Open-label, randomised multicentre study of CAMPATH-1H versus basiliximab induction treatment and sirolimus versus tacrolimus maintenance treatment for the preservation of renal function in patients receiving kidney transplants

CAMPATH, Calcineurin inhibitor reduction and Chronic allograft nephropathy (the 3C Study)

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CAMPATH, Calcineurin Inhibitor Reduction and Chronic Allograft Nephropathy (The 3C study)

DOES CAMPATH INDUCTION ALLOW REDUCED EXPOSURE TO CALCINEURIN INHIBITORS?

The major challenge facing the kidney transplantation community at present is improving the long-term survival of grafts. Although there has been significant progress in reducing acute rejection in the last decade, there has been no concomitant increase in the long-term survival of kidney transplants.

The use of calcineurin inhibitors (CNIs; ciclosporin and tacrolimus) has improved acute rejection rates, but also probably contributed significantly to chronic allograft nephropathy (the commonest cause of late graft loss). Efforts have been made to reduce exposure to these agents, but these are often hampered by an increased incidence of acute and chronic rejection. CAMPATH (Alemtuzumab) is a powerful induction agent which might reduce the requirement for calcineurin inhibitors.

DOES DELAYED CONVERSION TO SIROLIMUS ALLOW COMPLETE WITHDRAWAL OF CALCINEURIN INHIBITORS?

Sirolimus is a novel immunosuppressant that acts via a separate pathway to calcineurin inhibitors and is not nephrotoxic. There is evidence that using sirolimus to reduce calcineurin inhibitor exposure may improve graft function, but using sirolimus from the time of transplantation is poorly tolerated and associated with an increased acute rejection rate. Potentially, converting to sirolimus after six months could allow calcineurin inhibitors to be avoided in the long-term, but without the peri-operative side-effects of sirolimus.

A further advantage of the combination of CAMPATH and sirolimus is a possible tolerogenic effect. The combination may induce '*prope'* (almost) tolerance, whereas calcineurin inhibitors are known to interfere with this.

TWO RANDOMISATIONS PROVIDES AN EFFICIENT METHOD OF ANSWERING TWO IMPORTANT QUESTIONS

Patients will be randomly allocated to receive either CAMPATH or 'standard' induction with basiliximab (an interleukin 2 receptor antagonist) at the time of their transplant surgery. All patients will then receive tacrolimus and mycophenolate, and those assigned to basiliximab will also receive a reducing course of corticosteroids, which can be withdrawn at the local investigator's discretion. These two groups can then be compared to investigate the benefit of CAMPATH induction.

Six months after transplantation, patients will be randomly allocated, irrespective of their induction therapy allocation, to either remain on tacrolimus-based maintenance therapy for the duration of the study, or switch to a sirolimus-based regimen. Comparison of patients in these two groups will test the hypothesis that sirolimus-based maintenance therapy preserves graft function better than CNI-based therapy.

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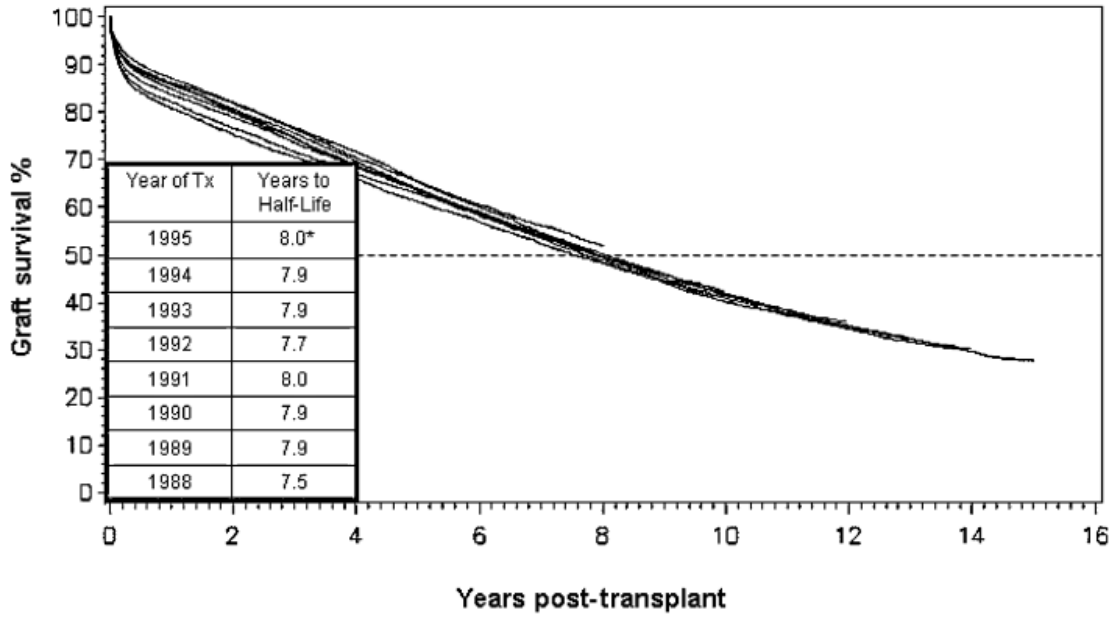
1 BACKGROUND AND RATIONALE

1.1 No improvement in long-term graft outcomes in the last decade

Kidney transplantation is well established as the best treatment modality for patients with end-stage renal failure (1). Despite significant advances in short-term graft survival over the past two decades, these have not been matched by improved long-term graft survival (Figure 1) (2). Long-term graft survival has many implications both for individual patients (who generally enjoy a better quality of life than when on dialysis) and for health care providers (after the initial cost surrounding the operation, the cost of maintaining a graft is less than that of dialysis). One-year graft survival rates are now over 90%, so there is considerable interest in strategies that can maximise the life-span of renal transplants.



* years to 51.8% survival (maximum follow up reached)

Figure 1. Kaplan-Meier overall graft survival, by year of transplant, for first cadaveric transplants 1988-1995.

1.2 Chronic allograft nephropathy is the major cause of late graft failure

There are many potential causes of late graft failure, the most common of which is chronic allograft nephropathy (CAN) (3). This is believed to be the end-result of graft damage of a wide variety of types, including preservation damage, rejection, calcineurin inhibitor (CNI) toxicity, hypertensive vascular disease and viral infection. Functional studies significantly underestimate the incidence of histological graft injury, and one study found that 94% of grafts had histological evidence of CAN at one year (4). This same study concluded that much of the chronic damage is due to calcineurin inhibitor toxicity

(Figure 2), even though the levels of these drugs had been maintained within the target range. For this reason many recent studies have focussed on reducing exposure to calcineurin inhibitors, and these have generally shown that this strategy produces better long-term outcomes (e.g. one year graft function) (5).

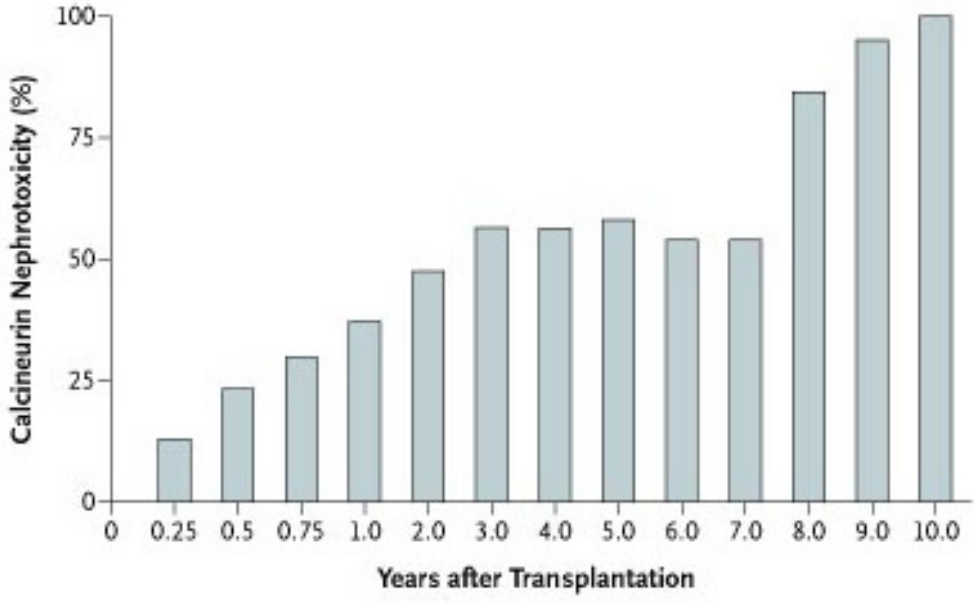


Figure 2. The prevalence of calcineurin inhibitor toxicity in a biopsy-based study of chronic allograft nephropathy in 120 kidney-pancreas transplant recipients. 52 biopsy specimens were analysed at 5 years, and 16 specimens at 10 years.

1.3 Sirolimus can be used to reduce calcineurin inhibitor exposure

Sirolimus is a macrocyclic lactone, and has a different mechanism of action to CNIs. It reduces cellular proliferation via the mammalian target of rapamycin (mTOR) pathway. It is not nephrotoxic, which is a major potential advantage over CNIs.

Sirolimus has been used in a number of different immunosuppressant strategies. It can replace either the CNI (6) or antimetabolite (7) (i.e. azathioprine or mycophenolate) in standard 'triple therapy', or it can be given with a reduced CNI dose (8).

A recent meta-analysis of the use of sirolimus analysed 27 randomised trials involving sirolimus in any of the above strategies (as well as high-dose versus low-dose sirolimus) (9). Eight trials including 750 participants evaluated sirolimus versus CNI. These trials varied in length from 3 months to 2 years, and used different outcome measures for graft function (serum creatinine or estimated glomerular filtration rate [eGFR]). The overall results suggested that allocation to sirolimus therapy was associated with improved graft function as compared to CNI (ciclosporin in six trials, tacrolimus in two trials). Those that used serum creatinine as the primary measure of graft function (four out of eight trials) reported a weighted mean difference of -18.31 µmol/L (95% confidence interval, -30.96 to -5.67) (i.e. 18 µmol lower in the sirolimus group). Those that used eGFR as the primary measure of graft

function (three out of eight trials) reported an improvement of 14.94 mL/min (95% CI, 9.33-20.55) in the sirolimus group. The mean length of follow-up in these trials was one year.

There was no difference in acute rejection rates, graft loss or mortality, although the trials were not powered to examine this. However, the analysis is complicated by a significantly greater incidence of changing patients from the protocol treatment in the sirolimus group (to standard CNI-based therapy) (relative risk versus control arm 1.82 [95% CI, 1.09 – 3.03, p=0.04]). Therefore the observed difference between allocation to sirolimus and CNI may be an underestimate of the true effect, although publication bias would operate in the opposite direction (i.e. the observed effect is partially attributable to the publication of trials with positive – and not negative – results).

Since the publication of this meta-analysis, two further studies have presented their results. Firstly, the Spare-the-Nephron study compared elective conversion to a sirolimus/mycophenolate based regimen with continued calcineurin inhibitor/mycophenolate based maintenance immunosuppression (10). 305 patients were randomised at a mean interval of 117 days after transplantation. Conversion to sirolimus was associated with a 7.4% improvement in calculated GFR (compared to a 1.3% improvement in the group assigned to remain on CNI/MMF; p=0.027). Secondly, the CONCEPT study randomised 192 kidney transplant recipients between remaining on ciclosporin-based regimen or switching to a sirolimus-based regimen at three months after transplantation. The patients allocated to sirolimus had better graft function at one year compared to the ciclosporin-allocated group (eGFR 68.9 versus 64.4 mL/min, p=0.017) with no significant excess of acute rejection (11).

Like all immunosuppressants, sirolimus has well-recognised side effects. These include hyperlipidaemia (both triglycerides and cholesterol, although the cholesterol fractions have not been reported) - this is easily managed but requires a higher rate of treatment with statins (9). Cytomegalovirus (CMV) infection was not increased in patients allocated to sirolimus therapy, but other infectious complications were not reported in the meta-analysis. Overall, about one quarter of patients allocated sirolimus discontinue it (12).

1.4 CAMPATH is a novel induction agent with potential benefit

The Campath-1 (Cambridge Pathology) series of rat monoclonal antibodies was first produced in the early 1980s in the Department of Pathology at the University of Cambridge, UK. The humanised variant of this antibody (Campath-1H - CAMPATH) was expressed in a rat myeloma cell line at Cambridge, and later in Chinese hamster ovary cells at the Wellcome Research Laboratories (13). Clinical investigations of CAMPATH have been conducted in patients receiving solid organ transplantation and in patients with haematological malignancies (14), autoimmune diseases such as rheumatoid arthritis (15), and multiple sclerosis (16).

The Campath-1 antigen (CD52) is an abundant molecule (approximately 5×10^5 antibody binding site per cell) that is present on at least 95% of all human peripheral blood lymphocytes and monocytes/macrophages (17). Although antibodies against CD52 are highly effective in depleting lymphocytes by complement and antibody-dependent cell-mediated cytotoxicity (ADCC), this does not appear to be the case for monocytes/macrophages. The antigen has been found on a subpopulation (<5%) of granulocytes but not on human erythrocytes, platelets or bone marrow stem cells. As a consequence, the therapeutic use of antibodies against CD52 does not compromise normal haematopoiesis.

Treatment with CAMPATH rapidly and almost totally depletes the peripheral lymphocyte population. Although B-cells start to re-populate after three months, the T-cell population remains severely depressed for up to 18-24 months (18).

1.4.1 Clinical experience in kidney transplantation with CAMPATH

CAMPATH was first studied in kidney transplantation as a potential treatment for acute rejection. In a small non-randomised pilot study of 12 patients, it was very effective but associated with severe infective episodes (19). The dosage was revised from seven daily 10mg doses to five daily 6mg doses, and no further severe infections occurred in the five patients who received the less potent regimen.

It has since been used as induction therapy in many centres. A group from Addenbrooke's Hospital, Cambridge, UK have recently reported a five year follow-up of patients treated with CAMPATH induction therapy (20). This retrospective comparison of 33 patients treated with CAMPATH and 66 contemporaneous controls showed no significant differences in graft function, graft loss or adverse events (i.e. infections and malignancy) between the two groups. However, the CAMPATH-treated patients received minimal steroids, half-level ciclosporin and no antimetabolite drugs.

CAMPATH is now widely used, and over 1500 transplants in the USA received CAMPATH induction in the two years 2003 and 2004 (21, 22). A multivariate retrospective analysis of the UNOS/OPTN (United Network for Organ Sharing/Organ Procurement Transplantation Network) database compared the effect of different induction therapies (IL-2 receptor antagonists, Thymoglobulin and CAMPATH) on the incidence of acute rejection in the first year post-transplant. In deceased donor transplants, the relative risk compared to CAMPATH induction of acute rejection was 1.51 ($p=0.001$) for no induction, 1.30 ($p=0.04$) for IL-2 receptor antagonist induction and 1.00 ($p=1.0$) for Thymoglobulin induction (21). These differences did not translate into a difference in graft survival or function in this analysis however.

There have only been four small randomised clinical trials involving CAMPATH. In a multicentre controlled study in Asia (23), 20 patients were randomly allocated to receive CAMPATH induction (two 20mg doses on days 0 and 1) followed by low-dose ciclosporin monotherapy, and 10 received no induction and full dose ciclosporin, azathioprine and steroids. After six months there was no difference in graft and patient survival, serum creatinine or acute rejection rates between the two groups.

The second randomised clinical trial was a three arm trial, with 30 patients in each arm, which was designed to compare three different induction agents (Thymoglobulin, CAMPATH and daclizumab) (24). All patients received maintenance immunosuppression with tacrolimus, mycophenolate and steroids, but the CAMPATH arm received half-dose tacrolimus, and no steroids after one week. An interim analysis (after a median follow-up of fifteen months) showed no difference in patient or graft survival, acute rejection rates or graft function.

In another recent study of 21 high risk (panel reactive antibody >20% or re-transplant) transplant recipients, CAMPATH induction followed by tacrolimus monotherapy was compared to Thymoglobulin induction followed by tacrolimus, MMF and steroids (25). The participants were followed for a median of 377 days, and no differences in graft function or survival were detected, although the study was not powered sufficiently to do so.

The most recently published study compared CAMPATH induction followed by tacrolimus monotherapy with standard triple therapy (tacrolimus, mycophenolate and steroids, notably without induction) in 131 patients (26). The primary outcome was biopsy proven acute rejection at six months,

and secondary outcomes included graft function at one year. No statistically significant differences were detected in either outcome, although the overall safety profile was similar.

All the aforementioned prospective studies were unable to detect plausibly moderate differences, either because they were non-randomised, too small, or both. Many commentators have acknowledged that a large study of CAMPATH is required (27).

1.4.2 Safety concerns with CAMPATH

Particularly in view of the early experience with CAMPATH when it was first used to treat rejection (19), there was concern about its potential risks of infection and malignancy when it was introduced as an induction agent. However, the published data so far have been reassuring on both these issues.

A group from Pittsburgh, USA, have examined 449 consecutive cases who received CAMPATH induction prior to solid organ transplantation (195 patients received a kidney transplant) (28). They assessed the type of bloodstream infections found in patients who had received CAMPATH, and compared it to bloodstream infections typically seen in other CD4 T-cell depleted states such as the acquired immunodeficiency syndrome (AIDS). They did not find a single case of an infection typical of CD4 T-cell depletion in those patients who had received CAMPATH.

A retrospective review of 49 patients treated with CAMPATH and 56 historical controls, (27 of whom had received basiliximab induction) reported no infection-related deaths among CAMPATH-treated patients, and, in fact, the CAMPATH treated group had a lower rate of infectious complications (16% versus 32%, p=0.061) (29).

Retrospective studies have also failed to find an increased risk of malignancy. Post-transplant lymphoproliferative disorder (PTLD) is a particular concern because it is virally-driven. However, a review of the OPTN database of 46,690 kidney transplant recipients actually showed that CAMPATH was associated with the lowest incidence of PTLD among the induction agents that had been used (30). It has been suggested that this may be because CAMPATH also depletes B-lymphocytes, from which PTLD develops.

There are no other data to suggest an increased risk of other malignancies following CAMPATH induction, although as it has only entered widespread use recently the length of follow-up is relatively short.

1.5 The combination of CAMPATH and sirolimus may induce *prope* tolerance

The combination of CAMPATH and sirolimus may enable exposure to CNIs to be reduced or eliminated. This could be favourable because CNIs are nephrotoxic and may interfere with tolerogenesis (31).

It is known that ischaemia-reperfusion injury occurring during organ implantation enhances the activation of the immune system (32). Depleting induction agents profoundly reduce the number of circulating lymphocytes capable of mounting an immune response during this period. It has been suggested that, by the time the peripheral lymphocytes return, the graft may have recovered from the injury and will therefore be immunologically quiescent (33).

An attempt to induce tolerance using CAMPATH alone was not successful (34), probably due to the persistence of monocyte/macrophages, NK cells and memory cells (which CAMPATH does not effectively deplete). However, studies examining the use of CAMPATH followed by monotherapy (either tacrolimus (35) or sirolimus (36)) have had encouraging results, consistent with (but not yet proving) the concept of donor-specific hyporesponsiveness suggested by Calne when he proposed the term *prope* (almost) tolerance (37).

Sirolimus also is potentially tolerogenic; it increases the number of CD4+ cells with regulatory activity (Treg cells) (38). Treg cells dampen the effector response to antigenic challenge and are a critical element of peripheral tolerance. Furthermore, in addition to its effects on tolerance-promoting Treg cells, sirolimus facilitates the deletion of effector alloreactive T cells (39).

Sirolimus also has potentially favourable effects on antigen-presenting cells. Semi-mature dendritic cells continuously transport and present self-antigen in T cell areas of lymphoid tissue, and they induce Treg cell formation by mechanisms distinct from those mentioned above. By up-regulating chemokine receptors on their surface, sirolimus has a pro-migratory effect on these cells which translates into increased Treg function (40).

In combination, CAMPATH and sirolimus have been demonstrated to induce donor-specific hyporesponsiveness, as assessed by *in vitro* tests (41). This is obviously encouraging, and merits further investigation.

1.6 The benefit of a short initial period of calcineurin inhibitor

Sirolimus exerts its immunosuppressive effect by interfering with cell replication. This leads to unwanted effects in the immediate post-operative period. Wound healing is delayed by sirolimus, and lymphocoeles are more frequent than with CNI-based immunosuppression (9). These problems can be avoided by using a CNI in this period, and then switching to sirolimus, at a later stage before the deleterious effects of CNI-based therapies become established.

Another reason to follow CAMPATH induction with CNI-based maintenance therapy for at least a short period is the atypical pattern of acute rejection seen with CAMPATH induction. Rejection episodes occur later than is normal with conventional immunosuppression, and the predominantly monocyte/macrophage infiltrate differs to that normally observed. Although cellular rejection following CAMPATH induction is easily reversible with steroids, humoral rejection is more frequent if CAMPATH is followed immediately by sirolimus: this has led the group pioneering this approach to recommend a short period of CNI treatment (36).

Low-dose tacrolimus has recently been established to be the optimal therapy following interleukin 2 receptor antagonist induction (5) and has also been used with considerable success following CAMPATH induction. In a 30 patient pilot study in Oxford of the protocol proposed for the experimental arm of this trial, one year death-censored graft survival was 100% and mean eGFR was 60 mL/min. There were two deaths: one was due to a perioperative myocardial infarction at the time of transplant, and the other was due to refractory PTLD. The one year acute rejection rate was 7.1%, with all rejection episodes occurring in the first six months. The initial protocol planned to withdraw MMF at one year, but following three episodes of acute rejection after MMF withdrawal, the protocol was altered to continue MMF at 250mg bd after one year. Since that change, there have been no further such late acute rejection episodes.

1.7 Two randomisations provides an efficient method of answering two important questions

The aim of the 3C study is to test two hypotheses:

- is CAMPATH-based induction therapy superior to standard IL-2 receptor antagonist-based induction?
- does a switch to sirolimus-based maintenance immunosuppression at six months post-transplant preserve graft function more effectively than remaining on CNI-based therapy?

In order to test the first hypothesis, all study participants will initially be randomly allocated to receive either CAMPATH- or basiliximab-based induction therapy. Patients allocated to receive CAMPATH induction (arm 1) will be given two doses of 30mg 24 hours apart. However, patients over the age of 60 will only be given the first 30mg dose (to avoid over-immunosuppression). For the next six months, these patients will receive low-dose tacrolimus (target level 5-7 ng/mL) and MPA (360mg bd)[1].

Patients who receive standard basiliximab induction (arm 2) will receive standard dose tacrolimus (target levels 5-12 ng/mL), mycophenolate (MPA, 540 to 720mg bd)[1] and corticosteroids (which will be reduced gradually to 5mg od, and withdrawn at the local investigator's discretion). These two groups (arms 1 and 2) can then be compared to compare the effect of a strategy of CAMPATH-based induction with a strategy based on standard IL-2 receptor antagonist induction.

In order to test the second hypothesis, six months after transplantation patients will be also be randomly allocated to either remain on tacrolimus-based maintenance therapy or to change to a sirolimus-based regimen. Patients allocated to tacrolimus will have a target trough tacrolimus level of 5-7 ng/mL, and those patients allocated to sirolimus will have a target trough sirolimus level of 6-12 ng/mL for the first six months, then reducing to 5-10 ng/mL. These two groups can then be compared to investigate whether or not sirolimus does preserve graft function better than low-dose CNI-based maintenance therapy.

[1] If mycophenolate sodium cannot be prescribed for local reasons, a dispensation can be made to allow mycophenolate mofetil to be substituted (see Appendix 9: Mycophenolate dosing for equivalent dosing).

2 PLAN OF INVESTIGATION

2.1 STUDY AIMS

The 3C study will include 800 patients aged over 18 years who have been listed for kidney transplantation. The two primary aims are to assess the differences:

- in biopsy-proven acute rejection among those allocated CAMPATH, low-dose tacrolimus and MPA versus basiliximab induction, standard-dose tacrolimus, MPA and corticosteroids as initial therapy (primary assessment at six months);
- in graft function among all those allocated tacrolimus versus sirolimus as maintenance therapy (primary assessment at two years).

Secondary objectives include an assessment of study treatments (i.e. CAMPATH versus basiliximab, and tacrolimus versus sirolimus):

- on graft-related outcomes (graft survival, chronic allograft nephropathy, rates of biopsy-proven acute rejection);
- on safety outcomes (episodes of infection, in particular opportunistic infections; malignancy; patient survival);
- on other events of interest (new onset diabetes after transplantation [NODAT], hyperlipidaemia, anaemia, leucopaenia, thrombocytopaenia, hypertension).

Tertiary objectives include assessment of the effects of study treatment on the primary outcomes within subgroups defined by various baseline characteristics (including assessment of graft function and survival in the two induction therapy groups).

2.2 TREATMENT COMPARISONS

Study medications will not be blinded as this will not be practical. The effects of CAMPATH on the lymphocyte count are readily apparent, and are not shared with basiliximab. Also, the necessity of therapeutic drug level monitoring in the maintenance phase will make it infeasible to blind the tacrolimus and sirolimus.

2.2.1 CAMPATH- vs basiliximab-based induction

At the time of surgery, patients will be allocated to receive either:

- CAMPATH induction, followed by low-dose tacrolimus (target trough levels 5-7 ng/mL) and MPA (360mg bd)[2]; or
- Basiliximab induction, followed by standard-dose tacrolimus (target trough levels 5-12 ng/mL), MPA (540 – 720mg bd)[2] and reducing-dose corticosteroids (which will be withdrawn at the discretion of the local investigator[see Section 4.2.1.1]).

2.2.2 Tacrolimus- vs sirolimus-based maintenance therapy

Six months after transplantation, patients will be allocated to receive:

- tacrolimus-based maintenance therapy (target trough level 5-7 ng/mL); or

sirolimus-based maintenance therapy (target trough level 6-12 ng/mL for first six months, then reducing to 5-10 ng/mL).The study treatments are outlined below in figure 3. Further details of the treatment schedules are given in Appendix 2: Detailed treatment

[2] See footnote on page 10.

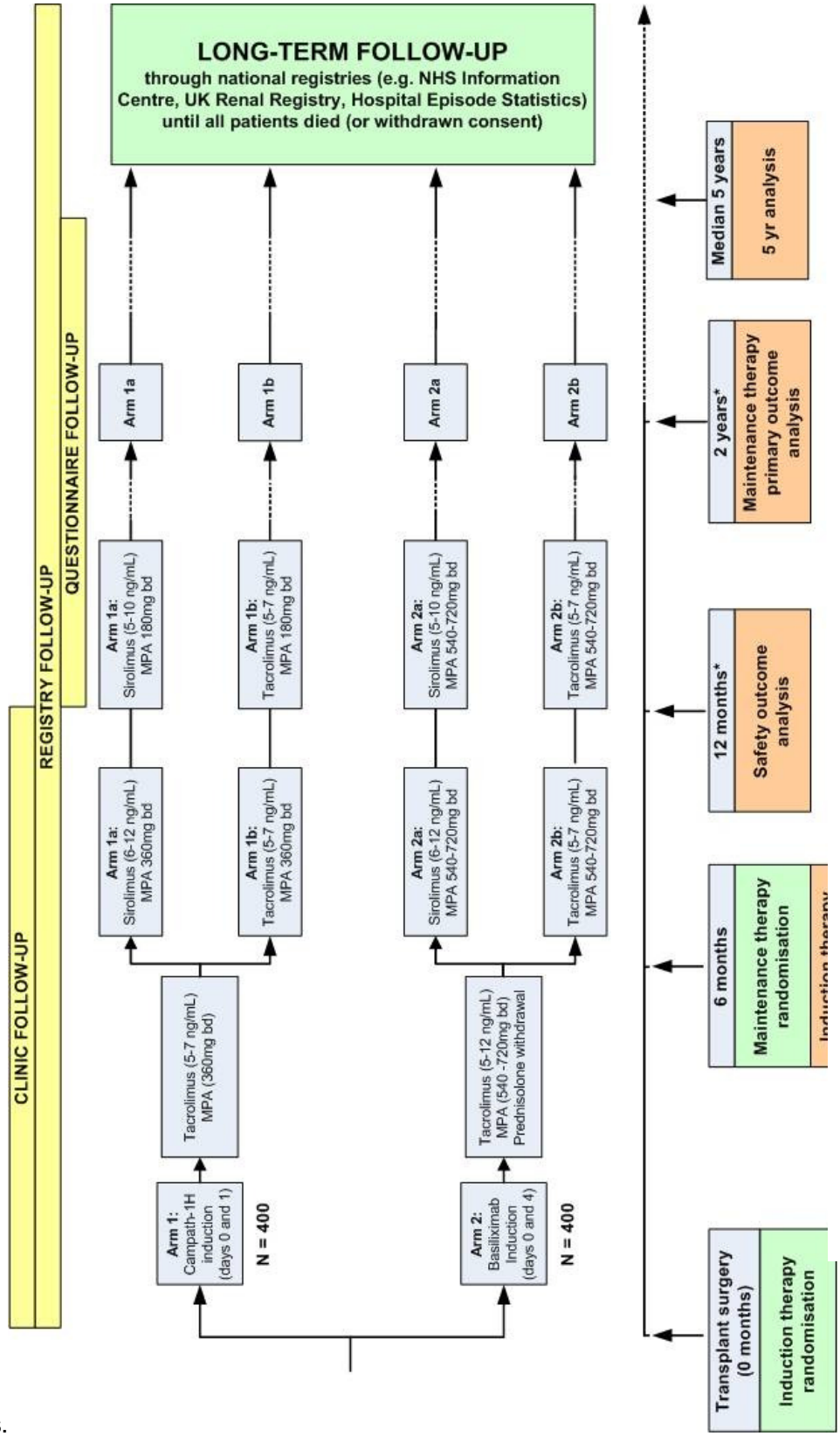


Figure 3: randomization scheme and timeline, including timing of primary assessment. For more detail of treatment schedules, see Appendix 2: Detailed treatment schedules. If mycophenolate sodium cannot be prescribed for local reasons, a dispensation can be made to allow mycophenolate mofetil to be substituted (see Appendix 9: Mycophenolate dosing for equivalent dosing).

schedules.

2.3 ASSESSMENT OF OUTCOMES

Two data analysis plans will be agreed by the Steering Committee. The first of these will describe detailed methods for the analysis of the comparison of the group allocated to CAMPATH induction with the group allocated to basiliximab induction. This will include the primary assessment of acute rejection at six months years. Comparisons at later time points may be possible, but they are potentially biased by the effect of the second randomisation.

The second will describe the detailed methods for the analysis of the comparison of the group of patients allocated to receive tacrolimus-based maintenance therapy with the group allocated to receive sirolimus-based maintenance therapy. This includes the primary assessment of graft function at two years, and secondary assessments of safety outcomes at one year.

2.3.1 Comparison of induction therapies

2.3.1.1 Primary assessment at six months

The primary comparison will involve an "intention-to-treat" analysis of the time to first biopsy proven acute rejection episode, in patients allocated to arm 1 (CAMPATH induction) and arm 2 (basiliximab induction).

2.3.1.2 Secondary assessments at six months

Secondary assessments will include "intention-to-treat" analyses of:

- all rejection episodes (biopsy-proven and presumed)
- steroid-resistant rejection
- delayed graft function
- safety measures e.g. incidence of serious infection
- graft and patient survival

2.3.1.3 Tertiary assessments at two and five years

The long-term effect of induction therapy will be assessed in terms of:

- graft outcomes: graft function and graft survival
- safety outcomes: incidence of serious infection and cancer
- other outcomes of interest including major vascular events (a composite outcome of non-fatal myocardial infarction, cardiac death, non-fatal or fatal stroke and arterial revascularisation)
- patient survival

Because such analyses are potentially biased by unequal numbers of patients undergoing the second randomization in the two induction therapy arms, these tertiary assessments will be interpreted cautiously and substantial allowance will be made for multiple hypothesis testing, the nature of the events and evidence from other studies.

2.3.2 Comparison of maintenance therapies

2.3.2.1 Primary assessment at two years

The primary comparison will involve an "intention-to-treat" analysis of the graft function (estimated by calculating GFR using the 4-variable MDRD [Modification of Diet in Renal Disease] equation) at two years after transplantation, in patients allocated to sirolimus-based maintenance therapy (arms 1a and 2a) and tacrolimus-based maintenance therapy (arms 1b and 2b).

2.3.2.2 Secondary assessments at two and five years

All secondary assessments will be conducted amongst all patients entering the maintenance therapy randomisation, and will be of the effects of maintenance treatment allocation (tacrolimus- versus sirolimus-based therapy) on safety outcomes and efficacy measures:

1. safety measures
   - incidence of serious infections (see section 4.1 for definition);
   - incidence of malignancy (including post-transplantation lymphoproliferative disorder);

2. efficacy measures
   - incidence of acute rejection (biopsy-proven, presumed and steroid resistant: see Appendix 1: Definitions for definition);
   - graft survival;
   - patient survival.
   - major vascular events (a composite outcome of non-fatal myocardial infarction, cardiac death, non-fatal or fatal stroke and arterial revascularisation)

2.3.2.3 Tertiary assessments at five years

In addition, some of the above comparisons will also be conducted in pre-specified sub-groups of patients, including:

- induction therapy allocation;
- men and women;
- cadaveric and living-related transplants;
- categories of HLA mismatch;
- white and non-white;
- first transplant and subsequent transplants;
- categories of sensitisation;
- categories of baseline graft function (eGFR <40, 40-60, >60 mL/min/1.73m^2);
- categories of baseline proteinuria (<30, 30-300, >300-1000, >1000 mg/day).

2.3.3 Safety analysis at one year

The relevant safety data from the induction therapy comparison at six months and the maintenance therapy comparison at one year will be included in this early safety analysis, once all patients have completed one year of follow-up.

2.3.4 Health Economic analysis

An economic analysis will be performed, with the objective of estimating average costs and effectiveness within each study arm, and the incremental cost-effectiveness of CAMPATH induction compared to basiliximab induction, and of tacrolimus-based maintenance therapy compared to

sirolimus-based maintenance therapy. The analyses will be performed primarily from a health service perspective, but additional information will be collected on employment status.

The time-horizon for the first comparison will be 6 months, in line with the primary trial outcomes, to include any differences in acute rejection rates but exclude any effects from the second randomisation. The time horizon for the second comparison will be at 2 years. In both cases additional lifetime analyses will be performed, based on registry data and an extrapolation model; this will be important to capture the long-term costs and effectiveness of the interventions and spill-over effects including the effect of reduced acute rejection rates and improved longer-term graft function on the availability of organs for others on waiting lists.

Costs will be based on measured resources used and appropriate national unit costs. Resources will include immunosuppressant and other drugs, nephrology out-patient visits, biopsies and tests, and all treatments related to adverse events. These will be obtained primarily from trial case report forms, including (from one year after transplantation) a simple 1-page questionnaire to patients requesting information on GP and other consultations, other health care use, days off work and current work status.

Outcomes in the economic evaluation will be based on quality adjusted survival. Quality of life will be measured using the EQ-5D administered at the discharge visit (for retrospective assessment of quality of life prior to transplant, and then at 3, 6, 9 and 12 months and annually thereafter.

2.4 DURATION AND DEFINITION OF END OF STUDY

Patients will be recruited into the study at the time of their kidney transplant surgery. All willing and able patients will be seen then, and at the time of their discharge from hospital after this index admission. The patients will be seen again at 1, 3, 6, 9 and 12 months after surgery: these visits will usually be scheduled to coincide with their routine clinical appointments. Following this, patients will be followed up by annual postal or web-based questionnaires until a median follow-up of five years. Patients who do not respond to two mail or e-mail requests for information will be contacted by telephone by a member of the 3C study team in order to complete the questionnaire. Patients will also be “flagged” with central registries (e.g. NHS Information Centre, UK Renal Registry, Scottish Renal Registry, Hospital Episode Statistics) for long-term follow-up (i.e. beyond the period of questionnaire follow-up and potentially for the lifetime of the patient) which will permit a reliable assessment of the long-term safety and efficacy of the study treatments. The study will therefore continue until all patients have died (or withdrawn consent for registry-based follow-up).

2.5 SAMPLE SIZE CALCULATIONS

The 3C study sample size is determined by the maintenance therapy allocation. A meta-analysis of the effect of conversion to sirolimus-based maintenance therapy showed an improvement in eGFR of 6.4 mL/min (95% CI 1.9-11) in the group assigned to sirolimus (12). The trials included in this meta-analysis varied in duration, with most patients followed for one year. If these differences could be maintained, it would be reasonable to anticipate a 10 mL/min difference in GFR two years after conversion to sirolimus (i.e. median follow-up at least 2.5 years after transplantation). Adherence to sirolimus therapy is likely to be around 75% (i.e. approximately 25% of patients allocated sirolimus in randomised trials discontinue it (12)) and this must be taken into consideration.

800 patients would have excellent power (>90%) with alpha =0.05 and good power (>80%) with alpha =0.01 to detect such a difference when allowance is made for the likely proportion of patients who are ineligible for the second randomisation and adherence with sirolimus.

800 patients would also provide good power (90%) with alpha =0.05 to detect a halving in the acute rejection rate at six months (from 15% to 7.5%), which is the primary comparison in the induction therapy comparison.

2.6 DATA AND SAFETY MONITORING

2.6.1 Interim analyses: role of the Data Monitoring Committee and Steering Committee

During the period of the study, interim analysis of serious adverse events, particularly those believed to be due to study treatment, will be supplied regularly (every three months during the first year of the study and six-monthly thereafter) in strict confidence to the Chairman of the independent Data Monitoring Committee. In the light of these analyses and the results of other relevant trials, the Data Monitoring Committee will advise the Steering Committee if, in their view, the randomised comparisons in the 3C trial have provided **both** (i) "proof beyond reasonable doubt"[3] that for all, or some specific types, of patient, induction with CAMPATH and/or maintenance therapy with sirolimus is clearly indicated or contraindicated in terms of a net difference in graft or patient survival; **and** (ii) evidence that might be reasonably expected to influence materially the patient management of many clinicians who are already aware of the main results of any other trials. The Steering Committee can then decide whether to modify the study, or to seek additional data. Unless this happens, the Steering Committee, the local lead investigators, the study participants, and all study staff (except those who provide the confidential analyses to the Data Monitoring Committee) will remain blind to the study results.

2.7 CENTRAL COORDINATION OF LOCAL CLINICAL CENTRES (HOSPITAL CLINICS)

2.7.1 Overview of study organisation

The 3C Trial will be coordinated by the Coordinating Centre based at the Clinical Trial Service Unit of Oxford University. The Coordinating Centre will be responsible for the administrative support of the Local Clinical Centres (LCCs). At each LCC, a Lead Investigator (a transplant surgeon or nephrologist) and a LCC Research Nurse (or, in some circumstances, medically qualified research fellow) will be responsible for the identification, recruitment and follow-up of study patients for the duration of the study. It is hoped that most of the transplant centres in the UK (23 currently) will participate.

2.7.2 Training and monitoring

The 3C Trial will be conducted in accordance with the principles of the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH-GCP) and relevant local, national and international regulations. Prior to initiation of the study at any LCC, the LCC clinic staff will be trained in the methods of the study and the LCC may be visited by a representative of the Coordinating Centre to ensure that the site has adequate facilities and resources to carry out the study. In addition, LCC Lead Investigators and LCC clinic staff will be provided with materials detailing relevant study procedures for LCCs ("Manual for Local Clinical Centre Procedures").

[3] appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but in general terms a difference of at least three standard deviations in an interim analysis of graft or patient survival would be needed to justify halting, or modifying the study prematurely. This criterion has the practical advantage that the exact number of interim analyses is of little importance.

During the study, representatives of the Coordinating Centre will visit all LCCs as required by needs of the centre and monitoring. The purpose of these visits will be to help LCC clinic staff to resolve any local problems with the study, to ensure that the study is conducted according to the protocol and Good Clinical Practice, and to review study records, data quality and the completeness of follow-up. A report of each visit will be prepared by the monitor and reviewed by the Coordinating Centre staff.

2.7.3 Supply of study materials

Equipment for the study clinics and all paperwork (e.g. study manuals, stationery) will be provided by the Coordinating Centre. Study treatments will not be provided (see section 3.13).

2.7.4 Data management

Patient data will be entered by clinic staff onto electronic Case Report Forms, which will then be forwarded to the coordinating centre. Data from these forms will be entered into computer databases at the coordinating centre, and stored on secure servers. Patient identifiable details will be stored for the purposes of flagging patients with central registries, but such details will be stored separately to all other data collected and linked by a unique study identifier.

2.7.5 Laboratory measurement of samples

All blood and urine test results required for the 3C study will coincide with those taken for routine clinical management. The Case Report Forms will include spaces for relevant results to be transcribed.

2.7.6 Source documents and archiving

As far as possible the CRF will be completed by direct interview with the participant. It may also be necessary to refer to the patient's hospital records (including electronic clinical, laboratory and pharmacy records). Furthermore, the additional information obtained on key variables from electronic records, reported outcome measures and other relevant events, death certificates and other information from national registries will also be source documents for the study. Investigators will permit trial related monitoring, audit or regulatory inspection providing direct access to source data/documents.

2.7.7 Funding

This study has been initiated and designed by the 3C Trial Steering Committee, and the data will be collected, analysed and published independently of the source of funding. Grants have been received from NHS Blood & Transplant, Novartis Pharmaceuticals UK Ltd and John Wyeth and Brother Ltd. The University of Oxford will act as the Sponsor in this study. This grant will support the central and local administration of the study, meetings and travel, study materials, and the necessary LCC clinic staff time. The cost of drugs (including study drugs) will not be funded by the 3C budget.

2.7.8 Indemnity

The University of Oxford, as Sponsor, will indemnify participating Research Subjects against any harm arising from their participation in this study. Furthermore, any harm arising from the provision of clinical treatment - and which would have arisen irrespective of the participation in this study - , and arising from negligence will be indemnified under the terms of the participating hospital trust's membership of Clinical Negligence Scheme for Trusts [or local variations for any hospitals participating from Scotland, Wales or Northern Ireland] administered by the NHS Litigation Authority.

2.7.9 Publications, reports and substudies

Draft copies of any manuscripts will be provided to all local lead investigators for review prior to their submission for publication. Papers will be written in the name of the 3C Trial Collaboration, with each individual investigator named personally at the end of the report (or to comply with medical journal requirements, in web-based material posted with the report).

Proposals for sub-studies on patients randomised into the 3C Trial will be welcomed, but must be approved by the Steering Committee before these begin. In considering such proposals, the Steering Committee will need to be satisfied that the proposed sub-study is of a high quality and that it will not compromise the main study in any way (for example by reducing the recruitment rate or compliance with study treatment).

3 SUMMARY OF PRACTICAL PROCEDURES

POTENTIALLY ELIGIBLE

- active on transplant waiting list, or live donor transplant planned
- aged over 18
- male or female

IDENTIFICATION AND INVITATION

- potentially eligible patients identified from local transplant waiting list
- agreement to screen such patients sought from consultants
- patient sent information about the trial

FIRST RANDOMISATION (prior to surgery)

- Written informed consent sought from eligible and willing patients
- Basic medical history and details of transplant recorded
- induction therapy randomisation: CAMPATH- or basiliximab-based induction
- Inform patient's nephrologist, transplant surgeon and GP of randomisation

DISCHARGE VISIT (and FOLLOW-UP VISITS at 1, 3, 6, 9 & 12 months)

- Further details of medical history and transplant
- Serious adverse events
- Current medication
- Clinical measurements and laboratory results

SECOND RANDOMISATION (at ~6 months after transplantation)

- check inclusion and exclusion criteria for second randomisation
- maintenance therapy randomisation: sirolimus- or tacrolimus-based maintenance therapy
- inform patient's nephrologist, transplant surgeon and GP of randomisation

FOLLOW-UP after one year after transplantation

- Annual postal questionnaire for serious adverse events, medications, other health care usage and quality of life (until median 5 year follow-up)
- Registry sources for laboratory results, survival and cancers (for duration of the study).

MONITORING OF SAFETY AND EFFICACY

- Central review of blood results and SAEs by Coordinating Centre
- Further details on relevant outcomes from hospital records sought by LCC clinic staff
- Relevant events confirmed and reviewed by central Outcomes Adjudication Panel

See also Appendix 6: Schedule of study procedures.

3.1 ELIGIBILITY FOR THE 3C STUDY

Patients are eligible for randomisation into the 3C Study if: (a) their nephrologist or transplant surgeon does not believe there is a definite indication for, or contraindication to, CAMPATH or sirolimus; and (b) all inclusion criteria are satisfied whilst no exclusion criterion applies.

3.1.1 Identification of potentially eligible patients

Potentially eligible patients will be identified by the LCCs from their transplant waiting list with their consultant's permission. Such patients will be sent a letter informing them of the trial or informed at routine outpatient clinic attendance (at centres where mailing everyone on the local waiting list is not feasible or desirable). Details of the 3C study will be provided with contact details for the local clinic staff should the patient have any questions. This will raise knowledge and awareness of the trial so that, when the patient is approached in the immediate pre-operative period, informed consent can more appropriately be requested.

3.2 FIRST RANDOMISATION VISIT

3.2.1 Assessment of relevant medical history and eligibility

This will be done after the patient has been called for transplant. If this is during the time when the clinic staff are working, they will discuss the trial with the patient. If it is outside of these hours, then the local lead investigator (or his/her designated deputy) will undertake this discussion.

There is no contraindication to entry into the 3C study on the basis of concomitant medication. All medications will be recorded at the discharge visit (see section 3.3.3 below).

3.2.1.1 Inclusion criteria for first randomisation

- men or women aged over 18 years;
- recipient of a first or subsequent kidney from a cadaveric or live donor;
- negative pregnancy test at randomisation, and agreement to use an acceptable form of contraception for duration of the study from women with child-bearing potential. Anuric (therefore unable to provide a urine specimen) female patients with child-bearing potential will be eligible, providing they agree to use an acceptable form of contraception.

3.2.1.2 Exclusion criteria for first randomisation

- Recipients of multi-organ transplants (eg kidney + liver, kidney + pancreas)
- previous treatment with CAMPATH;
- active infection, including HIV (antigen or antibody), hepatitis B (surface antigen), or hepatitis C (PCR) positivity;
- past history of anaphylaxis following exposure to humanised monoclonal antibodies;
- history of malignancy (with the exception of adequately treated non-melanoma skin cancer) diagnosed or recurred in the last five years;
- loss of previous kidney transplant within six months <u>not</u> due to technical reasons;
- medical history that might limit the individual's ability to take trial treatments for the duration of the study.

3.2.2 Invitation to participate in the randomised study and patient consent

Those patients who appear to be eligible will have the study explained to them by the study staff, using the Patient Information Sheet as a basis for discussion. Patients will be given a copy of the Patient Information Sheet and will have an opportunity to ask any questions and have a short time to think about their participation in the study. Patients will be discouraged from participating if it is thought unlikely that they would be willing and able to comply with follow-up for at least five years.

Eligible patients who agree to participate will be asked to provide written informed consent to enter the study, which will include consent for both the first and second randomisations and for long-term registry-based follow-up. One copy of the signed consent form will be filed in the patient's medical notes, one copy will be given to the patient and one copy will be stored in the site file.

3.2.3 Completion of case report form

The LCC staff will complete an electronic case report form which will include patient identifiable details (so that the patients can be "flagged" with national registries), the inclusion and exclusion criteria and basic details about their previous treatment (if any) for end-stage renal disease and the transplant itself.

3.2.4 Random allocation of study treatment

Eligible and consenting individuals will then be randomised to study treatments. The study IT system will minimise the randomisation with respect to important patient characteristics (42). The study staff will be informed which induction therapy the patient is to receive (CAMPATH- or basiliximab-based induction) and given the patient's unique study reference.

The randomisation procedure will use minimized randomisation in order to ensure that treatment groups are balanced with respect to prognostically significant variables. The minimization variables will be:

- Recipient age: ≤30, 31-59, ≥60 years old;
- Ethnicity: white, non-white;
- Type of transplant: cadaveric heart-beating donor, non-heart-beating donor, living donor;
- HLA mismatch: as per current UK Transplant protocol:
    - 0-0-0 (HLA A-B-DR) mismatch;
    - 0 DR mismatch and 0-1 B mismatch;
    - 0 DR mismatch and 2 B mismatch, or 1 DR mismatch and 0-1 B mismatch;
    - 1 DR mismatch and 2 B mismatch, or 2 DR mismatch and 0-2 B mismatch;
- patient sensitisation status: 'highly sensitized' defined, for example, by a positive cross-match with 85% of donors (reaction frequency >85%).
- centre

3.2.5 Reducing dose of CAMPATH in elderly

In view of concerns of over-immunosuppression in the elderly (and in particular the risk of post-transplant lymphoproliferative disorder in this group) the total dose of CAMPATH will be reduced in those ≥60 years old. These patients will <u>not</u> receive the second 30mg dose of CAMPATH.

3.2.6 Informing patient's GP

The local centre will write to inform the patient's GP of their entry into the study when they are discharged from hospital following their transplant if such information is not included in the standard discharge summary from the local hospital. The letter will include contact details for the local centre should the GP have any questions.

3.3 DISCHARGE VISIT

All patients will be seen again prior to their discharge from hospital after their index admission (i.e. the admission for transplant surgery). They will ideally be seen on the day of their discharge, or as close to it as is possible.

3.3.1 Further details of medical history and transplant

Further details of the patient's medical (and in particular, renal) history will be sought and noted on the CRF. In addition, details about the transplant which would not have been available at the time of randomisation will be noted.

3.3.2 Adverse events

Any serious adverse events that occur during the index admission will be recorded on a separate SAE case report form (see section 3.10.2). This will include a description of the event (including how it meets the criteria for being a serious adverse event), the date of onset and resolution. An assessment of relationship to study treatments will also be made, and if it is thought to be related (i.e. a Suspected Serious Adverse Reaction, SSAR) then it will be reported to the Coordinating Centre immediately.

Non-serious adverse events that are thought to be related to study treatment will also be recorded at this visit.

3.3.3 Discharge medication

A list of medication (including dosages and frequency) will be recorded.

3.3.4 Clinical measurements

The patient's blood pressure, height and weight will be recorded.

3.3.5 Laboratory results

The results of blood and urine tests from the day the form is completed (or the closest available results) will be recorded. This will include serum creatinine and albumin, full blood count, tacrolimus level and urine protein:creatinine ratio.

3.3.6 Health economic evaluation

Patients will be asked to complete an EQ-5D quality of life assessment retrospectively to evaluate their quality of life prior to the admission for transplantation.

3.4 FOLLOW-UP VISITS (at months 1, 3, 6, 9 and 12)

Following randomisation, patients are scheduled to be seen prior to discharge from hospital following the index admission, then at months 1, 3, 6, 9 and 12. These are timed to coincide as far as possible with the standard follow-up of these patients, so extra visits will be kept to a minimum. Telephone follow-up will be used should the patient be unable to attend a clinic follow-up.

3.4.1 Adverse events

All serious adverse events will be recorded on a separate SAE CRF. This will be similar to section 3.3.2 above. If the SAE is thought to be due to study treatment, it should be discussed immediately with the Clinical Coordinator or his/her deputy for expedited reporting (see section 3.10.3).

Non-serious adverse events should only be recorded if they are believed to be due to study treatment.

3.4.2 Current medication and clinical measurements

The patient's current medication will be recorded. Blood pressure will be recorded at all follow-up visits and weight recorded at 6 and 12 months..

3.4.3 Blood results

At each follow-up visit, results for creatinine and therapeutic drug level monitoring will be recorded, as well as a full blood count, lipid profile (total cholesterol, HDL-cholesterol and triglycerides if available) and urinary protein:creatinine ratio at certain visits. These will be copies of results of routine clinical tests requested by the managing team. See Appendix 6: Schedule of study procedures.

3.4.4 Health economic evaluation

Patients will be asked to complete an EQ-5D quality of life assessment and answer a brief questionnaire to capture details of health care usage, days off work and current work status (see section 2.3.4).

3.4.5 Steroid withdrawal in basiliximab-allocated patients

Patients allocated to basiliximab will have a reducing course of prednisolone prescribed (see Section 4.2.1.1). Prednisolone can be withdrawn completely at the discretion of the local investigator but should not be withdrawn between 5 and 7 months after surgery (i.e. around the time of the second randomisation).

3.5 SECOND RANDOMISATION (at ~6 months)

The second randomisation (between sirolimus- and tacrolimus-based maintenance therapy) can occur from 5 to 7 months post-transplantation (ideally to coincide with 6 month follow-up visit). The following exclusion critera will apply:

- proteinuria >800 mg/day (or protein:creatinine ratio >80 mg/mmol);
- biopsy-proven acute rejection (Banff score >1) in previous month.

(See section 3.5.1 for guidance on the management of patients who are not willing and eligible for second randomisation or whose local investigator does not consider it in their best interests.)

In order to minimise the impact of this exclusion criterion, investigators will be encouraged to monitor proteinuria and treat it appropriately (with ACE inhibitors and/or angiotensin receptor blockers) during the first six months post-transplant surgery.

The second randomisation will be stratified by induction therapy allocation and minimized by prognostically-relevant criteria including graft function, proteinuria and blood pressure.

Investigators should only randomise patients for whom they remain uncertain about the benefits of CNI-based or sirolimus-based maintenance treatment. Patients with reduced graft function (e.g. eGFR <40 mL/min/1.73m^2) will not be routinely excluded, but their events in this subgroup will be monitored closely by the independent Data Monitoring Committee.

Patients will be allocated to either remain on tacrolimus (all patients to have target trough level 5-7 ng/mL) or convert to sirolimus. The target trough level for sirolimus is 6-12 ng/mL for the first six months (patients who received basiliximab induction should be maintained at the upper end of this range) and thereafter 5-10 ng/mL.

3.5.1 Management of patients who do not enter maintenance therapy randomisation

Patients who do not have their maintenance treatment randomised should have their tacrolimus target trough level reduced to 5-7 ng/mL (unless their local investigator wishes to do otherwise).

3.6 FOLLOW-UP AFTER ONE YEAR AFTER TRANSPLANTATION

After their one year follow-up visit, all patients will be followed-up by ”flagging” with national registries (see below) and annual questionnaires (to capture information on serious adverse events, non-study medication, other health care usage, work status and a quality of life assessment). Flagging with central registries will allow long-term follow-up of all patients to reliably assess the long-term safety and efficacy of the study treatments.

3.6.1 Serious adverse events

Information about Serious Adverse Events will be collected from national registries (including but not limited to the Medical Records Information Service [MRIS], Hospital Episode Statistics, the UK Renal Registry, the Scottish Renal Registry). Further supporting documentation will be requested from the hospital where the event occurred if the SAE is:

- reported as being related to study treatment (and therefore a Suspected Serious Adverse Reaction [SSAR]; see section 3.10.1);
- a potential study endpoint (see Section 2.3).

3.6.2 Laboratory results

Relevant laboratory results will be obtained by a data feed from the Renal Registry and/or UK Transplant (or from LCC if necessary). Consent for this will have been obtained at the time of the initial surgery, and only relevant data will be requested from these sources.

3.6.3 Mailed questionnaires

All patients in the study will be sent an annual questionnaire (starting at around 2 years after transplantation) which will request details of any serious adverse events (in particular episodes of acute rejection) and current medication (including last recorded tacrolimus or sirolimus level). Questionnaires will be sent until a median follow-up of five years has occurred.

3.7 FOLLOW-UP AT REFERRING HOSPITALS

At certain centres it may be standard practice for patients to return to their referring hospital for routine follow-up after a certain period after transplantation. If this occurs prior to the second randomisation the investigator at the transplant centre remains responsible (after discussion with the local managing clinician) for the second randomisation and will inform the local physician of the treatment allocation. Follow-up forms will be completed by telephone by staff from the transplant centre with relevant laboratory results being sought from the local hospital.

3.8 DISCONTINUATION OF STUDY TREATMENT

Patients will be encouraged to adhere to study treatments during the study. However, the local investigator may decide to discontinue study treatment at any time in the interests of the patient’s health and well being. For example, study treatment may be temporarily or permanently discontinued for a particular patient if one of the following criteria are met:

- **serious** adverse event **thought likely to be due to study treatment;**
- conditions or procedures in which trial agents may be contraindicated (e.g. patients on sirolimus undergoing surgery where wound healing is a concern) (see section 4.3.1.1);
- pregnancy or any other situation where, in the opinion of the patient’s own doctors or the clinic staff, continuing study treatments would not be in the patient’s best interest.

If a patient wishes to discontinue from study treatment, he or she would still be followed-up for the duration of the study in the usual way. Complete follow-up of such data is essential as the analysis will be on an "intention-to-treat" basis.

3.9 WITHDRAWAL FROM STUDY

In accordance with ICH-GCP, a patient has the right to withdraw from the study at any time and for any reason, without prejudice to his or her future medical care by the physician or at the institution, and is not obliged to give his or her reasons for doing so. If patients are no longer willing to attend study clinics or complete questionnaires, then this data will be collected directly from the transplant centre and central registries, unless patients withdraw consent for this as well.

3.10 REPORTING ADVERSE EVENTS

3.10.1 Non-serious adverse events

Any non-serious adverse events that are believed to be due to study treatment will be recorded on the case report form. Other non-serious adverse events will not be recorded as they are common in this population and if not related to study treatment, analysis of such events is unlikely to yield useful information about the safety and efficacy of the study treatments.

3.10.2 Serious adverse events

Serious adverse events (SAEs) are defined as those adverse events that:

- result in death;
- are life-threatening;
- require in-patient hospitalisation or prolongation of existing hospitalisation;
- result in persistent or significant disability or incapacity;
- are a congenital anomaly or birth defect following maternal or paternal exposure;
- are important medical events in the opinion of the responsible investigator (i.e. any event that is not immediately life-threatening and does not result in death or hospitalisation but which may jeopardise the patient or may require intervention to prevent one or the other outcomes listed above).

In addition, the following events will be recorded as Serious Adverse Events in this protocol.:

- cancers (including post-transplantation lymphoproliferative disorder)
- transplant biopsies
- rejection episodes
- loss of graft
- opportunistic infections such as cytomegalovirus and BK virus
- deliberate overdose of study treatment

All reports of SAEs will be entered on the IT system and those that are potential study endpoints will be reviewed, verified and coded (see section 3.12). SAEs that are not considered due to study treatment will be processed in a manner that allows the Coordinating Centre to provide information on such events for regulatory processes in accordance with current timelines[4].

[4] Such as stipulated in the European Clinical Trial Directive (Article 18 of Directive 2001/20/EC).

Information about the occurrence of study outcomes and all other serious adverse events (SAEs) will be sought at scheduled visits (for the first year after transplantation) and from national and other registries (including, but not limited to the Medical Records Information Service [MRIS], Hospital Episode Statistics, the UK Renal Registry, the Scottish Renal Registry).

The following minimum information will be collected for all SAEs:

- the unique Study Identification Number (Study ID) for the participant;
- the unique SAE Form Identification Number (SAE Form ID);
- the time and date that the SAE Form is completed;
- the source of the report (e.g. patient, relative, friend, etc.);
- a description of the event (e.g. myocardial infarction, pneumonia);
- the reason for believing the adverse event to be serious (e.g. death, life-threatening, hospitalisation, disabling, congenital anomaly in offspring, other important medical condition);
- the date the event first occurred (or started);
- the place the event was diagnosed or was managed (e.g. hospital inpatient, hospital outpatient, GP surgery, etc.);
- the number of nights spent in hospital (where appropriate);
- the name of the place the event occurred (where appropriate);
- the name of the doctor responsible for the patient's care;
- the outcome at the time of reporting (e.g. death, ongoing, recovered, unknown);
- whether believed due to study treatment, and (if so), which study treatment(s) is suspected;
- details of person reporting the SAE.

3.10.3 Immediate reporting of any serious adverse event believed to be due to study treatment

All SAEs considered to be due to study treatment with a reasonable probability (i.e. Suspected Serious Adverse Reactions: SSARs) are to be reported immediately [5] to a Coordinating Centre clinician. The Coordinating Centre clinician will then record standard information including patient study number, the identity of the person reporting the event, a narrative description of the event and the reasons for possible attribution of the event to study treatment. All such reports will be assessed promptly by the Clinical Coordinator (or his deputy) who will seek any necessary additional information, and review the event's seriousness, relatedness (preferably in discussion with the reporting party) and expectedness (see section 3.10.4). The Clinical Coordinator will provide a report of all SSARs (regardless of treatment allocation), to the Chairman of the Data Monitoring Committee at regular intervals (see section 2.6) and to the responsible Research Ethics Committee and regulatory authority in the trial Development Safety Update Report.

For those SSARs that are unexpected (Suspected Unexpected Serious Adverse Reaction, SUSAR), the Coordinating Centre will provide a report to all relevant Ethics Committees, Investigators, host NHS institution and appropriate regulatory authorities to comply with the expedited reporting requirements for SUSARs[6].

3.10.4 Expected study treatment related adverse events

These are those defined by the documents for each study drug listed below.

[5] ideally while the patient is still with clinic staff. Failing that, it should be discussed within 12 hours of being reported.

[6] Such as stipulated in the European Clinical Trial Directive (Article 18 of Directive 2001/20/EC).

3.10.4.1 CAMPATH

Summary of Product Characteristics dated 04 June 2009.

3.10.4.2 Basiliximab

Summary of Product Characteristics dated 16 December 2008.

3.10.4.3 Tacrolimus

Summary of Product Characteristics dated 26 May 2009.

3.10.4.4 Sirolimus

Summary of Product Characteristics dated 14 May 2009.

3.11 Out-of-hours assistance

If doctors at collaborating centres have any questions, they will be able to contact an on-call doctor from the coordinating centre by calling 0800 585323 (Freefone). Such help is available 24 hours a day, seven days a week.

If patients in the study (or those considering entering the study) have any questions relating to the study, they can also call 0800 585323 (Freefone) and speak to a coordinating centre doctor.

3.12 CONFIRMATION AND VERIFICATION OF STUDY OUTCOMES

3.12.1 Confirmation of all deaths and relevant non-fatal serious adverse events

The LCC clinic staff will seek additional information from the hospital records and other appropriate sources about SAEs that are of particular interest. Such SAEs include those reported as acute rejection, graft loss, opportunistic infections (such as CMV and BKV), malignancy (including PTLD) or other hospital admissions.

The Coordinating Centre will also seek, from the Medical Records Information Service and other relevant sources, the certified cause of death for all patients randomised into the study. For each death reported, the LCC clinic staff will seek additional information from the hospital records and other appropriate sources.

3.12.2 Central verification of study outcomes

A Central Outcomes Adjudication Panel will review the specified causes of all deaths and all serious adverse events.

3.13 STUDY TREATMENTS

There are four Investigational Medicinal Products (IMPs) in the 3C study: CAMPATH and its comparator basiliximab, and sirolimus and its comparator tacrolimus. Further details of the doses used of these study treatments are given in Appendix 2: Detailed treatment schedules.

3.13.1 Supply of study treatments

All study treatments are marketed products and will be purchased by the host institution for use in the trial.

3.13.2 Labelling of study treatments

Basiliximab, sirolimus and tacrolimus all have a license for renal transplantation and are used regularly as specified by this protocol. They will be prescribed by the patient's managing doctor and labelled in accordance with the requirements for a dispensed medicine. These IMPs are exempt from the labelling requirements and will be labelled according to the Clinical Trials Regulation 46(2) of SI 2004 No. 1031.

CAMPATH does not have a license for kidney transplantation but will be prescribed by the patient's managing doctor. It will be labelled by the sites according the Medicine for Human Use (Clinical Trials) Regulation 46(1). An example label is shown in Appendix 7: Specimen label for CAMPATH.

3.13.3 Storage of study treatments

A stock of CAMPATH will be stored on the transplant ward in a refrigerator and a daily temperature log kept. This stock will be labelled (with labels provided by the coordinating centre [see Appendix 7: Specimen label for CAMPATH]) and maintained by the host pharmacy to ensure that availability of CAMPATH does not limit recruitment into the study. Other study treatments will be stored according to local practice.

3.13.4 Modification of dose of study treatments

The doses of study drugs to be used in the 3C study are outlined in Appendix 2: Detailed treatment schedules. However, if a patient cannot tolerate the specified dose, then it can be altered at the discretion of the local investigator. Any adverse events that lead to dose modification (or discontinuation of the study treatment) will be recorded, as will the current dose of study treatment.

3.13.5 Accountability of study IMPs

CAMPATH requires a full accountability record to be maintained at the site. The other IMPs (basiliximab, tacrolimus and sirolimus) are exempt from the accountability requirements because they are being used under license on the prescription of a doctor. Furthermore, it would not be feasible to maintain such records for the duration of the study given the wide variety of routes by which participants receive such treatments around the UK.

4 APPENDICES

4.1 Appendix 1: Definitions

Acute rejection:

Biopsy-proven:	Rise in serum creatinine ≥20% above baseline accompanied by consistent changes on allograft biopsy (43)
Presumed:	Rise in serum creatinine ≥20% above baseline, treated as acute rejection either without biopsy or with inconsistent biopsy findings.
Steroid-resistant:	Failure of graft function to return to baseline following treatment of acute rejection with pulse of steroids.

Anaemia (44):	Hb <13 g/dL in men, <12 g/dL in women;
Severe:	Hb <11 g/dL in men, <10 g/dL in women.
Leucopaenia:	Total white count <3.0 $x10^9$ cells/$mm^{3.}$
Neutropaenia:	Absolute neutrophil count <2.0 $x10^9$ cells/$mm^{3;}$
Severe:	Absolute neutrophil count <1.0 $x10^9$ cells/$mm^{3.}$
Thrombocytopaenia:	Platelet count <75 $x10^9$ cells/$mm^{3.}$
CMV syndrome:	Suggestive symptoms AND positive laboratory assay (e.g. CMV antigen, CMV PCR).
Invasive CMV disease:	Histological evidence (or suggestive radiological findings) of CMV tissue infection, in presence of suggestive symptoms or signs and positive laboratory assay.
BK virus infection:	Positive blood PCR for BK virus.
BK nephritis:	Histological evidence of BK virus infection of graft (e.g. positive staining for SV40 antigen) and positive blood PCR for BK virus.

New-onset diabetes after transplantation (NODAT) (45):
Fasting blood sugar >7 mmol/L; or
Random blood sugar >11 mmol/L; or
New use of oral hypoglycaemic agent or insulin

4.2 Appendix 2: Detailed treatment schedules

4.2.1 Induction treatments:

	Day 0	Day 1	Day 2	Day 3	Day 4-	Month 6	Month 12
ALLOCATION TO CAMPATH INDUCTION							
	TRANSPLANT SURGERY						
Tacrolimus				2mg bd PO	Target trough level 5-7 ng/mL	**See 4.2.2**	
Steroids	500mg IV methylprednisolone pre-reperfusion						
CAMPATH[7]	30mg IV[8] prior to re-perfusion (within 15-30 minutes of methylprednisolone)	30mg IV 24h after first dose (unless patient >60 years old)					
Other	10mg chlorphenamine IV 15-30 mins prior to CAMPATH	10mg chlorphenamine IV 15-30 mins prior to CAMPATH 1g paracetamol 30-60 mins prior to CAMPATH					
MPA[9]	360mg pre-op PO	360mg bd PO				360mg bd PO	180mg bd PO
ALLOCATION TO BASILIXIMAB INDUCTION							
	TRANSPLANT SURGERY						
Tacrolimus	0.05 - 0.1mg/kg bd PO	Target trough level 5-12 ng/mL				**See 4.2.2**	
Steroids	500mg IV methylprednisolone pre-reperfusion	15-20 mg pre-op PO, then reduced (see section 4.2.1.1)					
Basiliximab	20mg 1h pre-op IV				20mg IV		
MPA[9]	540 - 720mg pre-op	540 - 720mg bd				540 - 720mg bd	540 - 720mg bd PO

[7] See Appendix 8 for guidelines for administration of CAMPATH

[8] If a centre wishes to administer CAMPATH subcutaneously they may do so in all their patients.

[9] If mycophenolate sodium cannot be prescribed for local reasons, a dispensation can be made to allow mycophenolate mofetil to be substituted (see Appendix 9: Mycophenolate dosing for equivalent dosing).

4.2.1.1 Basiliximab patients: Prednisolone withdrawal

Corticosteroid withdrawal in the basiliximab arm should begin no later than two weeks after transplantation. The dose should be reduced according to local practice. Reduction beyond 5mg daily would require specific approval from the local investigator, but should not occur between 5-7 months after transplantation (ie, around time of second randomisation). Examples of where complete prednisolone withdrawal may be not be appropriate, include a pre-existing condition that is controlled by steroids (e.g. SLE), recent rejection episode, failure to tolerate other immunosuppression at full-intended doses, a non-favourably matched highly sensitised recipient / re-transplant.

4.2.2 Maintenance treatments

	Month 6: Day +0	Day +1	Day +2	Day +3	Day +4
ALLOCATION TO SIROLIMUS MAINTENANCE					
Tacrolimus	Stop after evening dose[10]				
Sirolimus		3mg od (unless <60 kg when 2 mg od)	3mg od (unless <60 kg when 2 mg od)	3mg od (unless <60 kg when 2 mg od)	3mg od (unless <60 kg when 2 mg od)
					Trough level (target 6-12 ng/mL reducing to 5-10 ng/mL from 12 months post-transplantation)
MPA[11] (for patients in Campath arm)	360mg bd (reduced to 180mg bd from 12 months post-transplantation)				
MPA[11] (for patients in basiliximab arm)	540 - 720mg bd				
ALLOCATION TO TACROLIMUS MAINTENANCE					
Tacrolimus	Target trough level 5-7 ng/mL				
MPA[11] (for patients in Campath arm)	360mg bd (reduced to 180mg bd from 12 months post-transplantation)				
MPA[11] (for patients in basiliximab arm)	540 - 720mg bd				

[10] If once-daily tacrolimus is used, stop after morning dose

[11] If mycophenolate sodium cannot be prescribed for local reasons, a dispensation can be made to allow mycophenolate mofetil to be substituted (see Appendix 9: Mycophenolate dosing for equivalent dosing).

4.2.3 Living donor transplant recipients

If it is standard practice at a centre to commence immunosuppression prior to transplantation in recipients of living donor organs then this is acceptable and should be done according to local practice. In this instance it would not be necessary to stop tacrolimus for the first two post-operative days if allocated to receive CAMPATH-based induction therapy.

4.2.4 Concomitant medications

4.2.4.1 Infection prophylaxis

All patients are to receive prophylactic treatment according to current local practice for:

- Pneumocystis carinii pneumoniae
- Oral fungal infection
- CMV

4.2.4.2 Thrombosis prophylaxis

Thrombosis prophylaxis should be commenced in all patients at the time of transplantation according to local practice.

4.2.4.3 Dose modifications for neutropaenia

Absolute neutrophil count (x 10^9/mm^3)	**MPA[12] dose**			
	Year 1 Induction therapy		**After year 1** Induction therapy	
	Campath	Basiliximab	Campath	Basiliximab
> 2.0	360mg bd	540-720mg bd	180mg bd	540-720mg bd
1.0 – 2.0	180mg bd	180mg bd	180mg od	180mg bd
< 1.0	stop	stop	stop	stop

This protocol does not specify any contraindicated medications, but local investigators will remain responsible for the management of the participant including all concomitant medications. The treatment schedules above are intended for guidance for the local investigator, and the detailed implementation remains their responsibility and will not be monitored by the coordinating centre.

4.3 Appendix 3: Transplant management

4.3.1 Clinical management decisions

As far as possible, general management of the patient (including rejection episodes, medical and surgical complications, side effects of treatments) remains the responsibility of the doctors responsible for patient care, and this study does not impose unnecessary restrictions on such clinical management decisions.

[12] If mycophenolate sodium cannot be prescribed for local reasons, a dispensation can be made to allow mycophenolate mofetil to be substituted (see Appendix 9: Mycophenolate dosing for equivalent dosing).

In the case of rejection episodes, a biopsy would be strongly encouraged but not required by this protocol.

4.3.1.1 Surgery

Patients who are receiving sirolimus and require a surgical procedure may, if their managing consultant wishes, stop sirolimus up to one week prior to the procedure and use tacrolimus instead until two weeks after the procedure. At this point they should then switch back to sirolimus therapy (according to the procedure outline above in section 4.2.2).

4.4 Appendix 4: Study personnel

Sponsor: University of Oxford
University Offices, Wellington Square,OXFORD OX1 2JD

Co-principal Investigators: Professor Peter Friend (Chief Investigator)
Professor Colin Baigent
Dr Paul Harden
Dr Martin Landray
Dr Richard Haynes (clinical coordinator)

Steering committee: Professor Peter Friend (Chair)
Professor Colin Baigent
Prof James Neuberger
Dr Martin Landray
Dr Paul Harden
Dr Richard Haynes
Mr Sanjay Sinha (Oxford)
Mr Hany Riad (Manchester)
Mr Chris Watson (Cambridge)
Dr Neil Sheerin (Newcastle)
Mr Argiris Asderakis (Cardiff)
Dr Peter Rowe (Plymouth)
Mr Keith Rigg (Nottingham)
Miss Laura Buist (Glasgow)
Mr Murat Akyol (Edinburgh)
Mr Chidambaram Nathan (Sheffield)
Dr Chas Newstead (Leeds)
Mr Abdul Hammad (Liverpool)
Dr Paramit Chowdhury (Guy's)
Dr Gareth Jones (Royal Free)
Mr Paul Gibbs (Portsmouth)
Prof Sunil Bhandari (Hull)
Mr Carmelo Puliatti (Royal London)
Miss Nithya Krishnan (Coventry)
Prof Iain Macdougall (King's)
Dr Adnan Sharif (Birmingham)
Dr David Lewis (CTSU)
Dr Will Herrington (CTSU)
Ms Kathy Jayne (CTSU)
Dr Michael Lay (CTSU)
Mr Alex Baxter (CTSU)

Data monitoring committee:
Prof Peter Morris (chair)
Dr Keith Wheatley
Dr Daniel Abramowicz

Statisticians (non-voting): Dr Jonathan Emberson and Ms Lisa Blackwell

4.5 Appendix 5: Organisational structure and responsibilities

Principal Investigators

The Principal Investigators have responsibility for:

- the design and conduct of the 3C study
- preparation of the protocol and subsequent revisions
- preparation of Standard Operating Procedures
- design, testing and documentation of all computer systems
- managing the coordinating centre
- organising meetings of the 3C Steering Committee
- publication of study reports

The Chief Investigator has overall responsibility for these.

Steering Committee

The Steering Committee is responsible for:

- agreement of the final protocol
- reviewing progress of the study and, if necessary, agreeing to changes to the study protocol and/or Standard Operating Procedures to facilitate the success of the study
- reviewing new studies that may be of relevance to the 3C study

Data Monitoring Committee

The independent Data Monitoring Committee is responsible for:

- reviewing interim data from the 3C study according to the schedule set out in the Protocol
- advise the Steering Committee if, in their view, the randomised comparisons in the 3C study have provided **both** (i) "proof beyond reasonable doubt" that for all, or some specific types, of patient, use of CAMPATH and/or sirolimus is clearly indicated or clearly contraindicated; **and** (ii) evidence that might reasonably be expected to influence materially the patient management of many clinicians who are already aware of the main results of any other trials.

Coordinating Centre

The Coordinating Centre is responsible for the overall coordination of the 3C study. Its functions include:

- study planning, organisation of Steering Committee meetings
- contractual issues with Local Clinical Centres and budget administration
- design and maintenance of 3C study IT system
- provision of study materials
- assistance with local Research Governance applications
- auditing and monitoring progress of the study and Local Clinical Centres
- responding to technical, medical and administrative queries from the Local Clinical Centres
- liaison with the Data Monitoring Committee, regulatory and other outside agencies
- training Local Clinical Centre staff
- organisation of meetings of collaborators

Local Clinical Centres

The responsibilities of the Local Clinical Centre (LCC) lead investigator and LCC research staff will include:

- obtaining local Research Ethics Committee and Research Governance approval (aided by the Coordinating Centre)
- provision of access to appropriate hospital computer systems
- identification and recruitment of suitable patients
- liaising with consultant colleagues in LCC and referring hospitals
- conducting clinic procedures in accordance with the protocol and standard operating procedures
- dealing with routine enquiries from patients and their families
- obtaining appropriate information to confirm potential study endpoints

4.6 Appendix 6: Schedule of study procedures

		Visit							Questionnaire			
		Transplant surgery	Discharge	Month								
				1	3	6	9	12	24	36	48	60
Check eligibility		•										
Medical history		•	•									
Informed consent		•										
First randomisation		•										
Second randomisation						•						
Quality of life assessment			•	•	•	•	•	•	•	•	•	•
Laboratory results	Renal function*		•	•	•	•	•	•	•	•	•	•
	Lipid profile†		•			•		•				
	FBC§		•		•	•	•	•				
	Immunosuppressant level		•	•	•	•	•	•	•	•	•	•
	Urine protein:creatinine ratio		•	•	•	•	•	•				

* serum creatinine
† total cholesterol, HDL-cholesterol and triglycerides
§ including white cell differential

4.7 Appendix 7: Specimen label for CAMPATH

For Clinical Trials Use Only
CAMPATH, Calcineurin inhibitor reduction and Chronic allograft nephropathy
(The 3C Study)

EudraCT number 2008-008553-27
Sponsor: University of Oxford, UK (Freefone 0800 585323)

For use in trial: to be given intravenously as specified in trial protocol

Chief investigator: Professor Peter Friend

Patient name and identification number Date of supply

4.8 Appendix 8: Guidelines for administration of CAMPATH

4.8.1 Intravenous administration

Available As: 30mg/1mL vial (alemtuzumab, MabCampath)

Beware: No more that ONE vial per dose is required for this indication. If you believe that more than ONE vial is required, please contact the coordinating centre (0800 585323) to discuss the dose.

Reconstitution:

N/A

Administration:

Add required dose to 60mL sodium chloride 0.9% and administer intravenously over 2 hours. Invert syringe to mix gently. CAMPATH should be administered under the supervision of a doctor. In order to assure complete delivery of CAMPATH, the line will be flushed with a minimum of 10mL 0.9% sodium chloride following completion of the CAMPATH infusion. To do this, attach another syringe to the syringe pump and administer the solution at the same rate as the previous infusion

During storage vials must be protected from light. Once diluted, infusion does not need to be protected from light during 2 hour administration time. However if not being administered immediately after dilution the infusion should be protected from light until it is administered, otherwise the antibody may start to deteriorate.

If infusion related side-effects occur infusion time can be increased up to 8 hours from time of reconstitution of CAMPATH for infusion.

Compatible Infusions:

Sodium Chloride 0.9%.

Cautions/Side Effects:

Exercise caution in the handling and preparation of the solution. Gloves must be worn. There is no information relating to the use of CAMPATH in pregnancy. As a precaution the company recommend it should not be handled by women who are pregnant or planning pregnancy. However the use of gloves will ensure there is no drug contact to staff. Safety glasses should be worn to avoid exposure in case of breakage or spillage.

Side-effects common with the first dose and include fever, rigors, headache, nausea, vomiting, rash, urticaria, pruritis, dyspnoea, hypotension and diarrhoea. Infusion related fever commonly begins 5-6 hours after infusion starts. More severe reactions such as bronchospasm and severe hypotension have been seen in a few instances and late hypotension can occasionally be a problem.

Thrombocytopenia most common during weeks 2-4 and neutropenia common between weeks 4-8.

Treatment of side-effects:
Side effects can be controlled by slowing or temporarily stopping the infusion.
Administer PRN medication (paracetamol PO, chlorphenamine IV, hydrocortisone IV) if necessary.
Nebulised salbutamol may be required for bronchospasm.
If hypotension develops, hydration with normal saline is indicated, unless contraindicated based on underlying cardiac status.

Other information:
The CAMPATH will be administered as soon as possible post-reperfusion of the transplanted organ, once patient is haemodynamically stable. In some circumstances administration may be delayed until patient is in theatre recovery.

- IV methylprednisolone 500mg will be administered intraoperatively prior to organ reperfusion.
- 15-30 minutes before administer CAMPATH give 10mg IV chlorphenamine.
- Give CAMPATH 30mg IV over 2 hours once patient is haemodynamically stable. CAMPATH infusion should be completed within 3 hours post organ reperfusion. Where the patient is unstable and the administration is delayed, CAMPATH infusion must be complete within a maximum 6 hours after organ reperfusion.

4.8.2 Subcutaneous administration

First dose

- Methylprednisolone 500mg IV prior to reperfusion of first organ
- Chlorphenamine 10mg IV 15-30minutes before SC alemtuzumab
- Immediately after first organ reperfusion give 30mg/1mL SC CAMPATH

Second dose (24 hours later)

- No pre-medication with steroid or antihistamine is generally necessary (but can be given at local investigators discretion)
- CAMPATH 30mg/1ml SC post transplant, 24 hours after first dose.

Side effects with SC CAMPATH: local irritation (e.g. erythema, rash) at the injection site is common.

4.9 Appendix 9: Mycophenolate dosing

The following table gives the equivalent doses of mycophenolate sodium and mycophenolate mofetil (which can be used if MPA is not available).

Mycophenolate sodium	**Mycophenolate mofetil**
180 mg	250 mg
360 mg	500 mg
540 mg	750 mg
720 mg	1000 mg

4.10 Appendix 10: References

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