taking part in the trial have reached the 12-month post-transplantation time-point. Analyses will be performed on an intention-to-treat basis.

| | Treatment group | | | | Total |
|----|-------------------------------|----------------------------|----------------------------|----------------------------|-------|
| | Control early Control late | Early RIPC Control late | Control early Late RIPC | Early RIPC Late RIPC | |
| SD | (i) 12.9 | 69 | 69 | 69 | 276 |
| | (ii) 13.9 | 80 | 80 | 80 | 320 |
| | (iii) 14.9 | 92 | 92 | 92 | 368 |

Table 1. Sample size calculations for comparison of GFR assuming 10% higher GFR on RIPC (for 80% power and allowing for 15% dropout).

This schedule of randomisation gives us adequate power for the primary end-point, whilst retaining useful power for secondary analyses. The trial will provide greater than 80% power if the difference is greater than 4.73 ml/min/1.73m² (as might be expected if the effects of early and late RIPC combine multiplicatively i.e. a 21% increase compared to no RIPC), the SD is lower than anticipated or dropouts are lower than 15%.

Further, some allowance for the possibility of a moderate interaction will be made whereby the impact might be to lessen the anticipated effect when comparing (i) the arms receiving late RIPC vs not receiving late RIPC, and (ii) the arms receiving early RIPC vs not receiving early RIPC. To allow for this possibility the aim is to recruit 100 patients in each of the four arms (400 in total).

6.4.2 Secondary analyses

A large interaction between early and late RIPC on mean GFR is not expected although this will be assessed. For instance, if it appears as though there is no additional benefit of receiving both early and late RIPC together over receiving either early or late separately, a secondary analysis will be undertaken combining all RIPC arms together vs. the control arm receiving no RIPC. A trial of this size gives well over 90% power to detect a 15% difference in the time for creatinine to halve post-transplantation at 80% power (time for creatinine to halve is 9.4 hours with an SD of 3.4 hours). For urine and plasma cytokine analyses, the sample size will allow the detection of a 20% difference in cytokine levels. For tissue analysis no power calculation is possible; much of the analysis will be qualitative and there are no quantitative human data. We anticipate that 8-10 tissue samples will be harvested from each limb of the study. Similarly, for T cell alloreactivity, we anticipate that samples will be obtained for 8-10 donor/recipient pairs.

6.5 Randomisation

Recruits will be allocated at random in a 1:1:1:1 ratio to control (sham RIPC), early RIPC (immediately pre-operatively), late RIPC (24 hours pre-operatively) and dual RIPC (RIPC 24 hours and immediately pre-operatively) groups.

6.6 Study duration

The anticipated duration will be 5 years. The intervention phase will last 80 minutes over a 24 hour period. Data collection will occur for up to 5 years after recruitment.

6.7 Study objectives

6.7.1 Primary objective

To determine if RIPC improves glomerular filtration rate of the transplanted kidney in adults 12 months after undergoing living-donor transplantation.

6.7.2 Secondary objectives

To determine the effects of RIPC on

1. Rate of fall in creatinine in the first 72 hours after transplantation
2. Inflammatory response to surgery in the first 5 days after transplantation
3. Protein expression in kidney parenchyma samples using histochemistry
4. Protein activation and expression in renal vasculature using immunoblotting
5. Kidney fibrosis 6 months after transplantation
6. Alloreactivity of T cells in the first 18 months after transplantation
7. Patient outcomes 2-5 years after transplantation using renal registry data

6.8 Study endpoints

6.8.1 Primary endpoint

Glomerular filtration rate (GFR) 12 months after transplantation using iohexol clearance

6.8.2 Secondary endpoints

1. Time for serum creatinine to fall by 50%
2. eGFR 3 months after transplantation
3. White cell count, CRP and plasma IL6, interferon gamma and TNF alpha before, and 1-3 days after surgery (donors)
4. White cell count, CRP and plasma IL6, interferon gamma and TNF alpha before, and 1-5 days after surgery (recipients)
5. RIPC-induced protein expressional changes in renal tissue [analysis in biopsy material; protein kinase C (epsilon isoform; activated/membrane-bound fraction), superoxide dismutase (MnSOD), cyclo-oxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), heat shock proteins (HSP) 27/72, reperfusion injury salvage kinases (PI3K-Akt and MEK1/2-ERK)]
6. Renal graft cortical tubulointerstitial fibrosis at 6 months (digital analysis of Sirius red staining in biopsy material)
7. Incidence of delayed graft function (either the need for dialysis in the first 7 days after transplantation or serum creatinine levels increase, remain unchanged, or decrease less than 10% per day in three consecutive days in the first week after transplantation)
8. T cell activation, cytokine synthesis and proliferation in response to donor cells
9. Incidence of acute rejection during the first 12 months after transplantation
10. Serum creatinine and eGFR 2 to 5 years after transplantation
11. 3 month, 12 month, and 2 to 5 year graft survival
12. 12 month, and 2-5 year patient survival

6.9 Trial treatments

The intervention is non-pharmacological. Patients will be randomised into 4 groups: control (sham RIPC), early RIPC, (immediately pre-operatively, late RIPC (24 hours and immediately pre-operatively) and dual RIPC (early and late RIPC; *figure 5*).

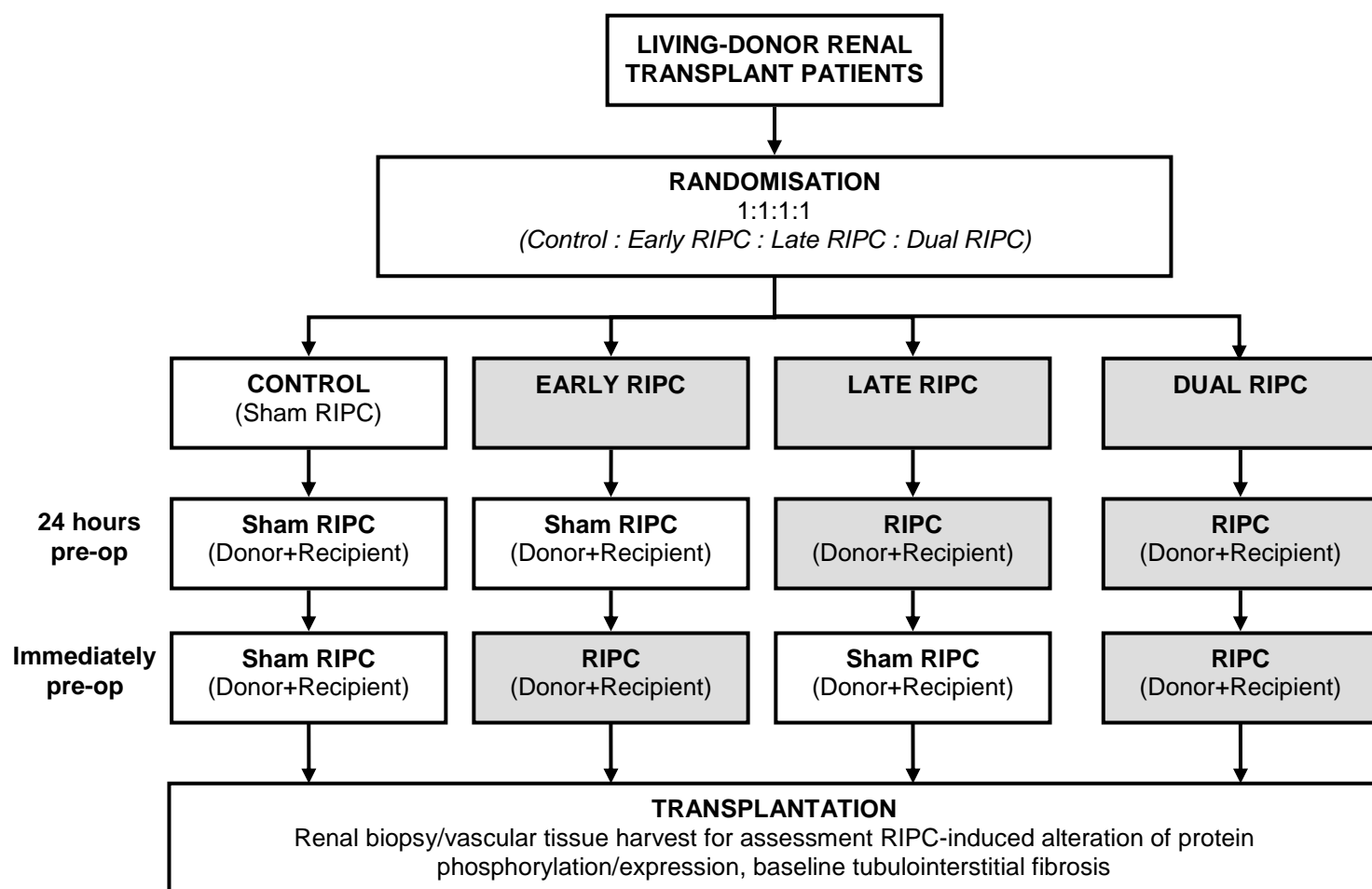


Figure 5 Randomisation and RIPC schedules

6.9.1 24 hours before surgery

Two of the groups will undergo active treatments (late and dual) and two will undergo sham RIPC (control and early). Active treatment will consist of four 5-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg above systolic blood pressure. The inflations will be separated by 5-minute periods when the blood pressure cuff will be deflated. Placebo treatment (sham RIPC) will consist of three 5-minute inflations of a blood pressure cuff on the upper arm to 40 mmHg. The inflations will be separated by 5-minute periods when the blood pressure cuff will be deflated and will also occur 24 hours before surgery (*figure 5*).

6.9.2 Immediately before surgery

The group randomised to early RIPC and dual RIPC will undergo an RIPC stimulus immediately before surgery to activate early phase RIPC. Patients randomised to the control group or late RIPC will receive sham RIPC immediately pre-operatively (*figure*

5). The active or placebo sequences will be initiated before induction of anaesthesia and completed in advance of the initiation of surgery.

There will be no other interventions.

6.10 Criteria for discontinuation

6.10.1 Individual subject

1. Patients are free to choose to withdraw from the trial at any time.
2. Operative complications that directly influence graft revascularisation.

6.10.2 Trial

Unexpected safety issues on the advice of the Data Monitoring Committee

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Inclusion criteria

1. Patients undergoing living donor transplantation
2. Patients aged 18 years and above

7.2 Exclusion criteria

1. 0,0,0-mismatched renal grafts (no mismatch in HLA-A/B/DR antigens between donor and recipient)
2. Patients on ATP-sensitive potassium channel opening or blocking drugs
3. Patients on ciclosporin
4. Patients with a known iodine sensitivity (who cannot undergo iohexol clearance studies)
5. Patients with ABO incompatibility
6. Any patient requiring HLA antibody removal therapy

7.3 Assignment and randomisation number

This will be done on a web-based service through the Trials Co-ordination Group at the London School of Hygiene and Tropical Medicine.

7.4 Method of blinding

A research nurse at each study site will remain blinded to the allocation of patients to either real or sham RIPC. The preconditioning procedure will be performed by an investigator who is not involved in sample collection or data analysis.

7.5 Emergency un-blinding

The benign and short-term nature of the intervention makes this an unlikely event. This will be overseen by the Trials Co-ordination Group at the London School of Hygiene and Tropical Medicine.

7.6 Subject withdrawal criteria

Withdrawal from the study will be uncommon, because of the benign nature of the intervention, its application within a single 24 hour period, and the embedding of follow-up within routine clinical care

7.6.1 Criteria for withdrawal from study

Withdrawal of consent by the patients
Complications of surgery that result in failed anastomosis

7.6.2 When and how to withdraw subjects from the trial

This can happen at any time pre-, peri- or post-operatively. There are no specific procedures required.

7.6.3 Replacement of withdrawn subjects

If a subject withdraws from the study before randomisation he/she will be replaced with another subject. The sample size allows for up to a 15% drop-out rate during the trial.

7.6.4 Follow-up of subjects withdrawing from study

Patients who are randomised but withdraw before the intervention will undergo standard clinical care according to local protocols. If patients undergo the intervention but subsequently withdraw, they will undergo standard clinical care. Patients will be encouraged to allow data and samples that have been collected before withdrawal to be used in the analyses. However, if consent to use data/samples is also withdrawn, then these will be discarded. All patients participating in the study will undergo routine post-transplant follow-up. Patients withdrawing from the study will continue to be followed-up by their local transplant team. There should be no need for further follow-up from the research team.

8 TREATMENT REGIMENS

8.1 Immunosuppressive therapy

In order to make the outcome analysis as straightforward as possible, patients will follow the same immunosuppressive protocol. The immunosuppression, agreed by all participating centres, will comprise:

8.1.1 Prednisolone

Patients will receive methylprednisolone and/or prednisolone according to local practice. All patients will be commenced on not less than 20mg/day, reducing to no more than 7.5 mg/day by three months.

8.1.2 Anti-CD25 (Basiliximab)

This will be administered according to the manufacturer's recommendations, 20mg iv pre transplant followed by 20mg iv on day 4.

8.1.3 Mycophenolate/Azathioprine

All patients will receive mycophenolate or Azathioprine. Mycophenolate will be administered as mofetil (CellCept) starting at a dose of at least 1gm/day, or mycophenolate sodium (Myfortic) starting at a dose of at least 720mg/day. Azathioprine will be administered at a starting dose of Azathioprine 2mg/kg

8.1.4 Tacrolimus

All patients will receive tacrolimus (first dose pre-operatively). Target concentration will be according to local practice.

8.2 Concomitant therapy: antimicrobial and anti-thrombotic prophylaxis

Anti-microbial and anti-thrombotic prophylaxis will be in accordance with local practice. It is anticipated that subjects will receive prophylaxis against pneumocystis carinii pneumonia, oral candida albicans, and cytomegalovirus (CMV donor positive, recipient negative).

There will be no alterations to routine treatment.

8.3 Anaesthesia

There will be no changes from routine practice.

9 STUDY PROCEDURES AND ASSESSMENTS

9.1 Informed consent

Subjects will be informed about the study in advance of being admitted for surgery. Recruitment of subjects will be initiated in the outpatient setting.

9.2 Baseline data

9.2.1 *Recipient*

All recipients will have a full medical history taken and a clinical examination as part of usual care. The following are to be recorded:

1. Weight
2. Height
3. Gender
4. Ethnicity – Caucasian, Asian, Afro-Caribbean, other
5. Date of birth
6. Blood Pressure
7. Date when started dialysis (or creatinine if pre-dialysis)
8. Type of dialysis at time of admission: Pre / Haemodialysis/ Peritoneal dialysis
9. Creatinine, urea, albumin pre-transplant

9.2.2 *Donor*

The following donor information will be recorded from the UK Transplant or Eurotransplant organ donor form:

1. Weight
2. Height
3. Gender
4. Age
5. Ethnicity – Caucasian, Asian, Afro-Caribbean, other
6. Blood Pressure
7. Creatinine, urea, albumin pre-transplant

8. GFR (MDRD equation)

9.3 Day before transplantation (day 0)

9.3.1 Donor

1. Pre-transplant serum creatinine
2. Plasma and urine for cytokine analysis
3. Blood for DNA and RNA analysis

9.3.2 Recipient

1. Pre-transplant serum creatinine (but after any pre-transplant haemodialysis)
2. Plasma and urine collected for cytokine analysis
4. Blood for DNA and RNA analysis
3. Blood for lymphocyte isolation

9.4 First 7 days post-transplantation

9.4.1 Donor

1. Plasma and urinary inflammatory indices at day 1, 2 and 3 after surgery.
2. Collection of vascular biopsy samples

9.4.2 Recipient

1. Serum creatinine; 4, 8 and 24 hours and then daily up to 72 hours post-operatively (first collection at recovery)
2. Plasma and urinary inflammatory indices at day 1, 2 and 5 after surgery.
3. Collection of vascular biopsy samples
4. Delayed graft function (serum creatinine levels increase, remain unchanged, or decrease less than 10% per day in three consecutive days in the first week after transplantation)
5. Peri-operative transplant biopsies; redundant tissue to be processed for immunohistochemistry

9.5 3 and 12 months post-transplantation

The following are to be measured/recorded at 3 months and 12 months for each kidney recipient:

1. Weight
2. Biochemistry (creatinine, urea, albumin)
3. eGFR at 3 months, iohexol clearance at 12 months
4. Date of last dialysis
5. Total number of dialysis episodes and their nature (haemodialysis or peritoneal dialysis)
6. Date commenced tacrolimus
7. Kidney biopsy at 6 months (Leiden only); redundant tissue to be processed for immunohistochemistry
8. Blood samples for T cell alloreactivity studies (donor and recipient)

9.6 Every admission in the first 12 months post-transplantation

The following are to be recorded at every admission during the first post-operative year for each kidney recipient:

1. Admission date
2. Indication for admission
3. Discharge date
4. Dialysis episodes while inpatient (and nature; haemodialysis or peritoneal dialysis)
5. Acute rejection episodes and their treatment
6. Date of death (if applicable)
7. Date of graft loss (if applicable)

9.7 1-18 months post-transplantation (Royal Free Hospital only)

1. Lymphocyte preparation at 1, 3, 6, 12 and 18 months from donor and recipient

9.8 Subsequent follow up

Serum creatinine (for estimated GFR calculation using the MDRD equation), graft and patient survival data will be obtained annually for years 2 to 5 following transplantation. Subjects will continue follow-up at their local transplant centre as per local protocol. There is no additional requirement for study visits.

9.9 Study procedures table: donor

| | Study visits | | | | | | | | | | | | |
|--|--------------|----------|----------|-----------|-------------------------------|---|---|---------------------------------|---|---|----|----|--|
| | Pre-op | | | | Post-op | | | | | | | | |
| | | | | | Visit identified by study day | | | Visit identified by study month | | | | | |
| CLINICAL ASSESSMENTS | screen | baseline | Pre RIPC | Post RIPC | 1 | 2 | 3 | 1 | 3 | 6 | 12 | 18 | |
| Informed consent | x | | | | | | | | | | | | |
| Review of inclusion/exclusion criteria | x | | | | | | | | | | | | |
| History and physical examination | x | | | | | | | | | | | | |
| Weight, height | x | | | | | | | | | | | | |
| Demographics | x | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| LABORATORY ASSESSMENTS | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| Urea, creatinine, albumin | | x | | | | | | | | | | | |
| Plasma cytokines | | x | | | x | x | x | | | | | | |
| Urine cytokines | | x | | | x | x | x | | | | | | |
| Blood lymphocyte ¹ | | x | | | | | | x | x | x | x | x | |
| Plasma biomarkers | | | x | x | | | | | | | | | |
| Blood for DNA/RNA | | | x | x | | | | | | | | | |
| | | | | | | | | | | | | | |

¹ Royal Free Hospital only

Study procedures table: recipient

| | Study visits | | | | | | | | | | | | | | | | |
|--|--------------|-----------|----------|-----------|--------|-------------------------------|---|---|---|---------------------------------|---|----|----|----|----|----|----|
| | Pre-op | | | | Per-op | Post-op | | | | | | | | | | | |
| | screen | Base line | Pre RIPC | Post RIPC | | Visit identified by study day | | | | Visit identified by study month | | | | | | | |
| | | | | | 1 | 2 | 3 | 5 | 1 | 3 | 6 | 12 | 18 | 24 | 36 | 48 | 60 |
| CLINICAL ASSESSMENTS | | | | | | | | | | | | | | | | | |
| Informed consent | x | | | | | | | | | | | | | | | | |
| Review of inclusion/exclusion criteria | x | | | | | | | | | | | | | | | | |
| History and physical examination | x | | | | | | | | | | | | | | | | |
| Weight, height | x | | | | | | | | | x | | x | | | | | |
| Demographics | x | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| LABORATORY ASSESSMENTS | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| Urea, creatinine, albumin | | x | | | | x | x | x | | x | | x | | | | | |
| Plasma cytokines | | x | | | | x | x | x | | | | | | | | | |
| Urine cytokines | | x | | | | x | x | x | | | | | | | | | |
| Blood lymphocyte ¹ | | x | | | | | | | x | x | x | x | x | | | | |
| Plasma biomarkers | | | x | x | | | | | | | | | | | | | |
| Blood for DNA/RNA | | | x | x | | | | | | | | | | | | | |
| Tissue samples | | | | | x | | | | | | | | | | | | |
| Iohexol clearance | | | | | | | | | | | | x | | | | | |
| eGFR | | | | | | | | | | x | | x | | x | x | x | x |
| Biopsy for fibrosis ² | | | | | | | | | | | x | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| CLINICAL OUTCOMES /SAFETY | | | | | | | | | | | | | | | | | |
| Dialysis episodes | | | | | | | | | | x | | x | | | | | |
| Routine clinical data | | | | | | | | | | | | x | | x | x | x | x |

¹ Royal Free Hospital only ² Leiden only

9.10 Laboratory techniques

9.10.1 *GFR assessment at 12 months*

1.2g iodine/2.59g ml of iohexol (5 ml Omnipaque 240) will be administered intravenously and blood samples taken at 5, 120, 180, and 240 min after dosing. Heparinised blood (5 ml) will be centrifuged at 400g for 10 minutes and a 2 ml sample of supernatant aspirated and collected in labelled eppendorf. Heparinised plasma will be stored at -70°C/-80°C, until analysis, with GFR calculated using the iohexol clearance rate between 120 and 240 min. Results will be corrected for body surface area.

9.10.2 *Immunoblotting*

Vascular tissue will be homogenised in buffer containing peptidase inhibitors, electrophoresed on an SDS-polyacrylamide gel and transferred to nitrocellulose (as previously described (62)). Antibodies (Calbiochem, Invitrogen and Dako) against the components specified in section 4.4.1 will be used to probe membranes for protein. G6PDH immunoblotting will be used as a control. We will aim to collect 8-10 tissue samples from donors and recipients in each of the control, early, late and dual RIPC limbs of the trial for qualitative analyses.

9.10.3 *Immunohistochemistry*

5µm cross-sections will be obtained from formalin fixed, paraffin embedded renal biopsy tissues. Sections will be prepared for immunohistochemistry by dewaxing and rehydrating using xylene and alcohol. Antibodies for immunohistochemistry will be obtained from Serotec (MnSOD, HSP27) or Abcam (COX-2, iNOS). Diaminobenzidine (0.06% [w/v], DAKO) will be used to provide visualization of immunoreactivity with methyl green counterstaining. Isotypic and pre-adsorbed staining controls will also be included to demonstrate antibody selectivity and specificity.

9.10.4 *Kidney graft fibrosis*

Cortical tubulointerstitial collagen deposition will be assessed by Sirius red staining of tissue slices, using digital analysis software (63;64). Analysis will be performed at baseline and at 6 months following transplantation; graft fibrosis at 6 months will be expressed relative to baseline graft fibrosis.

9.10.5 *ELISA assessments*

ELISA (R+D Systems) will be used to measure interleukin 6, interferon gamma and TNF alpha in plasma (donors and recipients) and urine (recipients) before (plasma), 1-5 days after surgery (58). Urine and heparinised blood (5 ml) will be centrifuged at 400g for 10 minutes and a 2 ml sample of supernatant aspirated and collected in labelled eppendorfs. Urine and heparinised plasma will be stored at -70°C/-80°C, until analysis at UCL.

9.10.6 *T cell alloreactivity (Royal Free Hospital only)*

Host lymphocytes will be isolated from blood taken pre-transplant and at 1, 3, 6, 12 and 18 months post transplant. Alloreactive T cells will be stimulated by co-incubation with

cells from the peripheral blood of the specific donor. These will be monocyte-derived dendritic cells, cytokine-stimulated mononuclear cells and EBV-transformed lymphoblastoid cell lines (61), to represent decreasing levels of allostimulator capacity. These assays will test the capacity of host T cells to respond to “directly” presented alloantigens. 9-colour flow cytometry (FCM) will simultaneously measure activation (CD69/CD138), cytokine synthesis (intracellular interleukins, TNF alpha and interferon gamma), cell proliferation (BRDU-uptake) and cell surface phenotype (CD3, CD4, CD8, CD45RA, CCR7). This allows the comparison of cell surface phenotypes of cells with different functional characteristics.

9.10.7 Genomic and/proteomic analyses

Blood (10mls) will be taken before RIPC (or sham RIPC) at t-24. A second sample will be taken at t=0 after completion of the second RIPC (or sham RIPC) stimulus. A 5 ml EDTA sample will be stored -70°C/-80°C, until analysis at UCL. A 5 ml heparinised sample will be centrifuged at 400g for 10 minutes and a 2 ml sample of supernatant aspirated and collected in an eppendorf. Heparinised plasma will be stored at -70°C/-80°C, until analysis at UCL.

10 EVALUATION OF RESULTS

10.1 Response criteria

10.1.1 Biochemical measures

These categorical variables will be measured as described above.

10.1.2 Delayed graft function

Delayed graft function will be diagnosed by the need for dialysis in the first 7 days after transplantation or when serum creatinine levels increase, remain unchanged, or decrease less than 10% per day in three consecutive days in the first week after transplantation (65).

10.1.3 Acute rejection

Rejection will be defined in three ways:

1. *Biopsy proven rejection* will be defined as any rejection grade according to the Banff criteria (66) of the histopathological appearances of a needle core biopsy of the transplant kidney.
2. *Clinical acute rejection* will be defined as any biopsy proven or biochemical rejection which is treated with pulsed methyl prednisolone.
3. *Steroid resistant rejection* will be defined as a rejection episode that doesn't respond to a three day course of pulsed methyl prednisolone and which requires anti-thymocyte globulin or muromonab-CD3 therapy.

10.1.4 Survival (patient and graft)

These will be measured from the date of transplant and will be reported for all deaths and graft failures both due to rejection and due to all causes. The cause of death or graft failure is thus to be recorded in all instances.

10.1.5 Graft function

Graft function will be defined by “*formal*” GFR measured by iohexol clearance 12 months post-transplantation, and “*estimated*” GFR using the Modification of Diet in Renal Disease (MDRD) equation (at 3 months and annually 2 to 5 years post-transplantation) (67).

11 ASSESSMENT OF SAFETY

11.1 Definitions

This is not a trial of an investigational medicinal product. Therefore, by definition all untoward occurrences will be adverse events rather than adverse reactions. Safety assessments will be from time of randomisation to completion of follow up for recipients, and from time of intervention to discharge for donors.

11.1.1 Adverse event

This is defined as any untoward medical occurrence affecting a patient which does not necessarily have a causal relationship with the RIPC stimulus. The terms “*mild, moderate or severe*” are used to describe the intensity of a specific event or reaction. This is not the same as “*serious*” which is based on patient/event outcome or action criteria as defined below. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of RIPC whether or not considered related to the technique.

11.1.2 Serious adverse event (SAE)

Any untoward medical occurrence/effect that:

1. Results in death.
2. Is life-threatening.
3. Requires hospitalisation or prolongation of existing inpatient’s hospitalisation.
4. Results in persistent or significant disability or incapacity.

“*Life-threatening*” in the definition of a serious adverse event refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

11.1.3 Unexpected adverse event

This is defined as an adverse event, the nature or severity of which is not consistent with an expected consequence of RIPC.

11.2 Expected adverse events (recognised to be caused by the RIPC stimulus)

The benign nature of the RIPC stimulus excludes there being any expected serious adverse events. The following are expected non-serious events in response to the RIPC stimulus and will be recorded on the Case Report Form. They do not need to be reported to the Trials Co-ordination group (see section 11.4).

1. Transient (1-2 minutes) pain, paraesthesia
2. Skin petechiae caused by cuff inflation

11.3 Expected serious adverse events related to usual clinical care

These events are recognised complications of renal transplantation and related interventions. They will be recorded on the Case Report Form but do not need to be reported separately on an SAE form (see section 11.4).

1. Delayed graft function

2. Acute rejection
3. Renal artery thrombosis
4. Renal vein thrombosis
5. Post-operative infection
6. Graft loss
7. Haemodialysis, peritoneal dialysis, or haemofiltration
8. Admission for removal of ureteric stents
9. Admission for removal of a peritoneal dialysis catheter
10. Admission for protocol kidney biopsy or for biopsies of kidneys with suspected rejection
11. Admission for any other recognised complication of renal transplantation

The following events are recognised complications of routine clinical care and for the purposes of this trial will not be designated as SAEs. They do not need to be reported.

1. Complications of surgery (other than those listed in 11.3)
2. Complications due to administration of anaesthetic agents
3. Known adverse effects of immunosuppressive and other drugs used in routine clinical care

11.4 Reporting unexpected adverse events

Investigators will make their reports of all unexpected adverse events, whether serious or not, to the Trials Co-ordination Group, London School of Hygiene and Tropical Medicine.

11.4.1 Unexpected Serious Adverse Events (SAE)

SAEs (other than those described in 11.3) should be reported to the Trial Coordination Group within 7 days. The report should include an assessment of causality by the Principal Investigator (see section 11.4.4). The Chief Investigator will be responsible for the prompt notification of findings that could adversely affect the health of subjects or impact on the conduct of the trial. Notification of confirmed unexpected SAEs will be to the Sponsor, the Research Ethics Committee and the Data Monitoring Committee (DMC). All deaths will be reported to the sponsor irrespective of whether the death is related to transplantation or is an unrelated event.

11.4.2 Unexpected Non serious adverse events (NSAE)

Unexpected non-serious adverse events should be evaluated by the Principal Investigator. This should include an assessment of causality and intensity (see section 11.4.4) and reports made within 14 days. The Trials Co-ordination Group will keep detailed records of all unexpected adverse events reported. Reports will be reviewed by the Chief Investigator to consider intensity, causality, and expectedness. As appropriate these will be reported to the sponsor, the DMC and the Ethics Committee.

11.4.3 Assessment of intensity

Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.

Severe: Significant impairment of functioning; the subject is unable to carry out usual

activities and/or the subject's life is at risk from the event.

11.4.4. Assessment of causality

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the adverse event and administration of the intervention.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the adverse event and administration of the intervention.

Unlikely: A causal relation is improbable and another documented cause of the adverse event is most plausible.

Unrelated: A causal relationship can be definitely excluded and another documented cause of the adverse event is most plausible.

12 STATISTICS

12.1 Study statistician

Mr Tim Clayton, Trials Co-ordination Group, London School of Hygiene and Tropical Medicine.

12.2 Statistical methods to be employed

A detailed statistical analysis plan will be produced prior to unblinding of any data. However, the primary analyses will be comparisons of mean GFR at one year after transplantation between (i) the two arms receiving late RIPC compared to the two arms not receiving late RIPC (ii) the two arms receiving early RIPC compared to the two arms not receiving early RIPC. A large interaction between early and late RIPC on mean GFR is not expected although this will be assessed by using a regression model and fitting an interaction term between the two treatments (early and late RIPC). If a large interaction exists, a secondary analysis will be undertaken as described in 6.4.1. Differences in means (continuous variables) and proportions (categorical variables) will be calculated together with 95% confidence intervals. Significance tests will also be calculated using t-tests and chi-squared tests. The primary analyses will be conducted on an intention to treat basis.

12.3 Interim analyses

The DMC (section 18.2) will perform an interim safety analysis at least annually.

12.4 Number of subjects to be enrolled

We estimate that 400 subjects will need to be recruited (*see section 6.4 for power calculation*).

12.5 Procedure to account for missing or spurious data

All subjects randomised to the study will be analysed on an intention to treat basis as well as according to the actual pre-treatment that the kidney received. Data will be validated and the data analysis will take appropriate account of missing values.

12.6 Definition of the end of the trial

The trial shall be considered finished when the last patient recruited reaches the 5-year follow up point. At that point notification of closure of the study will be sent to the Research Ethics Committee.

12.7 Criteria for the termination of the trial

The trial will terminate once the last patient recruited reaches 5 year follow-up.

13 DIRECT ACCESS TO SOURCE DATA / DOCUMENTS

Local investigators shall ensure that all study data are available for trial related monitoring, audits, and research ethics committee review.

14 ETHICAL CONSIDERATIONS**14.1 Consent**

All patients will freely give their informed consent to participate in the study. A patient may decide to withdraw from the study at any time without prejudice to their future care.

14.2 Declaration of Helsinki and ICH Good Clinical Practise

The study will conform to the spirit and the letter of the declaration of Helsinki, and in accord with the ICH Good Clinical Practice Guidelines.

14.3 Ethical committee review

An appropriately constituted research ethics committee will review the study protocol, and a favourable opinion required before study commencement. Copies of the letters of approval are to be filed in the study files at each centre.

15 DATA HANDLING AND RECORD KEEPING

Electronic data will be returned to the London School of Hygiene and Tropical Medicine. Data will be kept for 15 years following completion of the study. The use of the data from the study will be controlled by the chief investigator and the Trials Co-ordination Group at the London School of Hygiene and Tropical Medicine. A signed hard-copy of the RIPC intervention sheet will be kept at each centre and copied to the Trials Co-ordination Group at the London School of Hygiene and Tropical Medicine.

16 INSURANCE

Centres will be covered by NHS indemnity for negligent harm providing researchers hold a contract of employment with the NHS, including honorary contracts held by academic staff. Medical co-investigators will also be covered by their own medical defence insurance for non-negligent harm.

17 PUBLICATIONS POLICY

It is our intention to disseminate the results of the study as widely as possible. This is likely to be through a publication in a peer-reviewed transplant journal, and through presentations at National and International Transplantation conferences. Publications will follow the CONSORT guidelines. Authorship will follow international guidelines.

18 RESEARCH GOVERNANCE

18.1 Trial Steering Committee

University College London will be the sponsor of this study. The Trial Steering Committee (TSC) will include independent experts (Professor Tom Meade (Chair), Adam McClean, John Forsyth and Lisa Burnapp Consultant nurse, living donor transplant) and trial investigators, including Raymond MacAllister, Stavros Loukogeorgakis, Mark Harber, Chris Watson and Rosemary Knight. Two consumer representatives will also be appointed. The TSC will oversee arrangements for ethics committee application, and recruitment of research staff. Once the study is underway, it will meet 6 monthly to review recruitment rate and consider protocol amendments. It will be responsible for drafting the final report and submission for publication.

18.2 Data Monitoring Committee (DMC)

This consists of three independent members with expertise in ischaemic preconditioning, renal medicine, clinical trials and statistics. They are Rajesh Kharbanda, Alan Jardine and Joan Morris. A pre-enrolment meeting will be held by the DMC to establish its charter. Subsequently the DMC will meet at least annually to review data. The DMC will primarily focus on patient safety while also reviewing operational issues such as recruitment.

19 REFERENCES

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