CAMPATH INDUCTION IN RENAL TRANSPLANTATION

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Abstract

Kidney transplantation significantly improves quality of life and survival in patients with advanced renal failure and it has become the treatment of choice. Long-term immunosuppressants following transplantation are associated with an increased risk of infection, malignancy and diabetes. Therefore, transplant physicians endeavour to reduce long-term maintenance of the immunosuppression burden and aim to reduce the risk of developing these complications. This has been made possible in the last decade with the introduction of biological induction agents, namely Campath 1H.

Campath (Campath-1H) is a humanised rat monoclonal antibody directed against the CD52 antigen, which is one of the most abundant antigens on human lymphocytes. Activation of CD52 antigen causes profound cell lysis and cytokines release. This was thought to be crucial in the mechanism of developing a partial tolerance in transplant recipients.

A great interest has developed in its use in transplantation since the first reported use of Campath-1M in solid organ transplantation in the late 1980s and humanised Campath 1H in 1998. Campath had been used as an induction agent together with varying maintenance regimes with different results.

In this thesis, I am going to present a brief history of kidney transplantation and a literature review on the use of Campath in transplantation. I will follow by presenting the results and discussions on clinical studies that I developed during my study: the pilot study of Campath induction with tacrolimus monotherapy; CamTac study - a randomised controlled study; the prospective long-term outcomes study; the retrospective cohort dose-finding study; further studies developed to examine the incidence and risk related to long-term immunosuppression – causes of graft loss, long-term allograft pathology, infection, malignancy, haematological profile; and a detailed study on lymphocyte proliferation and rejection following Campath induction.

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Declaration

I hereby declare that this thesis is my own work and effort. Any published and unpublished work of others has been acknowledged in text and a list of references is given

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1 Introduction

The incidence of end-stage renal failure [ESRF] in the United Kingdom has been slowly rising over 10 years. The UK renal registry estimated that the incidence of ESRF in the UK has risen from 566 per million populations [pmp] in 2002 to 794 pmp in 2009 (1). Treatment options in patients with ESRF are somewhat limited. Death is almost inevitable without treatment. There is a substantial improvement in survival with each treatment option; however, it comes with significant trade-offs.

Haemodialysis is the commonest renal replacement modality; (1) Patients are required to attend a specialised unit for dialysis three times a week, and duration of each session is approximately four hours. Even with this length of dialysis, this form of renal replacement therapy [RRT] only managed to offer a minimal renal support. This form of RRT has a detrimental impact on a patient's quality of life (2), as well as increased risk of infection, bleeding from anticoagulation required for dialysis, cardiovascular risk, and amyloid.

Peritoneal dialysis is an alternative to haemodialysis; this form of RRT requires patients to perform dialysis at home using pre-prepared fluid. This is a viable option for patients who seek RRT away from hospital settings; however, this form of dialysis requires patients' ability to commence dialysis as well as a considerate amount of storage space for the disposables. Furthermore, there is growing evidence that this form of dialysis is associated with incurable complications in the long term (3).

Either of the dialysis modalities provides bare minimum renal support to patients with ESRF, and has an adverse impact on patients' quality of life because of the nature of dialysis.

Kidney Transplantation from either a living or deceased donor would be a good alternative to dialysis in patients with ESRF. Transplantation improves mortality, quality of life and general well-being. However, this is not without risk and complication, which I will discuss further.

Patients with ESRF have a substantially poor mortality. The renal registry highlighted a 50% 5-year survival rate in patients aged between 55 and 64. (1) NHS statistics showed that patients with chronic kidney disease have an almost 100-fold increase in mortality. It has long been established that transplantation improves mortality in the long term (4). Data from the UNOS database shows 5-

year patient survival of 85% after transplantation compared to the dismal survival of 67% reported in the renal registry.

1.1 History of renal transplantation

Renal transplantation is widely accepted as the treatment of choice in patients with advanced renal failure; it had been shown that transplantation significantly improves patients' quality of life and long-term survival. The effort and experimentation in development in organ transplantation started in the early 1900s and the development of clinical transplantation remains one of the most fascinating stories in clinical medicine and immunology.

The concept of transplanting organs between human beings and animals was not alien in historic literature. In Homer's The Odyssey, in the 9th century BC, he described an animal Chimera weaved together by supernatural forces with a lion, goat and dragon. A Chinese literature in the 4th century BC described Tsin Yue-Jen, a surgeon who switches the hearts of two soldiers – one had a strong spirit but weak will and the other had a weak spirit but strong will. A Roman Catholic story from 3rd century AD described St Cosmos and Damian replacing a patient's gangrenous leg with one from a recently deceased Ethiopian man. Although these stories are fictional, they certainly spark the curiosity of clinicians and the public towards transplantation.

The first documented transplantation was by James Blundell (5, 6). A British obstetrician transfused 4 ounces of blood from a man to his wife; during the time, a postpartum haemorrhage was common and associated with a marked mortality. He then attempted a transfusion in 10 other women, of which 5 survived. During his experiment, Blundell observed an immediate transfusion reaction with a few transfusion recipients. It is now clear that this is due to a circulatory antibody against a different blood group and this problem remains a major barrier in solid organ transplantation.

Experimentation on organ transplantation continues. The first successful bone transplantation was reported in 1878, attempts on bone marrow transplantation were made in 1896 and the first successful skin graft was in 1881 (7).

In 1902, Hungarian surgeon Emerich Ullmann and French surgeon Alexis Carrel began separately experimenting with kidney transplantation in dogs. Ullmann transplanted a dog's kidney into another dog's neck, whereas Carrel transplanted the dog's own kidney onto its neck. Although Ullmann's experiment failed soon after transplantation due to rejection, their experiments established a successful way for blood vessel anastomosis to be achieved. Carrel continued his experiment further by transplanting between dogs and discovered that transplantation between animals inevitably fails; he termed this effect "biological incompatibility", the most important barrier in transplantation, and this becomes the foundation of modern immunology.

There were many unsuccessful attempts to transplant kidneys between humans and animals and between humans between the early 1900s and mid-1950s. The first successful kidney transplantation in humans was performed between identical twins in 1954 in Boston, led by Joseph Murray (8). The donor and recipient were 23-year-old twins, one of whom was suffering from advanced glomerulonephritis. The transplant was successful: the recipient returned to work and led a normal life. Murray reported a further 6 successful cases of living donor transplantation.

However, transplantations were limited to homozygote twins until the idea of immunosuppression emerged. Murray attempted transplantation in a patient after she had received a high dose of total body irradiation as immunosuppression in 1958; although the surgery was successful and subsequent histological examination did not show any rejection, the patient died of thrombocytopenia following bone marrow failure induced by radiation. This resulted in a reduction in radiation doses and led to the first successful transplantation between non-identical twins in 1959, between siblings in 1960 and between non-siblings in 1961. Total body radiation was shown to be an effective immunosuppressant; however, the radiation also produced profound bone marrow aplasia and subsequent high risk of infection (9). This practice was soon abandoned following the discovery of Azathioprine (10).

It became possible to transplant a kidney from a deceased donor following the introduction of azathioprine and steroids in 1962(11), and the combination of Azathioprine and steroids became the

mainstream immunosuppressive regimen for almost 20 years until the introduction of cyclosporine. Together with azathioprine and steroids, the triple therapy immunosuppressive protocol had revolutionised solid organ transplantation and became the golden standard until the early 21st century (12).

1.2 Maintenance immunosuppression in renal transplantation

Overcoming an alloimmune response to an allograft following transplantation was, and still is, a major challenge for solid organ transplantation. Carrel first reported the inability to transplant allografts between animals in the 1900s in his experiment and labelled this "biological incompatibility" (12). Total body radiation was tried 60 years following the first animal experiment and successful transplantation between non-homozygotic individuals became reality. However, this drastic treatment is associated with high infective risk and mortality. A newer and more targeted treatment has been developed over the last few decades with the improved understanding of the role of the immune system in allograft recognition and rejection.

The immune system identifies foreign material via the Major Histocompatibility Complex [MHC] antigens, and it is called the Human Leukocyte Antigen [HLA] system in humans. MHC is located in chromosome 31 and covers a region of about 3.6mbp. The HLA complex is divided into three regions: Classes I, II & III. Both Class I and II are involved in an alloimmune response, whereas Class III encodes components of the complement systems. Each human expresses six MHC Class I alleles and six to eight MHC Class II alleles, and these antigens are present on most cell surfaces and are used to encode donor and recipient materials.

Any mismatch between donor and recipient HLA increase risk of rejection. Recipient T lymphocytes sense donor alloantigens via antigen-presenting cells which then undergo clonal expansion and subsequent activation of B lymphocytes after transplantation. An activated T lymphocyte will then migrate to a transplanted organ, causing invasion of renal tubules and tubilitis. Inflammatory

cytokines produced by T cells, in turn, cause aggregation of T lymphocytes and further damage to allografts with apoptosis.

Immunosuppression can be achieved by deleting lymphocytes, diverting lymphocyte traffic or blocking a lymphocyte response pathway (13). One ought to remember that apart from the desired therapeutic response to reduce the recipient response to a donor antigen, an immunosuppressant also causes an undesirable effect of increased risk of infection and malignancy by reducing the recipient response to harmful antigens.

1.2.1 Prednisolone

Prednisolone is a synthetic corticosteroid with predominant glucocorticoid and low mineralocorticoid activity. It was first isolated in 1950 and became commercially available in the 1960s. Together with the development of azathioprine, prednisolone allowed effective immunosuppression and led to successful renal transplantation between non-siblings and organs from deceased donors.

The mechanism of prednisolone on the immune system is complex. Prednisolone irreversibly binds intracellular glucocorticoid receptors, which then dimerise and interact with cellular DNA which either induces or inhibits gene transcription.

The overall effects of prednisolone are anti-inflammatory and immunosuppressive. This is achieved by inhibition of cytokines and cell adhesive molecule synthesis.

Although prednisolone has proven to be efficacious in renal transplantation, long-term exposure is associated with severe and irreversible side effects, including: Cushing's syndrome, weight gain and obesity, osteoporosis, glaucoma, cataract, diabetes, depression, hypertension and avascular necrosis. These side effects greatly increase cardiovascular risk and mortality in the long term.

Because of these side effects, the use of steroids in renal transplantation had been declining and steroid-sparing protocols became feasible after the introduction of monoclonal antibody induction.

1.2.2 Mycophenolate mofetil

Mycophenolate mofetil [MMF] is a prodrug that releases mycophenolic acid, which inhibits inosine monophosphate dehydrogenase, a key enzyme in purine synthesis. By preventing purine synthesis, it stops T and B cell proliferation following cell activation. MMF was considered as an improved version of azathioprine because therapeutic actions are similar in both drugs. In fact, MMF has largely replaced azathioprine because of the improved side-effect profiles.

MMF is free from transplant toxicity. Its principal non-immune toxic effects are gastrointestinal and hematologic. There was a suggestion that CMV and viral infection are more prevalent in MMF-treated patients, although these were thought to be dose-related (14).

1.2.3 Calcineurin inhibitors

Tacrolimus and its predecessor, Cyclosporine, revolutionised solid organ transplantation and became the mainstream of immunosuppressant.

Cyclosporine is a prodrug which binds with cyclophilin, an intracellular protein of the immunophilin family, forming a complex that inhibits activation of Calcineurin and that intercepts signals from activated MHC receptors on T cells, and subsequent cell activation (15). The pharmacological effect is similar to tacrolimus: tacrolimus binds immunophilin, an FK 506-binding protein, and creates a complex which, in turn, inhibits Calcineurin.

Long-term exposure of cyclosporine is associated with nephrotoxicity (16), hypertension, hyperlipidaemia, gingival hyperplasia, hirsutism, tremor, new onset diabetes after transplantation and haemolytic uraemic syndrome.

Side effects of tacrolimus are similar to those of cyclosporine but are less frequent and less severe (17, 18). Long-term tacrolimus is associated with nephrotoxicity, although less severe (19, 20), and a higher risk of new onset diabetes after transplantation (21).

Calcineurin inhibitors had been an important element in long-term immunosuppression following a renal transplant for the last 3 decades, and the use of tacrolimus is slowly rising because of the efficacy and improved side-effect profiles. There had been attempts to withdraw Calcineurin

inhibitors, mainly cyclosporine, following transplantation. A recent meta-analysis suggested that this did not improve allograft survival, but rather increased risk of rejection after withdrawal (22).

Other non-CNI-based immunosuppressive therapy failed to demonstrate superior outcomes. The biggest study, to date, comparing tacrolimus, high- and low-dose cyclosporine, and rapamycin maintenance combined with daclizumab induction and prednisolone showed that patients receiving the tacrolimus-based regimen had the highest allograft function and the lowest rejection rate at 1 and 3 years (23, 24). This suggested that tacrolimus should be used as the key maintenance immunosuppressant in renal transplantation and that a low dose should be used to reduce the potential long-term nephrotoxicity associated without increased risk of rejection.

1.2.4 Rapamycin

Rapamycin engages FKBP12 to create complexes that inhibit the intracellular mTOR protein; this, in turn, blocks signal 3 by preventing cytokine receptors from activating the cell cycle. This results in preventing T and B cell proliferation following activation.

The principal non-immune toxic effects of rapamycin include hyperlipidaemia, thrombocytopenia, and impaired wound healing, aggravation of proteinuria, mouth ulcers, skin lesions, pneumonitis, precipitated nephrotoxicity when used with Calcineurin inhibitors, haemolytic–uremic syndrome, and hypertension.

The benefit of rapamycin is its independency from calcineurin and it was thought that it could be used as a replacement for tacrolimus and could avoid long-term associated side effects. It had been hypothesised that rapamycin has antineoplastic (25) and arterial protective effects (26), hence there being a big interest in rapamycin-based immunosuppressive protocols despite the side-effect profile and lack of evidence supporting its impact on transplant outcomes (23, 24).

1.2.5 Induction antibodies

Induction antibodies have been used in renal transplantation since the 1960s to prevent acute rejection, particularly during the early period after surgery. A steroid-sparing regimen became feasible with the combination of induction antibodies and more potent therapy. Different induction

antibodies had been demonstrated to reduce the risk of rejection in the first year and to improve allograft survival (27-30). Therefore, the use of induction antibodies had been rising in the last decade and had risen up to 81.5% of all renal transplants in the US in 2008 (31).

Currently, only a small number of induction antibodies are used in renal transplantation:

- Non-depleting CD25/IL2 monoclonal antibody [basiliximab, dadizumab]
- Lymphocyte-depleting polyclonal antibody [Antithymocyte globulin]
- Lymphocyte-depleting monoclonal antibody [Campath]

1.3 Campath in renal transplantation

1.3.1 History of Campath

Campath [alemtuzumab] is a humanised rat monoclonal antibody directed against the CD52 antigen, which presented on all blood mononuclear cells and the male reproductive tract.

CD52 antigen-targeted antibodies were first discovered in the 1980s by Hale et al. during the research on bone marrow transplantation, aiming to develop an antibody to remove donor lymphocytes prior to transplantation (32). CD52 antigen is one of the most abundant antigens on human lymphocytes. It was estimated that lymphocytes have about 450,000 CD52 molecules per cell, accounting for 5% of the cell surface area. Apart from the high density, CD52 is also present in most human lymphocyte subtypes, and in most stages of cell differentiation apart from plasma cells, CD52 is also present in monocytes, macrophages and eosinophil. Apart from the haematological compartment, CD52 antigen can also be found in the epithelial lining of the male reproductive tract – epididymis, vas deferens and seminal vesicles (33). Although the physiological significance of the CD52 antigen is still under investigation, it is observed that once the CD52 antigen is stimulated, it causes profound lymphopenia by cell lysis and marked cytokine releases. The effect of this lymphopenia was thought to be lasting for months. A previous report suggested that the B lymphocyte count returns to normal after 3-12 months, whereas a subset of T cells remained

suppressed for up to 3 years (34). It was thought that this transient lymphopenia induced by Campath makes this a very useful benefit in organ translation (35), which I will discuss further.

Other short-term effects reported with Campath include depression of granulocyte or platelet count and elevation of liver function. The mechanisms of these effects are unclear (33).

Although Campath agglutinates an inactive sperm in vitro, there are no reproductive complications reported in the literature. That might be due to the poor penetration of immunoglobulin through the reproductive tract and this never reached a significant concentration to cause substantial effects in vivo (33).

The first Campath antibody [Campath-1M] was created from rat hybridomas in an attempt to produce an antibody that lyses lymphocytes in humans by fixing human complements (36). However, it was later noted that the efficacy of Campath-1M was poor and that it showed a tendency to aggregate. It was later demonstrated that the IgG subclass was the best rat isotype antibody in recruiting cell-mediated cytotoxicity in humans; therefore, a second class of Campath [IgG2b Campath 1G] was developed (37). This IgG alternative to the original Campath-1M was shown to be efficacious in bone marrow transplantation (38). Albeit its efficacy, Campath-1G remains a rat IgG antibody that might induce an anti-globulin reaction in repeat dosing. A humanised equivalent is then developed – Campath-1H. Campath-1H is a human IgG1; this subclass was shown to be most efficacious in activating cell-mediated cytotoxicity in humans and in complementing a reaction.

1.3.2 PROPE tolerance

Campath induces a transient lymphopenia immediately after administration and this effect can last up to 3 years. Calne et al. proposed that this transient lymphopenia is important in inducing tolerance in a transplanted allograft (35). He proposed that with the absence of an aggressive T cell response to a transplanted allograft early after transplantation, it allows engagement of the immune system to adapt to a transplanted allograft and to generate a state of “operational” tolerance (39). It was proposed that the presentation of donor MHC peptide without the supporting reaction of recipient T cells renders failure on the host response to the donor antigen challenge, together with

migration of donor antigen-presenting cells to recipients' lymphatic systems. These reduce the reaction in recipient alloreactive lymphocytes in the long term; this theory was later named the "Window of Opportunity of Immune Engagement" (WOFIE) (40). This theory was supported in animal model studies done by Knectle et al. (41): his group demonstrated a prolonged allograft survival in primates that was treated with T cell ablation using immunotoxin and intrathymal injection of donor lymphocytes.

In Calne's original human experiment, their group administered a 10-day course of Campath 1M in transplant recipients with high-dose cyclosporine. However, this study showed a superior allograft survival with an excessive incidence of infection (42). Their follow-up experiment published 7 years later using Campath 1H and low-dose cyclosporine (35) demonstrated excellent clinical results with a marked reduction in maintenance of immunosuppressants by the conventional standard.

These early studies certainly demonstrated an interesting property of the potential tolerance-inducting property of Campath.

1.3.3 Clinical studies

The development of Campath was originally targeted to avoid a 'graft versus host' disease in bone marrow transplantation (37). And because of its powerful lymphopenic ability and potential ability in inducing transplant allograft tolerance, it has started being used in solid organ transplantation.

The first published study on using Campath in solid organ transplantation was based on using Campath 1 (36); subsequently, a larger study was performed by Friend et al. using a 10-day course of Campath 1M followed by high-dose cyclosporine maintenance (42). The results showed a superior allograft survival in patients treated with Campath 1M, however, associated with an unacceptably higher risk of infection. Campath 1G (43) had also been used in treating allograft rejection.

Compared to Campath 1M, Campath 1G and Campath 1H were substantially more efficacious and the first report on using Campath 1H in renal transplantation was published by Calne et al. In this pilot study, 13 patients were given two doses of 20mg Campath 1H followed by low-dose cyclosporine maintenance. This was a radical approach in maintenance of immunosuppression in the

days when conventional therapy consisted of high-dose cyclosporine, azathioprine and prednisolone maintenance. All 13 allografts were functioning during the relatively short follow-up between 6 and 11 months. And only 1 patient experienced transplant rejection and required restarting azathioprine and prednisolone. These results were encouraging and further supported his theory on WOFIE and prope tolerance (35, 44, 45).

A follow-up report consisted of the original 13 and an extra 18 patients with a longer mean follow-up of 21 months. This report demonstrated 29/31 allografts functioning at 12 months. 6 of 31 patients experienced rejection and all reversed with the reintroduction of azathioprine and steroids. The incidence was "comparable" to conventional therapy. These two studies were the founding studies on the use of Campath and prope tolerance. This idea of prope tolerance and ability to reduce the long-term immunosuppression burden was certainly attractive to clinicians.

1.3.4 Campath as tolerance-inducing agent?

Both studies showed recipients still being able to reject a donor kidney; despite concurrent immunosuppression with Cyclosporine followed by Campath, complete tolerance is not achieved. Kirk (46) argues that the failure of achieving tolerance was perhaps related to timing of administration of Campath. Campath was administered after transplantation in the Calne studies compared to non-human primate trials. Furthermore, he argued that the concurrent use of cyclosporine also inhibits the donor immune adaptive capacity. Li and Wells suggested that interaction between remaining T cells is crucial for tolerance induction in vitro and that these were prevented by cyclosporine in the early clinical studies (47, 48).

In order to evaluate the effect of Campath on inducing complete tolerance further, Kirk et al. attempted the first study in renal transplantation using Campath induction without the use of a maintenance immunosuppressant (46). Seven patients received 3 to 4 doses of Campath intravenously at 0.3mg/kg pre-operatively and no maintenance immunosuppressant. All patients underwent a surveillance biopsy at day 0, day 7 to 14 and at months 1, 6, and 12, as well as when it was clinically indicated. This study confirmed that Campath rapidly depletes peripheral and lymph

node lymphocytes. All patients had excellent allograft function initially; however, 6/7 patients experienced rejection within the first four weeks after transplantation despite complete lymphopenia, and 1 patient developed recurrent focal segmental glomerular sclerosis soon after transplantation. Interestingly, the biopsy and RNA transcript material suggested that rejections observed were mediated by monocytes and macrophage lineage cells. This pilot study confirmed that Campath alone was not sufficient to induce transplant tolerance and this leads to a further study to examine whether tolerance induction is achievable by controlling the initial response with monocytes following Campath. In the second study, Kirk et al. used the same protocol with an addition of Deoxyspergualin (49); Desoxyspergualin is a polyamine-like antibiotic known to prevent NFB nuclear translocation in monocytes and macrophages. These then subsequently inhibit activation of these cells, thereby reducing antigen presentation. 5 patients were given 4 doses of 0.3mg/kg Campath, 1 pre-operatively and 3 doses post-operatively. All patients underwent a similar surveillance biopsy protocol. All 5 patients had excellent allograft function for the first 2 weeks after transplantation; however, all patients experienced clinical rejection within 4 weeks post-transplantation despite profound lymphocyte depletion and thrombocytopenia and neutropenia caused by deoxyspergualin. This study showed that Campath alone is not sufficient to induce complete tolerance and thus would require an adjunct immunosuppressant.

The nature of monocyte-mediated rejection after Campath was supported by a different group and was observed in patients receiving a maintenance immunosuppressant (50).

1.3.5 Campath with tacrolimus, mycophenolate mofetil and steroids maintenance

The combination of calcineurin inhibitors, anti-proliferative agent and steroids had been the golden standard for transplant immunosuppressants for decades (51). The results of using Campath and conventional triple therapy are best described in this retrospective study by the Wisconsin group (52). In this study, the group investigated the efficacy of Campath induction compared to anti-CD25 induction, Thymoglobulin, OKT3, ATG and no induction antibodies. The authors of this paper put patients receiving OKT3, ATG or no induction antibodies in the same group.

126 patients received 2 doses of 30mg Campath at Day 0 and Day 1 compared to 799 patients receiving anti-CD25 monoclonal antibodies, 160 receiving thymoglobulin and 156 others. All patients received a calcineurin inhibitor – either cyclosporine or tacrolimus, mycophenolate mofetil or steroids – for life.

The incidence of acute rejection is significantly lower in the Campath group, particularly for those who experienced delayed graft function [defined as the need for dialysis within the first week of transplantation]. Short-term allograft survival was superior in this group compared to the other 3 groups.

Despite the prolonged lymphopenia, this study also showed that the times to first infection in the Campath group are not different from other induction agents under the conventional triple therapy protocol. It was unclear whether the nature and rate of infection were different from this paper.

These single-centre retrospective results were encouraging. However, as with all retrospective studies, results are subject to bias on changes in practice over time.

1.3.6 Campath with tacrolimus and mycophenolate mofetil maintenance

Long-term steroid exposure is associated with development of diabetes after transplantation, increased cardiovascular risk, weight gain and osteoporosis; therefore, transplant clinicians endeavour to avoid the long-term use of steroids. Ciancio et al. from the University of Miami first reported the use of Campath induction in a steroid-sparing protocol which consisted of tacrolimus and mycophenolate mofetil maintenance in 2004 (53). In this retrospective study, 44 patients received Campath at 0.3mg/kg at day 0 and day 4, followed by a low dose of tacrolimus with a target level between 5 and 7ng/mL. 29/44 patients received a transplant from a deceased donor and there is a predominance of non-Caucasian recipients [69%]. It reported 100% patient and allograft survival at 1 year. Compared to the reports with Sirolimus maintenance (54, 55), patients receiving tacrolimus and mycophenolate mofetil maintenance have a substantially lower incidence of rejection: 1/40 recipients experienced rejection in the first 6 months after transplantation and 3

further cases were reported between 6 and 12 months. Furthermore, only 1 of 4 cases of rejection was antibody-mediated under this protocol.

4/40 patients required hospitalisation during follow-up, 1 case with atypical pneumonia and one with infected lymphocele. No CMV or BK infection had been identified.

The results of this study were consistent with a later and larger study comparing Campath with basiliximab in a steroid-sparing regimen (56). In this single-centre study, Kaufman et al. evaluated the results of 123 patients receiving a single dose of 30mg Campath compared to a historic control group of 155 recipients receiving basiliximab induction. Both groups received a steroid-sparing protocol consisting of tacrolimus and mycophenolate mofetil maintenance. 1- and 3-year patient and allograft survival were similar between the two groups. The acute rejection rate in the Campath group was reported as 14.9% at 1 year and was similar to the basiliximab group. It appeared that the timing of rejection was somewhat different, those receiving basiliximab rejects early compared to the Campath group. The median day to rejection in the Campath group was 153 days compared to 10 days in the basiliximab group. There was a suggestion that the nature of rejection in the Campath group appeared to be less severe – 77% Banff 1a or 1b compared to 61% in the basiliximab group.

The overall nature and incidence of infection were very similar between the two groups. CMV infection occurs in 4% of patients receiving Campath compared to 5% in those receiving basiliximab. Varicella, herpes infection and BK nephropathy were equally uncommon between the groups. There was a single case of fungal pneumonitis in the Campath group. The incidence of malignancy was also similar between the 2 groups. Two cases of post-transplant lymphoproliferative disorder [PTLD] and 2 other malignancies were reported in each group.

This medium-term retrospective study demonstrated that a steroid-sparing regimen with Campath is feasible and is not associated with an increased risk of rejection.

The early randomised controlled studies investigating the effect of Campath induction with a steroid-sparing tacrolimus and mycophenolate mofetil maintenance regimen, compared to the conventional triple therapy protocol with steroid maintenance and other induction, were performed

by the Miami group (57-59). The first study comprising 90 patients receiving a deceased donor (57, 58) and the second study consisting of 38 patients receiving living donor transplantation (59) were randomised into 3 groups: Thymoglobulin induction, Campath induction and anti-CD25 induction [daclizumab]. Recipients in the Campath group received 2 doses of Campath at 0.3mg/kg at day 0 and day 4, followed by a steroid-sparing maintenance protocol consisting of tacrolimus and mycophenolate mofetil. Patients in the Thymoglobulin and daclizumab group also received long-term maintenance steroids along with tacrolimus and mycophenolate mofetil. Patient and allograft survival were similar between the three groups. Incidences and timings of rejection were similar between the three groups in the first 6 months and in the long term. The nature of rejection was also similar: there was no suggestion that antibody-mediated rejection was more prevalent. Results were similar between the living donor and deceased donor studies. Although the incidence of rejection was similar between the 3 groups, curiously, patients received Campath induction and no maintenance steroids appear to have a higher number of indicative transplant biopsies with histological features associated with chronic allograft nephropathy. These were not consistent with later studies (60).

Risks of infection were similar. 8/30 deceased donor recipients and 3/13 living donor recipients in the Campath group required hospitalisation for infective causes, and were not different from the other two groups. The nature and incidence of infection were not documented in the paper; with the spare use of steroids, the incidence of new onset diabetes after transplantation was lower in the Campath group. During the course of the study, mycophenolate mofetil was withheld in 30-50% of patients in the Campath group because of leucopenia.

Although these studies were grossly underpowered, these do confirm that a steroid-sparing protocol is feasible with Campath induction.

1.3.7 Campath with Calcineurin inhibitor monotherapy

Campath induction was first used in renal transplantation in combination with low-dose cyclosporine monotherapy (42, 61, 62). In the first study in 1989, Friend and Calne used a 10-day course of

Campath 1M in combination with high-dose Cyclosporine monotherapy, showing recipients having a significantly lower incidence of acute cellular rejection after having received Campath 1M. However, the treatment is associated with a higher incidence of infection which was likely due to the high dosage.

Their follow-up study using the humanised form of Campath, Campath [Campath 1H], showed a similar benefit in outcomes with a much lower dose of the drug (61). In this study, 31 deceased donor transplant recipients were given 2 doses of 20mg Campath at day 0 and day 1. This was followed by low-dose cyclosporine maintenance. Allograft and patient survival were reported with a little number of rejections in the short term. Only 4 cases of cellular rejection and 1 antibody-mediated rejection were observed in the original study.

A subsequent long-term follow-up study (63) confirms that the efficacy of Campath persisted. Watson et al. reported the 5-year follow-up report in the original cohort. 5-year patient and allograft survival were comparable to those receiving conventional triple therapy. The hazards of rejection were similar between the two groups, although there are few cases of late rejection in the group receiving Campath and low-dose Cyclosporine, which was not observed in the control. Risk of malignancy and infection were comparable to the control group.

This pivotal pilot study demonstrated that Campath induction was safe and allows using a medium dose of cyclosporine monotherapy without an increased risk of complications.

There were two randomised studies investigating the use of Campath with calcineurin inhibitor monotherapy.

Vathsala et al. published a small randomised controlled study comparing 20 patients receiving 2 doses of 20mg Campath with low-dose cyclosporine maintenance with 10 patients having no induction and conventional triple therapy maintenance consisting of full-dose cyclosporine, azathioprine and steroids in Asia (64). The result from this small trial supports findings from a previous cohort study: the simple regimen using Campath induction and low-dose cyclosporine is

feasible and comparable to conventional triple therapy, although this study is grossly underpowered statistically.

The second and bigger randomised controlled study explored the effect of the same regimen but using tacrolimus, a newer form of calcineurin inhibitor (65). 65 patients were randomised to receive 2 doses of 20mg Campath at day 0 and day 1, followed by a conventional dose of tacrolimus; 66 patients were randomised to receive no induction agents with maintenance therapy of tacrolimus, mycophenolate mofetil and steroids. Results were encouraging: biopsy-proven rejection was lower in the Campath group compared to the triple therapy control group [20% vs 32%], albeit it did not reach statistical significance. Graft survival and allograft function were similar between the 2 groups. This study showed that Campath induction with tacrolimus monotherapy is at least as efficient as the triple therapy and that steroid maintenance is not necessary.

1.3.8 Campath with rapamycin monotherapy maintenance

There was concern that long-term calcineurin inhibitors cause irreversible nephrotoxicity and graft failure (16, 19, 66, 67). Ojo et al. examined the UNOS database and demonstrated that 16.5% of patients with a non-renal organ transplant have a degree of renal failure (67); they suggested that this is partially contributed by the use of calcineurin inhibitors (66). In terms of a renal allograft, an early study published in 2004 based on a cyclosporine-based regimen suggested that at least 80% of renal transplant surveillance biopsies express features associated with calcineurin inhibitor toxicity at 10 years (16).

Although it was thought that tacrolimus has a less long-term toxicity event compared to Cyclosporine (20, 68, 69), there is a trend in the transplantation circle to avoid or spare the use of long-term cyclosporine as well as tacrolimus. Therefore, different centres have attempted to use Campath with other immunosuppressants.

The use of Campath with Sirolimus maintenance was first proposed by Knechtle et al. (55) in an attempt to avoid using a long-term calcineurin inhibitor and steroids. They were hoping to avoid long-term complications of graft fibrosis and calcineurin inhibitor toxicity (19), as well as other side

effects associated with long-term steroid exposure. In their pilot study, they reported results of 29 primary renal transplant recipients undergoing living donor transplantations in their centre. The first 24 patients received only 20mg of Campath at day 0 and day 1 after operation and rapamycin, and the remaining 5 patients received a modified Campath regimen of 20mg at day -1 and day 0, as well as a supplement dose of thymoglobulin and 2 weeks of steroids. Patient and allograft survival were comparable to their experience with the conventional therapy. In the first 24 patients, 6 patients experienced acute rejection, of which 4 cases were early antibody-mediated rejection at days 6, 11, 17 and 147. Furthermore, 1 of these 4 antibody-mediated rejections resulted in graft loss. The remaining two cases of rejection were cell-mediated and also occurred early after transplantation at days 8 and 120. In view of the high incidence of rejection, this group modified the Campath regimen . Despite the addition of thymoglobulin and steroids, 2 of 5 patients receiving this modified protocol experienced predominantly antibody-mediated rejection early after transplantation at days 24 and 30. All patients rejected were converted from rapamycin monotherapy to tacrolimus, mycophenolate mofetil and Prednisolone maintenance (70).

Infective and malignancy complications were unclear from these reports, but there was no difference reported from previous experience. Albeit not being statistically significant, there is a trend suggesting that patients being treated with this protocol experienced a known complication associated with rapamycin – the rise of cholesterol and triglycerides levels resulted in 60% of patients starting on lipid lowering therapy, problems with lymphoceles and wound healing, and mouth ulcers.

1.3.9 Campath with rapamycin and mycophenolate mofetil maintenance

The previous report by Knechtle et al. showed that rapamycin monotherapy maintenance is not sufficient to prevent rejection in a renal transplant following Campath induction (55). This raised a question on whether the poor outcome was a result of rapamycin or simple inadequate immunosuppression with a single agent; this was particularly important in an effort of CNI sparing in renal transplantation. Flechner et al. attempted to answer this question in their pilot study (54). In

this pilot study, 22 patients were given 2 doses of 30mg Campath at day 0 and day 1 after transplantation. All patients received a steroid-sparing regimen consisting of rapamycin and mycophenolate mofetil maintenance. Patient survival was comparable to conventional therapy; however, 1-year allograft survival was substantially poorer at 86.3%. 1 patient died with adult respiratory distress syndrome [ARDS] and one other patient had immunosuppressants withdrawn as a result of ARDS. The incidence of biopsy-proven rejection was also high at 36% [8/22], with 6 of the 8 rejection cases occurring within 90 days of transplantation.

During the course of the study, 2 [9%] patients had ARDS, of which 1 died and 1 had immunosuppressants withdrawn, 2 [9%] patients had significant CMV viraemia, 4 [18%] patients had an oral ulcer and 2 [9%] had a peri-vaginal ulcer.

This study reported a higher-than-expected incidence of acute rejection compared to other observational studies using a CNI-based regimen (52, 61, 62), thereby suggesting that CNI is crucial in long-term immunosuppression following Campath.

1.3.10 Calcineurin inhibitor sparing following Campath

Results from the clinical studies demonstrated that Campath itself does not induce transplant tolerance and that maintenance immunosuppression is required to avoid acute rejection despite lymphopenia (46, 49). We also observed that maintenance immunosuppression has a huge impact on a transplant outcome. The incidence of acute rejection was low in patients treated with a calcineurin inhibitor-based immunosuppression protocol, regardless of whether maintenance mycophenolate mofetil or steroids were used (52, 56, 57, 61, 63). On the contrary, the incidences of acute rejection in the rapamycin maintenance regimes were unacceptably high, even with the concurrent use of mycophenolate mofetil (54, 55). These suggest that a Calcineurin inhibitor is essential in an immunosuppressive regimen with Campath.

The Pittsburgh group has attempted weaning off tacrolimus following Campath induction in hope of this allowing immune engagement to promote graft tolerance (71, 72) and to avoid early post-transplantation over immunosuppression and late toxicity related to immunosuppression.

205 living donor transplant recipients were included in their study (73, 74); all patients received 30mg of Campath immediately after operation, followed by a conventional dose of tacrolimus monotherapy. Allograft survival was 98.1% at 1 year and was comparable to their historic control. The incidence of acute rejection was significantly lower compared to their historic control group at 1 year [10.7% vs 21.3%, $p<0.005$]. Their centre protocol aimed at starting space weaning on tacrolimus in stable patients at 100 days after transplantation – start by reducing tacrolimus from twice daily to once daily dosing, then every other day after 1 to 4 months. Further weaning was then continued thrice weekly, twice weekly, once weekly and was stopped. Weaning was attempted in 76% of patients from this cohort and only 50% of patients attempted to remain on space weaning. 26.6% of patients who had attempted weaning developed acute rejection, and 18.5% de novo donor-specific antibodies, which has a significant impact on allograft survival (75). Apart from the increased risk of rejection after weaning, the nature of rejection also appeared to be more severe. This tacrolimus weaning protocol is also associated with an excessive allograft loss to rejection.

A second trial on stopping calcineurin inhibitors was attempted by the Wisconsin group; their group previously presented a successful result using Campath induction with maintenance therapy with Calcineurin inhibitors, mycophenolate mofetil and steroids (52). In this randomised controlled study, 40 stable patients were randomised to either continue or stop Calcineurin inhibitors 2 to 16 months after transplantation, concurrent with mycophenolate mofetil and steroid maintenance. Only 19 of 20 patients underwent calcineurin inhibitor withdrawal, of which 20% required anti-rejection treatment after withdrawal and 3 were biopsy-proven. There were no new cases of rejection observed in the control group. 3/8 surveillance biopsies in the withdrawal group showed features with acute alloimmune activities, whereas no control group did.

These 2 studies both suggested that tacrolimus withdrawal in clinically stable patients is associated with an increased risk of rejection afterwards. Considering that tacrolimus is significantly less toxic compared to its predecessor (20, 68, 69), the risk of tacrolimus withdrawal following Campath certainly outweighs its benefits.

1.3.11 Lymphocyte repopulation following Campath

Results from the clinical studies demonstrated that Campath is a powerful lymphopenic agent and that repopulation of lymphocytes varies depending on the subsets (46, 49, 52, 54, 62, 76). Campath itself does not induce transplant tolerance, and maintenance immunosuppression is required to avoid acute rejection despite lymphopenia (46, 49). We also observed maintenance immunosuppression having a huge impact on transplant outcomes. The incidence of acute rejection was low in patients treated with a calcineurin inhibitor-based immunosuppression protocol, regardless of whether maintenance mycophenolate mofetil or steroids were used (52, 56, 57, 61, 63). On the contrary, the incidence of acute rejection in the rapamycin maintenance regimes was unacceptably high, even with the concurrent use of mycophenolate mofetil (54, 55).

The difference in efficacies between different immunosuppression maintenance may be due to the functional nature of a residual lymphocyte following depletion. Pearl et al. underwent a study to investigate the phenotype and functioning characteristics of residual lymphocytes following lymphodepletion of Campath (77). Blood samples were drawn and analysed using flow cytometry prior to depletion and weekly for 4 weeks in 5 patients during the original tolerance-induction study (49); samples from patients who received rabbit anti-thymocyte globulin or daclizumab were acted as controls. This study showed almost complete lymphodepletion with naive T cells and regulatory T cells after Campath, but with a single phenotype (CD3+ CD4+ CD45RA- CD62L CCR7-). This depletion-resistant subset was thought to be a memory-effect T cell. These cells expand quickly following depletion and were uniquely prevalent during rejection. Interestingly, these memory T cells were also resistant to steroids, rapamycin and deoxysepergualin in vitro, but were uniquely sensitive to calcineurin inhibitors. These data might explain the reasons why Campath is not pro-tolerant and why calcineurin inhibitors are crucial for transplant immunosuppression maintenance following Campath induction.

1.3.12 Other indications of Campath

Campath was originally prepared and first utilised in allogeneic transplantation to prevent graft versus host through in vitro purging of T-cell lymphocytes from bone marrow prior to reinfusion (37, 78). However, its ability to induce profound lymphodepletion has allowed it to be used in many auto-immune diseases, including rheumatoid arthritis (79-81), multiple sclerosis (82-85), ocular inflammatory disease (86), graft versus host disease (87, 88), and haematological malignancies, e.g. non-Hodgkin lymphoma (89, 90) and leukaemia (91-93), besides organ transplantation.

1.3.13 Complications with Campath

Infection

It was thought that the powerful lymphodepleting ability of Campath might result in high risk of infection (42). Previous reports have suggested poor patient survival being associated with its use in patients with multiplying relapsed vasculitis exposed to Campath 1-H during the 1990s (94), and with reports of fatal fungal infections in kidney/pancreas transplant recipients exposed to multiple doses (95-97).

There was a concern of a slightly higher incidence of CMV infection in the earlier report using Campath (65). Subsequent clinical studies did not show a significantly different incidence and timing of CMV disease or other infections following Campath induction (52, 54-57, 98) in the short term. A long-term follow-up study with Campath is sparse in the literature; the 5-year, long-term follow-up pioneer study in Calne's group did not show a substantial difference in the risk of infection in the Campath group (63), albeit it had a small number of patients. Moreover, the effect of Campath on the risk of infection appears to be dose-related in both transplantation and rheumatological literatures, which used up to 400mg of Campath per treatment (79, 96, 99). The incidence of infection was higher in patients given a high dose of Campath 1M in the pioneer study in the 1980s (42). This is supported by a recent study presented by Nath et al. (96). They evaluated the effect of Campath on fungal infection, showing those who received Campath as maintenance therapy in their pancreas transplant programme having a fold rise in the incidence of systemic fungal infection [3.6%

vs 9.2%], and systemic fungal infection in these patients was associated with high mortality – 3/8 [38%] patients died during on-going treatment for their fungal infection. The incidence of fungal infection appears to be low in cases when Campath doses are low and are used as induction only (97, 98).

A further report from Pittsburgh suggested that patients who received Campath as a supplement with concurrent treatment of rejection are associated with a 3.5-fold increase in the risk of opportunistic infection (99).

These data strongly suggest that Campath 1-H should not be used in repeated, multiple-dosing regimens or in individuals already carrying a heavy burden of immunosuppression. However, an extensive body of reports, both cohort studies and randomised prospective trials, is now available to show that when used at total cumulative doses of between 30 and 60 mg, delivered intravenously (IV) or subcutaneously as induction therapy at the time of renal transplantation, Campath 1-H is a safe and effective therapy.

Autoimmune disease

Apart from the infective risk, higher risk of autoimmune disease following repeat doses of Campath had been reported in the multiple sclerosis literatures (100). Patients with multiple sclerosis were treated with a substantially higher dose of Campath compared to transplant recipients, ranging from 12-30mg daily for 5 consecutive days followed by an annual treatment consisting of 3 days of 12mg daily treatment. 22.2% of patients in this cohort developed autoimmune disease following Campath, often at 12-18 months after treatment. Graves' disease was the commonest autoimmune disease [31/52 cases], and other haematological, renal and dermatological autoimmune diseases were also observed. However, the author did not elicit a dose-related effect in MS.

Autoimmune disease following Campath in solid organ transplantation is rarely reported. There was only one case reported in the literature. An open letter by Kirk et al. (101) reported 1 patient developing Graves' disease 4 years after transplantation, during which she received 4 doses of Campath at 1.2mg/kg which is substantially higher than protocols in other units.

1.4 Problems with long-term immunosuppression

Long-term maintenance immunosuppression is crucial to prevent acute rejection and to maintain long-term allograft survival. However, chronic immunosuppression not only reduces the immune response of recipients to allografts, but also reduces a recipient's immune reaction to infection as well as the ability of immune surveillance which prevents growth and development of malignancies. It was also suggested that long-term immunosuppression reduces DNA repair at a molecular level and further increases risk in developing malignancies (102).

A recent report that analysed data from the UNOS registry suggested a cumulative incidence of malignancy up to 7.5% at 3 years, of which skin cancer was the most prevalent (103). This report also showed that transplant recipients have an increased risk in most types of malignancy. The incidences of the most common solid malignancies, e.g. lung, prostate, breast and cervical cancer, were only modestly increased. However, there is a marked increase in risk with skin cancer and lymphoma. It was estimated that the rates of skin cancer and lymphoma in transplant recipients were 90 and 37 times higher, respectively, compared to the general population after being adjusted for age. Furthermore, these findings were consistent throughout other registry data from Australia, New Zealand, Denmark and Norway. It was an interesting finding that long-term immunosuppression has a selective effect on skin cancer and lymphoma, which were both found to be linked to a viral pathology with human papilloma virus (104) and Epstein-Barr virus respectively (105).

This report also investigated the risk factors of developing malignancy following transplantation. Non-Caucasoids have a significantly reduced risk in developing any form of malignancy. In particular, Orientals and South Asians have an 89% reduced risk of skin cancer compared to Caucasians. A similar result was found in the Afro-American populations, of whom skin cancer risk was reduced by 94%.

Immunosuppression also has a substantial impact on the risk of malignancies: recipients on a tacrolimus-based immunosuppressant regimen have a 35% reduced risk of non-melanoma skin

malignancy, whereas those who received azathioprine maintenance increase the risk by 17%. Although there was a theoretical suggestion that rapamycin-based immunosuppression reduces the incidence of malignancies (25), data from this large registry study failed to support this (103).

The risk of skin malignancy is also 17% lower in recipients receiving induction antibodies; this might be confounded by the reduction in Azathioprine use following induction therapy. There was no suggestion of increased risk of other solid organ malignancies, compared to a previous report which was based on a large proportion of patients receiving anti-Thymoglobulin as induction (106).

As far as this data is concerned, patient survival certainly improves after transplantation (4). Furthermore, the risk of developing common solid organ malignancies was not higher compared to those remaining on a waiting list (103).

Besides the risk of malignancy, it has long been established that immunosuppression increases risk of infection (107, 108), that the pattern and nature of infection change over time after transplantation (108), and that they differ with immunotherapy used (109, 110).

Conventional teaching suggested that infections within the first 4 weeks following transplantation are mainly donor- or recipient-related, particularly bacteraemia as a result of wound infection, or related to anaesthetics and equipment used in invasive monitoring.

The risk of opportunistic infections, either from residual infections or reactivations of latent infections, was the highest in the first 12 months after transplantation. It was thought that patients were most heavily immunosuppressed during this period because of initial induction therapy as well as resulting escalation of immunosuppression following treatment of rejection, during which the rate was highest in the first year. Herpes virus, cytomegalovirus, hepatitis, tuberculosis and pneumocystis carinii pneumonia are common historically. As the excess immunosuppressive effects have worn off after the first 12 months, community-acquired infection becomes the commonest cause in transplant recipients (107, 108). The nature of infection has changed with the introduction of induction antibodies, which allows spare use of corticosteroids, as well as introduction of viral and antifungal prophylaxis after transplantation (109).

There is little in the literature describing the precise nature and rate of infection in recipients following transplantation.

Patients should not be dissuaded from transplantation because of malignancy and infection risk; instead, clinicians should endeavour to reduce these risks by limiting long-term immunosuppressive load and developing a vigilant surveillance programme.

2 Aim

The purpose of this thesis was to describe the experience of using Campath induction and tacrolimus monotherapy in renal transplantation in the Imperial College Kidney and Transplant Centre, Hammersmith Hospital.

This thesis consisted of different studies to examine various clinical aspects of the use of immunosuppression regime.

1] Our early experience with the regime based on a the short-term pilot study, the results were reported in Chapter 4 and a more detailed examination of long term results and causes of graft loss were reported in Chapter 6 and chapter 8

2] As a result of the encouraging experience with the pilot study, I developed a randomised controlled study to examine my hypothesis that the efficacy of this regime was non inferior to the conventional regime. Results of this RCT were described in Chapter 5

3] The ideal dosage of Campath used as induction was unclear, I set out to examine if the dosage of Campath should be adjusted for subjects weight in a retrospective cohort study and the results were described in Chapter 7

4] Apart from examine the efficacy of this regime, I also investigated the risk profile associated with this immunosuppression regimen. The risk of long term allograft pathology, haematological effects m infection, malignancy and metabolic effects were examined in Chapter 9,10 & 11 respectively

5] Campath is a powerful lymphodepletion agent, it was suggested that the rate of repopulation following Campath might be related to the occurrence of allograft rejection. I developed a mathematical model to examine this hypothesis and reported the results in Chapter 10.

3 Patients and Methods

3.1 Patients

The study data included in this thesis were based on patients receiving a kidney from a living donor or deceased donor from November 2002 in the Imperial College Kidney and Transplant Centre. Number of subjects varied between analysis in different chapters due to difference in timeline and design of the study. Patients who received a simultaneous kidney and pancreas transplant, as well as those undergoing desensitisation for blood group incompatibility and flow crossmatch positive transplant recipients, were excluded.

The key study group patients transplanted between received the minimalistic Campath induction with a tacrolimus monotherapy maintenance protocol. In some studies, the cohort of patients who received our historic immunosuppression regime based on daclizumab induction – the tacrolimus and mycophenolate mofetil maintenance protocol were acted as the control group.

All participants were followed up in the transplant clinics in the Imperial College Kidney and Transplant Institute.

3.2 Methods

This studies used in this thesis were self-funded and were reviewed and approved by the institutional review board.

3.2.1 Interventions

3.2.1.1 Campath group

Immunosuppression was started with Methylprednisolone at 500 mg and a loading dose of tacrolimus [0.5mg/kg] prior to surgery. The following operation – 30 mg of Campath [MabCampath, Roche] – was administered intravenously with hydrocortisone at 100mg and chlorphenamine at 10mg as premedication to prevent a cytokine reaction caused by Campath. Twice-daily tacrolimus was started orally at day 0 post-transplantation with a 12-hour trough target of 5-8mg/mL.

The patients also received our sparing protocol, consisting of hydrocortisone at 100mg intravenously or prednisone at 30mg orally twice daily between day 0 and day 3, then reduced to 30mg daily between days 4 and 6, and ultimately stopped.

3.2.1.2 daclizumab group

Immunosuppression was started with Methylprednisolone at 500 mg and a loading dose of tacrolimus [0.75mg/kg] and mycophenolate mofetil at 750mg prior to surgery. Following the operation, daclizumab [Zenapax, Roche] at 2mg/kg was administered intravenously. Twice-daily tacrolimus was started orally at day 0 post-transplantation with a 12-hour trough target of 8-12mg/ml, with twice-daily mycophenolate mofetil at 500mg and a target level of 1.2-2.5g/l.

All patients received our steroids sparing protocol, consisting of hydrocortisone at 100mg intravenously or prednisone at 30mg orally twice daily between day 0 and day 3, then reduced to 30mg daily between days 4 and 6, and ultimately stopped.

All patients with this protocol received a second dose of daclizumab at 2mg/kg on day 14 after transplantation.

The tacrolimus target was higher in the first year with this group, in which the 12-hour trough target was 8-12ng/ml in the first year, and reduced to 5-8 ng/ml after 1 year.

3.2.1.3 Antibiotics prophylaxis

Patients from both groups received Valgancidovir at 450mg daily for 3 months as cytomegalovirus infection prophylaxis regardless of donor or recipient cytomegalovirus status, as well as Co-Trimoxazole as Pneumocystis Carinii Pneumonia prophylaxis for 6 months.

Patients who had had a previous history of, or in close contact with others with tuberculosis and patients with a high risk of reactivation of latent tuberculosis were given lifelong tuberculosis prophylaxis in the form of Isoniazid at 150mg daily and pyridoxine at 50mg weekly to reduce the risk of neuropathy associated with treatment.

Campath induction was not used in patients with hepatitis because of safety concerns in the literature(112). All patients with hepatitis were given Lamivudine prophylaxis following daclizumab induction.

3.2.1.4 Rejection

Allograft rejection was diagnosed by a transplant biopsy and was treated with the steroids. Methylprednisolone at 500mg per day for 3 days followed with oral prednisolone at 30mg daily and was tapered to 10mg once daily in 3 months, with the addition of MMF [500mg twice daily] in the Campath group.

4 Pilot study results in Campath and tacrolimus monotherapy protocol

4.1 Introduction

Campath is a monoclonal antibody that targets CD52 receptors which are present on most circulating lymphocytes, and activation of CD52 receptors results in profound temporary lymphopenia.

Campath was first used in renal transplantation as an induction agent in combination with low-dose cyclosporine monotherapy (42, 61, 62) in 1989. Friend and Calne used a 10-day course of Campath 1M in combination with high-dose Cyclosporine monotherapy, showing recipients who received Campath 1M to have a significantly lower incidence of acute cellular rejection. However, the treatment was associated with a higher incidence of infection which was likely due to the high dosage.

Their follow-up study using the humanised form of Campath, Campath-1H, showed a similar benefit in outcomes with a much lower dose of the drug (61). Allograft and patient survival were reported with a little number of rejections in the short term. The subsequent long-term follow-up study (63) reported by Watson et al. confirms the efficacy of Campath. 5-year patient and allograft survival were comparable to those received in conventional triple therapy. The hazards of rejection were similar between the two groups, although there are several cases of late rejection in the group receiving Campath and low-dose Cyclosporine, which was not observed in the control. This pivotal pilot study demonstrated that Campath induction was safe and allows using a medium dose of cyclosporine monotherapy without an increased risk of complications.

Apart from cyclosporine monotherapy, Campath had been used in renal transplantation in combination with other maintenance immunosuppressant (52,54,57-58) . Short results from these studies were encouraging, showing a similar allograft survival and function and low rate of rejection, except when Campath was used along with rapamycin. It was proposed that the difference in rejection rate between different protocols were related to the differential effect of lymphodepletion with Campath(77). This depletion-resistant subset was thought to be a memory-effect T cell that

expands quickly following depletion and were resistant to steroids, rapamycin and deoxysepergualin in vitro, but were uniquely sensitive to calcineurin inhibitors. These data supports the crucial role of calcineurin inhibitor maintenance following Campath induction.

There is a trend in the transplantation circle to avoid or spare the use of long-term cyclosporine as well as tacrolimus, albeit it had been shown that calcineurin inhibitors were crucial for long-term transplant success. There was concern that long-term calcineurin inhibitors cause irreversible nephrotoxicity and graft failure (16, 19, 66, 67). Ojo et al. examined the UNOS database, demonstrating that 16.5% of patients with a non-renal organ transplant have a degree of renal failure (67) and suggesting that this was partially contributed by the use of calcineurin inhibitors (66). In terms of renal allografts, an early study published in 2004 based on a cyclosporine-based regimen suggested that at least 80% of renal transplant surveillance biopsies express features associated with calcineurin inhibitor toxicity at 10 years (16). However, it was thought that tacrolimus has a less long-term toxicity event compared to Cyclosporine (20, 68, 69).

Therefore, the Imperial College Kidney and Transplant Centre set out to investigate the feasibility of successful transplantation with lower-dose maintenance of tacrolimus. Thus, our unit developed the regimen of Campath induction with medium-dose tacrolimus in 2004, with the precedence of Calne et al. The difference with this regimen from previous protocols was not only a different induction agent, but also that it avoided the use of long-term mycophenolate mofetil and steroids. Further immunosuppression would only add in the face of allograft rejection. We anticipated that this minimalistic approach to maintenance immunosuppressants would translate into a reduction of long-term complications.

I discussed the short-term result from this cohort in this chapter, which led to development of our randomised controlled study (111).

4.2 Patients and methods

4.2.1 Patients

The study group consisted of 59 patients transplanted between August 2004 and November 2005 who received the minimalistic Campath induction with a tacrolimus monotherapy maintenance protocol. Our historic cohort of 101 patients who transplanted from November 2002 received the daclizumab induction – the tacrolimus and mycophenolate mofetil maintenance protocol acted as the control group.

All participants were followed up in the transplant clinics in the Imperial College Kidney and Transplant Institute.

Demographics

Table 4-1 Demographics

		Campath number [%]	**daclizumab number [%]**
Gender	F	25 [42.4%]	49 [48.5%]
	M	34 [57.6%]	52 [51.5%]
Ethnicity	Afro-Caribbean	6 [10.2%]	10 [9.9%]
	South Asian	16 [27.1%]	21 [20.8%]
	Caucasian	35 [59.3%]	67 [66.3%]
	Others	2 [3.4%]	3 [3.0%]
First transplant	Y	55 [93.2%]	94 [93.1%]
	N	4 [6.8%]	7 [7.3%]
Donor type	Deceased	27 [45.8%]	55 [54.5%]
	Living	32 [54.2%]	46 [45.5%]
Cold ischaemic time	Hours	17.9 $\pm$ 13.5	15.6 $\pm$ 13.2
Recipient age	Years	45.1 $\pm$ 13.1	45.1 $\pm$ 12.4
Donor age	Years	47.6 $\pm$ 14.4	46.5 $\pm$ 14.2
HLA mismatch		3.2 $\pm$ 1.8	3.2 $\pm$ 1.7
Follow-up	Months	70.5 $\pm$ 24.1	82.6 $\pm$ 30.2

Detailed demographics were listed in Table 4-1. 57.6% of patients transplanted in the Campath group were male and were comparable to the historic control of 51.5%. 37.2% of the cohort in this study were non-Caucasoid; this reflects the underlying ethnic composition in the catchment area of

the Imperial College Kidney and Transplant Centre. Proportions of non-Caucasoid transplanted in the two groups were similar. 10.2%, 27.1% and 3.4% of patients transplanted in the Campath group were Afro-Caribbean, South Asian and from other ethnic backgrounds, compared to 9.3%, 20.8% and 3.0% in the daclizumab group, respectively. Over 93% of patients transplanted in both groups had no previous solid organ transplantation. The proportion of living donor transplantation recipients was slightly higher in the Campath-treated group [54.2% in Campath group vs 45.5% in the daclizumab group]; this was likely reflecting a change of practice over the follow-up period as living donor transplantation, where feasible, has become a more favourable treatment for patients with end-stage renal failure. In the remaining patients who received a renal transplant from a deceased donor, the mean cold ischaemic time in the Campath group was 17.9 hours and was similar to the daclizumab-treated group at 15.6 hours. The average recipient and donor ages were indifferent between groups. The average recipient age was 45.1 years in both groups, and the average donor age was 47.6 years in the Campath group and 46.5 years in the daclizumab group. On average, both Campath and daclizumab groups have a 3.2 Human Leucocytes Antigen [HLA] mismatch between recipient and donor.

57/59 [96.6%] patients in the Campath group and 97/101 [96.0%] patients in the daclizumab group were under active follow-up in the transplant unit to date or until graft loss. 1 patient was transferred back to the local nephrology centre for follow-up at 13 months, and the remaining patients were transferred to other centres between 2 and 5 years after transplantation. Of the remaining 96% of patients under active review, the average follow-up in the Campath group was 70.5 months and was significantly lower than the historic daclizumab cohort of 82.6 months.

4.3 Statistics

The purpose of this study was to examine the short- and long-term outcomes in the original cohort of patients treated with the minimalistic immunosuppressive protocol.

4.3.1 Primary endpoint:

- Patient survival

4.3.2 Secondary endpoints:

- Patient survival with functioning graft
- Allograft survival after being censored for patient death with function
- Allograft function at 3, 6 and 12 months after transplantation
- Rejection-free survival
- Calcineurin inhibitor toxicity
- Transplant glomerulopathy
- Treatment survival

4.3.3 Outcome definition

Allograft failure was defined as the requirement of dialysis; the date of failure was the date of dialysis commencing.

Allograft function was assessed using serum creatinine and MDRD eGFR.

Allograft rejections, calcineurin inhibitor toxicity and transplant glomerulopathy were all biopsy-proven and classified by the Banff 2007 criteria.

4.3.4 Statistics analysis plan

This study was examined as the intention to treat analysis.

The Logrank method was used in comparison in survivals between the 2 groups, under an assumption that the underlying risk of failure and difference in hazards between groups were constant. The Weibull parametric model was used in cases where this assumption was violated and where the underlying risk of failure changes with time. The Cox semi-parametric model was used if the assumption of constant difference in hazards was violated.

The Kaplan Meier method was used to estimate cumulative survival over time. Patient survival, allograft survival and freedom from rejection, CNI toxicity and transplant glomerulopathy were examined using the same method.

Censoring for loss of follow-up or death with a functioning graft was assumed to be non-informative.

A mixed-effect model was used to estimate the difference in function at the baseline and difference in trajectory between groups on allograft function, as well as accounting for repeat measurements from the same patients over time. The point estimate on numeric outcomes on allograft function were tested using a Student's t test.

All statistical tests were performed as a two-sided test and were deemed to be statistically significant if the p value was <0.05.

Statistic tests were performed using Stata 12.0 (StataCorp, Texas).

4.4 Results

4.4.1 Patient survival

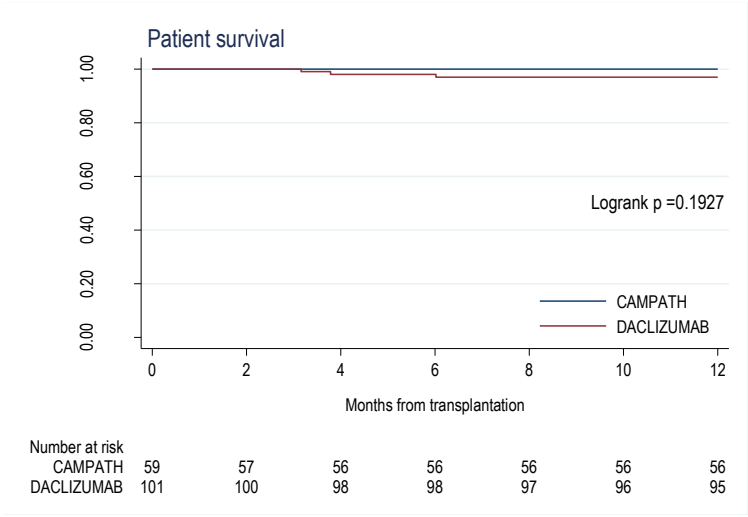

Figure 4-1 Twelve-month patient survival in pilot study

Figure 4-1 shows the twelve-month patient survival in this cohort. No patient loss was observed in the Campath group; therefore, there was cumulative patient survival of 100% at 12 months. There were three patient deaths in the daclizumab cohort. The first patient died with bone marrow failure and sepsis 3 months after transplantation. The second patient died at home unexpectedly at 4 months after transplantation; the cause of death was thought to be cardiac-related. The third patient died with overwhelming fungal sepsis 6 months after transplantation. As a result, cumulative patient survival in the daclizumab group was 100%, 98% and 97% at 3, 6 and 12 months after transplantation, respectively.

There was no statistical significance on patient survival between the groups [p=0.1927, Logrank].

4.4.2 Allograft survival

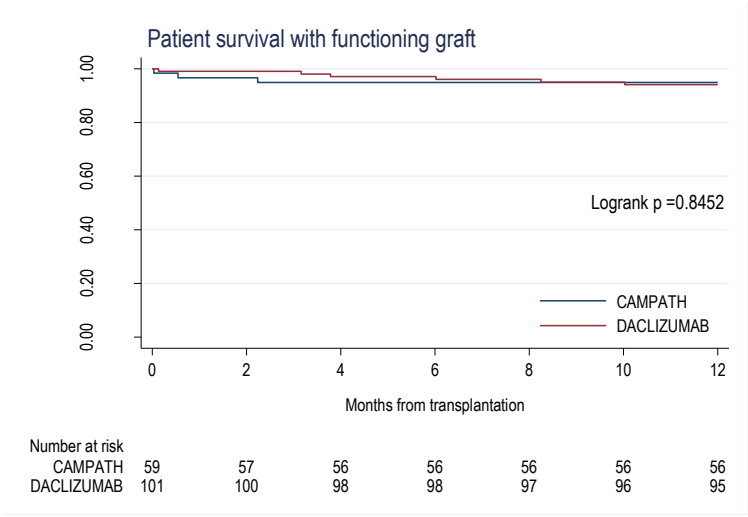

Figure 4-2 Twelve-month patient survival with functioning graft in pilot study

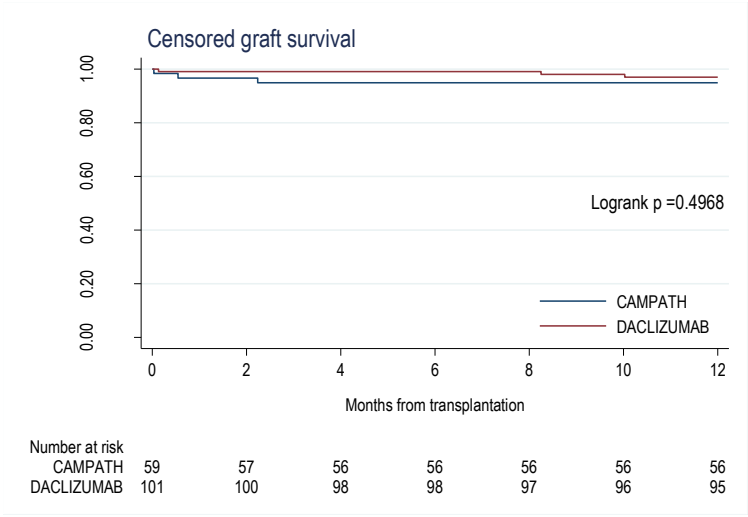

Figure 4-3 Twelve-month censored allograft survival in pilot study

Figure 4-2 and Figure 4-3 (above) show 12-month patient survival with a functioning graft and allograft survival after censoring with death with a functioning graft. There was no patient death in the first 12 months in the Campath group [Section 4.4.1]. 3 grafts were lost in total in the Campath group. One graft was lost due to technical failure. The second graft was lost soon after transplantation due to renal vein thrombosis in a recipient with a background of lupus nephritis and presence of pro-coagulant. The third graft was lost due to severe thrombotic microangiopathy at 2 months in a recipient with primary haemolytic uraemic syndrome; it was thought that the graft loss

was a result of antibody-mediated rejection because a later investigation confirms that this patient did not suffer from a familial form of haemolytic uraemic syndrome. Therefore, cumulative allograft survival was 94.9% at 3, 6 and 12 months in the Campath group, respectively.

The three graft losses were a result of patient death in the daclizumab group [Section 4.4.1]. Another 3 grafts were lost in this cohort. The first graft was lost as a result of transplant renal artery thrombosis soon after surgery in a patient with significant pro-coagulant. The second graft was lost as a result of chronic antibody-mediated rejection at 8 months; the third graft was lost at 10 months after transplantation with late presented graft pyelonephritis associated with the ileal conduit. Cumulative patient survivals with a functioning graft in this group were 99.0%, 97.0% and 94.1% at 3, 6 and 12 months, respectively. Cumulative allograft survivals after being censored for patient death with a functioning graft were 99.0%, 99.0% and 97.0% at 3, 6 and 12 months, respectively.

There were no statistical significant differences between the two groups in patient survival with functioning graft [p=0.8452, Logrank test] and allograft survivals after being censored for patient death with a functioning graft [p=0.4968, Logrank test].

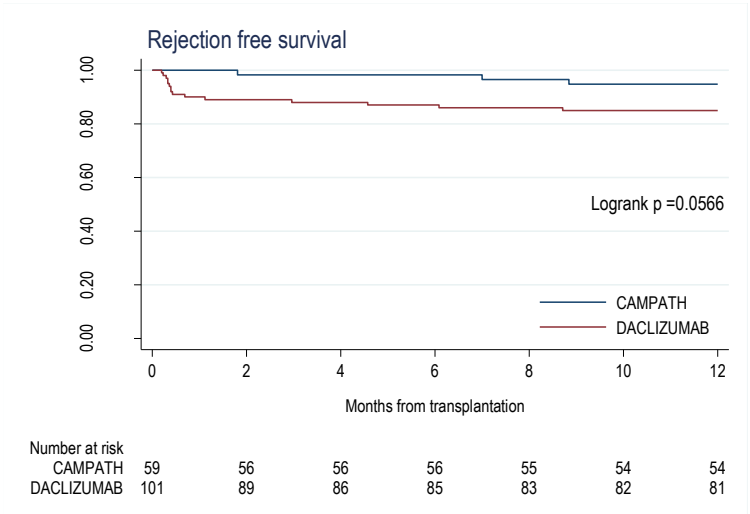

Figure 4-4 Twelve-month rejection-free survival in pilot study

Figure 4-4 (above) demonstrates a Kaplan Meier plot on cumulative freedom from rejection between the 2 groups in the first 12 months. There was a suggestion that risk of rejection was lower in the Campath group [p=0.0566, Logrank]. A more detailed examination of the data suggested that

risk of rejection reduces over time in this 12-month period [p<0.001, Weilbull model] and that it violated the baseline assumption of constant baseline risk with the Logrank model; therefore, an adjusted model was used. After being adjusted for changes of baseline risk over time, the model suggested that patients in the dadizumab group have a 3.2-fold increased risk of rejection; however, it did not reach statistical significant at 5% level [HR 3.2, 95% CI 0.92, 10.92; p=0.069].

3/59 [5.1%] patients in the Campath group experienced an episode of rejection in the first 12 months. 1 patient had severe antibody-mediated rejection at 2 months after transplantation which failed to respond to escalation of immunosuppression and resulted in graft loss. The remaining 2 rejections were cell-mediated rejection at 7 and 8.5 months, of which the latter was thought to be a result of non-compliance with immunosuppression.

15/101 [14.9%] patients experienced an episode of rejection in the first 12 months in the daclizumab group. 2 cases of pure antibody-mediated rejection were reported within the first month after transplantation. 11 cases of cellular rejection were reported in the control group, with an average time to rejection of 2.3 months. The remaining 2 cases of rejection were mixed antibody and cellular rejection developed within the first months of transplantation, of which one case developed chronic antibody-mediated rejection and resulted in graft loss at 8 months.

4.4.3 Allograft function

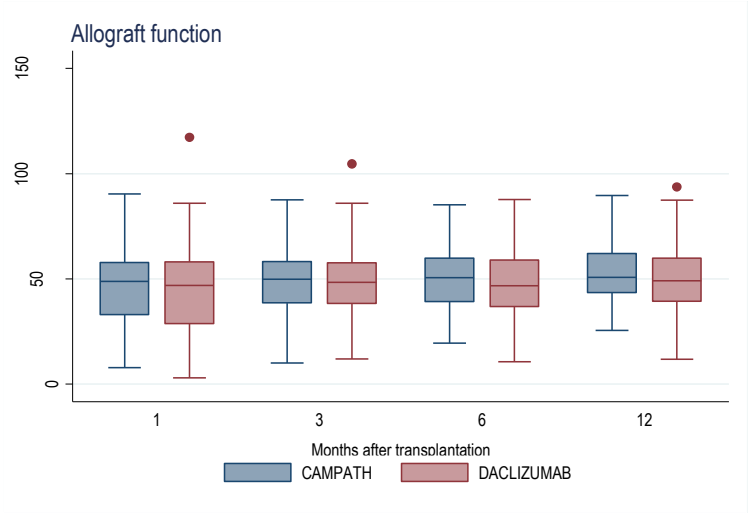

Figure 4-5 Twelve-month allograft function in pilot study [MDRD eGFR]

Figure 4-5 (above) demonstrates allograft function, measured by using MDRD eGFR, between the two groups. Allograft function was similar between the two groups at 1, 3, 6 and 12 months after transplantation.

MDRD eGFR was 45.8±18.1, 48.7±15.6, 50.0±14.8 and 52.1 ± 14.3 ml/min/1.73m^2 at 1, 3, 6 and 12 months in the Campath group, respectively, and it was 44.1±21.3, 47.3±16.2, 47.4±15.6 and 48.9±15.5 ml/min/1.73m^2 in the daclizumab group at the same timeline.

The longitudinal model showed that there was no significant difference between the groups in overall allograft functions [Average difference in function: -1.6 ml/min/1.73m^2, 95%CI -7.2, 4.0; p=0.574]. Moreover, the rates of changes in allograft function were not different between the two groups [Difference in rate of function changes per month: -0.17 ml/min/1.73m^2/month, 95%CI -0.63.0.28; p=0.451].

4.4.4 Calcineurin inhibitor toxicity

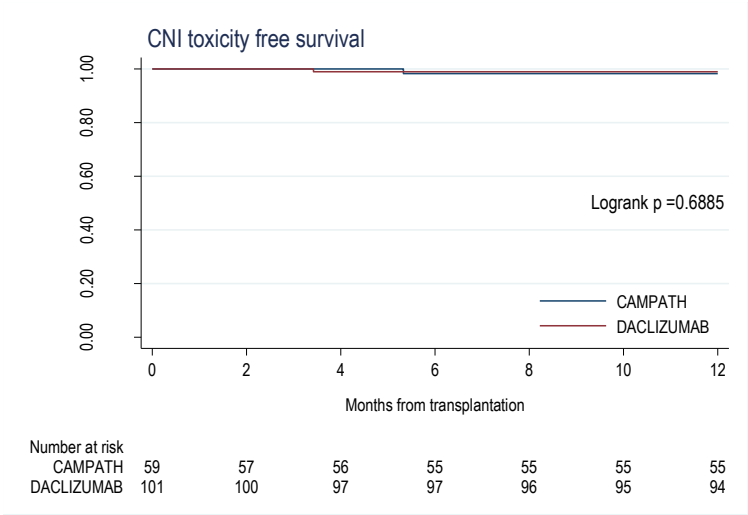

Figure 4-6 Twelve-month CNI toxicity-free survival in pilot study

Chronic calcineurin inhibitor toxicity was rare in the early phase after transplantation and this was illustrated clearly in Figure 4-6. CNI toxicity in this cohort was diagnosed on an allograft biopsy from clinical grounds during the investigation of allograft dysfunction. 2 patients were diagnosed with CNI toxicity in the first twelve months, 1 from the Campath-treated group at 5 months and 1 patient from the daclizumab group, diagnosed at 3.5 months after transplantation.

Cumulative CNI toxicity-free survivals were 98.2% and 99.0% at 12 months in the Campath and daclizumab group respectively. There was no statistical significance in the difference between the groups [p=0.6885, Logrank test].

4.4.5 Transplant Glomerulopathy

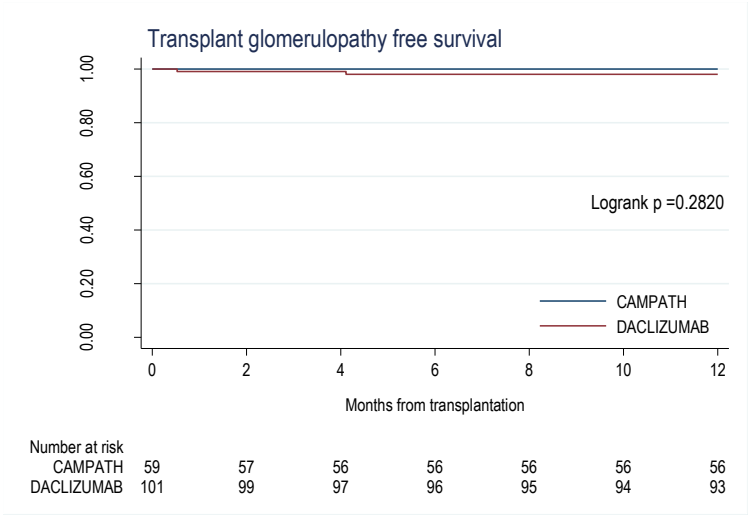

Figure 4-7 Twelve-month transplant glomerulopathy-free survival in pilot study

The incidence of transplant glomerulopathy was low in the first 12 months following transplantation. There were 2 cases of transplant glomerulopathy in the daclizumab group. The first case developed acute transplant glomerulopathy 2 weeks after transplantation; allograft function stabilised after optimising of immunosuppression and has remained functioning to date. The second case developed transplant glomerulopathy 3 months following treatment of antibody-mediated rejection. Allograft functions continue to deteriorate gradually despite escalation of immunosuppression. Moreover, the allograft failed at 38.4 months.

Cumulative transplant glomerulopathy-free survival was 100% and 98.0% at 12 months in the Campath and daclizumab-treated groups respectively. There was no statistically significant difference between the 2 groups [p=0.2820, Logrank].

4.4.6 Treatment survival

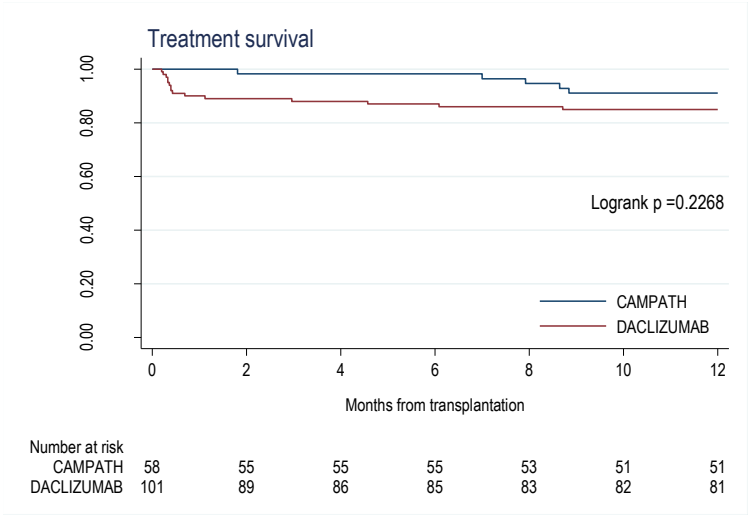

Figure 4-8 Twelve-month treatment survival in pilot study

Treatment survival was defined as a patient maintained on the treatment protocol. 54/59 [91.5%] patients in the Campath group remained in tacrolimus monotherapy in the first year. mycophenolate mofetil and steroids were added to 3 patients who experienced rejection. mycophenolate mofetil was added to 2 patients to facilitate further reduction in tacrolimus doses. Both patients received kidneys from elderly non-heart-beating donors, and subsequent transplant biopsies persistently demonstrated substantial scarring.

Conversely, 86/101 [85.2%] patients in the daclizumab group remained steroid-free at 1 year. All 15 patients received steroids at parts of the treatment of rejection.

Cumulative treatment survival in the Campath group was 98.2%, 98.2% and 91.1% at 3, 6 and 12 months, respectively, compared to 88.0%, 87.0% and 84.9% at the same timeline in the daclizumab group. There was no statistically significant difference between the 2 groups [p=0.2268, Logrank].

4.4.7 New Onset Diabetes after Transplantation

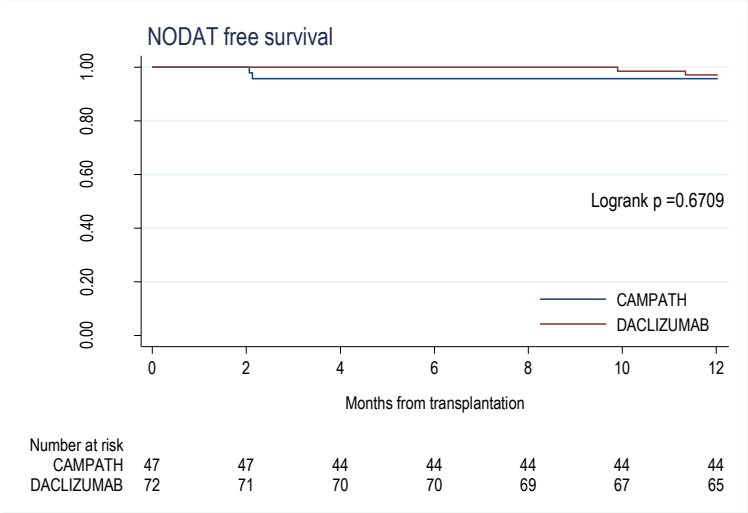

Figure 4-9 Twelve-month NODAT-free survival in pilot study

The incidence of new onset diabetes after transplantation [NODAT], defined as the de novo need for oral hypoglycaemics or insulin, was low at 12 months in both groups. 47 patients in the Campath group were not diabetic before transplantation, but 2 [4.3%] developed diabetes in the first 12 months. 2 of 72 [2.8%] patients in the daclizumab group who were non-diabetic prior to transplantation developed NODAT at 10 and 11 months after transplantation.

Cumulative NODAT-free survival in the Campath group was 95.7% at 3, 6 and 12 months, respectively, compared to 100%, 100% and 97.0% at the same timeline in the daclizumab group. There was no statistically significant difference between the 2 groups [p=0.6709, Logrank].

4.5 Summary

In this chapter, I reported the results of our pilot study, using Campath induction with tacrolimus monotherapy compared to our historic patient cohort receiving daclizumab induction, tacrolimus and MMF maintenance in 2004.

My study showed 1-year cumulative patient and graft survival were similar between the two groups. There were 3 graft losses and no patient deaths in the Campath group, compared to 3 graft losses and 3 patient deaths in the daclizumab group. The cumulative patient survivals with a function graft were 94.9% and 94.1% in the Campath and daclizumab groups respectively.

More importantly, the risk of rejection was substantially lower in the Campath-treated group despite the avoidance of MMF maintenance. It was estimated that patients in the daclizumab group experienced a 3.2-fold increased risk of rejection, albeit it did not reach statistical significance. The natures of rejections were similar between the two groups.

There were no differences in allograft function between the two groups or the trajectories of function over time.

One of the objectives of this pilot study was to establish whether it is feasible to reduce the tacrolimus dosage to lower the incidence of Calcineurin inhibitor toxicity. Our data showed that incidences of CNI toxicity were low with both tacrolimus protocols, and there was no significance between the two protocols at 12 months.

NODAT is associated with an increased cardiovascular risk in transplant recipients and results in lower graft survival, and tacrolimus was thought to be associated with an increased risk of NODAT. Our data showed that incidences of NODAT were similar between the two groups at 12 months despite a lower-dose tacrolimus in the Campath protocol benefitting with a lower incidence of NODAT.

91.1% of patients in the Campath group remained on tacrolimus monotherapy at 1 year.

Our pilot results were encouraging and demonstrated similar patient and allograft survival at 1 year with a trend of lower allograft rejection in the Campath-treated group. Therefore, we proceeded to a formal randomised controlled study and were discussed in the next chapter.

5 CamTac trial

5.1 Introduction

The combination of induction therapy and the use of tacrolimus-based regimens offer the promise of steroid-free maintenance regimens (113, 114), without an increased risk of rejection or graft failure, which were associated with steroid withdrawal from Cyclosporine [CsA] based regimens (115), or the associated the calcineurin-inhibitor toxicity related to aggressively dosed -based regimens (16, 116).

It has been suggested a substantial proportion of recipients are receiving more long-term immunosuppression than they require maintaining good, rejection-free, renal transplant function (117). Furthermore, long term immunosuppression was associated with an increased risk of developing malignancy (118), infection (107) and patients' death (119). Therefore, transplant physicians are considering regimens combining potent induction therapies with decreased intensity of long-term maintenance therapy.

Prospective randomised trials have demonstrated equivalent efficacy and safety of regimens using Campath induction without long-term steroids compared to classical CsA-based triple therapy (64), to tacrolimus/mycophenolate mofetil (MMF)/steroid triple therapy without induction (65), to induction with interleukin-2 receptor (IL-2R) MoAb or anti-thymocyte globulin with tacrolimus and MMF used in all three limbs (58), and in a small cohort comparing CsA with rapamycin monotherapy after Campath (121). Two large trials have compared induction with Campath with anti-thymocyte globulin or basiliximab followed by tacrolimus and MMF in both groups, with or without early steroid withdrawal, and both showed equivalent patient and graft survival with less rejection in the Campath arm (122, 123). The Pittsburgh group has taken the use of Campath further, reporting that the simple combination of Campath with low-dose tacrolimus maintenance monotherapy without steroids or MMF produces good outcomes, particularly in living donor transplantation (74), and this regimen has been shown to be as efficient as tacrolimus-based triple therapy without induction (65).

In our centre, the short-term experience using Campath induction and tacrolimus therapy without steroids or MMF maintenance was encouraging [Chapter 3], and there was a lack of formal randomised controlled studies to compare a conventional daclizumab induction/conventional-dose tacrolimus/MMF with this simple regimen. Therefore, we developed this randomised controlled study to examine the efficacy and safety of this regimen compare to our standard protocol.

In this chapter, I discussed our experience and results of this randomised controlled study.

5.2 Patients and methods

5.2.1 Patients

All patients receiving only a kidney transplant from a living donor or heart-beating deceased donor were invited to participate in this study. Recruitment was initiated in October 2005, and completed in April 2008

A total of 123 patients were randomised. The two arms were well balanced with no major differences in terms of live versus deceased donor transplantation, recipient gender, age, primary renal diagnosis, ethnicity, and first versus subsequent transplantation. The proportion of pre-sensitised patients was slightly higher in the daclizumab group [Table 5-1]

All participants were followed up in the transplant clinics in the Imperial College Kidney and Transplant Institute.

Table 5-1 CamTac: Demographics

	Campath n=82	**daclizumab n=41**
Recipient age [yrs.]	47.3+13.4	47.0+10.6
Donor age	44.3+13.2	48.4+12.0
Donor type		
Living donor	52 [63%]	25 [61%]
Deceased donor	30 [37%]	16 [39%]
HLA MM	3.3+1.7	3.4+1.9
Gender		
Female	28 [34.1%]	14 [34.1%]
Male	54 [65.9%]	27 [65.9%]
Ethnicity		
Afro-Caribbean	10 [12.2%]	7 [17.1%]
Asian	27 [32.9%]	10 [24.4%]
White	41 [50%]	21 [51.2%]
Others	4 [4.9%]	3 [7.3%]
Primary disease		
Glomerulonephritis	20 [24.4%]	9 [22%]
Diabetes	18 [22%]	6 [14.6%]
Polycystic kidney disease	10 [12.2%]	5 [12.2%]
Hypertension	6 [7.3%]	4 [9.8%]
Reflux nephropathy	5 [6.1%]	4 [9.8%]
Interstitial nephritis	1 [1.2%]	2 [4.9%]
Other	5 [6.1%]	0 [0%]
Unknown	17 [20.7%]	11 [26.8%]
First transplant	78 [95.1%]	36 [87.8%]
Pretransplant sensitisation status		
PRA 50%	4 [4.8%]	8 [19.5%]
PRA 85%	1 [1.2%]	3 [7.3%]

5.2.2 Methods

This study was a self-funded, institutional review board-approved and registered [Clinicaltrial.gov NCT00246129], single-centre prospective randomized comparison of Campath and low-dose tacrolimus monotherapy compared with daclizumab, tacrolimus and mycophenolate mofetil.

5.2.2.1 Trial design

This was a randomised controlled study aiming to look at the effect of a new treatment protocol, consisting of Campath induction with tacrolimus monotherapy maintenance, compared to the control protocol, consisting of daclizumab induction with tacrolimus and mycophenolate mofetil maintenance therapy.

This study was planned for non-inferiority in the treatment group in patient survival with a functioning graft at 1 year, compared to the control group. It was estimated that 1-year patient survival with a functioning graft in the control group would be 95% from the pilot study. The allocation ratio was 2:1 in Campath-to-daclizumab protocols. The reason for this allocation ratio was based on financial issues. The daclizumab protocol was substantially more expensive compared to the Campath protocol. The 2:1 ratio allocation would allow maximising the number of patients recruited for the funding available. This study was stratified for the type of donors because of the difference in graft outcomes between deceased and living donor transplantations. There were no changes to the trial design after the trial commenced.

5.2.2.2 Interventions

Trial group:

- Campath (MabCampath, Genzyme) at 30mg IV post-operatively.
- Tacrolimus [0.1mg/kg; target level 5-8 ng/ml] from Day 0.
- Methylprednisolone at 500mg IV pre-operatively.
- Prednisolone at 30mg bd Day 0-3, 30mg od Day 4-6, then stopped.

Control group:

- Daclizumab (Zenapax, Roche) at 2mg/kg at Day 0 and Day 14.

- Tacrolimus [0.15mg/kg; target level 8-12 ng/ml in first year, reduced to 5-8 ng/ml after 1 year] from Day 0.
- Mycophenolate mofetil [500mg twice daily; target level: 1.2-2.5 g/l].
- Methylprednisolone at 500mg IV pre-operatively.
- Prednisolone at 30mg bd Day 0-3, 30mg od Day 4-6, then stopped.

Allograft rejection was diagnosed by a transplant biopsy and was treated with steroids. Methylprednisolone at 500mg per day for 3 days, oral prednisolone at 30mg daily and was tapered to 10mg once daily in 3 months, and there was the addition of MMF [500mg twice daily] in the Campath group.

5.2.2.3 Outcomes

Primary endpoint:

- Patient survival with functioning graft at 1 year

Secondary endpoints:

- Patient survival at 1 year
- Allograft survival at 1 year after being censored for patient death with function
- Allograft function at 3, 6 and 12 months after transplantation
- Rejection-free survival at 1 year
- Allograft failure defined as the requirement of dialysis; the date of failure was the date dialysis commenced.
- Allograft function was assessed using serum creatinine and MDRD eGFR.

Allograft rejections were all biopsy-proven.

There were no amendments to study endpoints after the trial commenced.

5.2.2.4 Sample size

The sample size was calculated to reflect a non-inferiority of 10% in patient survival with a functioning graft at 1 year, with an estimated 1-year survival of 95% in the control group by independent statistics advice for the unit. It is estimated that the trial would require 80 subjects in

the treatment group and 40 subjects in the control group, based on a 2:1 randomisation. The power calculation was reviewed and approved by COREC prior to the study.

Interim analysis on allograft survival and function were undertaken after 50% of trial recipients had reached the primary endpoint. Peto rules were used in the trial-stopping boundary ($p<0.001$), as well as if there were clinical concerns with new treatments. Results on interim analysis were reviewed by an external reviewer from the Royal Free Hospital Renal Unit and blindly by the transplant research group.

5.2.2.5 Randomisation

Randomisation was performed with the method of random permuted block with a random block size between 6 and 9, with a randomisation ratio of treatment and control group of 2:1.

5.2.2.6 Concealment

Treatment allocation was concealed in an opaque envelope and prepared by staff members in the Leslie Brent Laboratory, who were independent to the study. In order to reduce bias, treatment allocation was pre-printed prior to study started and stored securely. The investigator was blinded to block sizes, and 20 sequential treatment allocations were used at one time. Different folders for deceased and living donors were used for stratification. Patient recruitments continued until both arms reached the minimum number required in the study design.

5.2.2.7 Implementation

Information sheets regarding the trial were given to patients in the last living donor clinic review prior to transplantation, in the living donor recipients. Due to the impossibility of predicting the offer of deceased donor organs, information sheets were only given to patients receiving a deceased donor transplant a few hours prior to the transplantation.

The randomisation sequence for 150 participants was generated prior to the start of the study by staff members in Leslie Brent Laboratory.

5.2.2.8 Consent

Consent for participation and treatment allocation were assigned by a junior investigator

[myself] or by duty transplant clinicians prior to transplantation.

5.2.2.9 Blinding

This was an open-label study involving different immunosuppression maintenance drugs, where drug levels required to be monitored carefully by clinicians, hence blinding was inappropriate.

5.3 Statistics

This study was examined as the intention to treat analysis; all patients had their induction therapy assigned by randomisation. Maintenance treatment might have changed as a result of complications and was classified as treatment failure. However, they remained in the randomised group, since the immunosuppressive effect of the initial treatment persists for a long period after transplantation.

The Logrank method will be used for comparison between patient, graft and rejection-free survival between the 2 groups. The Kaplan Meier method was used to estimate cumulative survival over time, and variance of cumulative survival was estimated using the Greenwood formula.

Censoring for loss of follow-up or death with a functioning graft was assumed to be non-informative.

Numeric outcomes on allograft function at 6 months, 1, 2 and 3 years will be tested using a Student's t test, as pre-determined in the study design. Due to the limited study number, no sub-group analysis was planned.

For the purpose of this project, a mixed-effect model was used to examine the difference in function over time between the 2 arms as well as the effect of rejection on graft function.

Statistic tests were performed using Stata 12.0 (StataCorp, Texas).

5.4 Results

Trial recruitment was initiated in October 2005 and completed April 2008, 123 patients underwent randomisation. The two arms were well balanced with no major differences in terms of live versus deceased donor transplantation, recipient gender, age, primary renal diagnosis, ethnicity, and first versus subsequent transplantation.

There were a higher portion of subjects in the daclizumab arm were sensitised against human leukocyte antigens defined as subject with calculated PRA more than 50%. Subject was sensitised in

the context of a previous transplant in two of four patients in the Campath arm and four of eight patients in the dadizumab arm. On single-antigen bead analysis, three of the sensitised patients in each arm had detectable donor-specific antibodies. This did not have a detectable effect on rejection or graft survival because none of the sensitised patients (calculated PRA >50%) in either arm suffered acute rejection during the first 2 years, and only one sensitised patient (in the Campath arm) suffered graft failure (due to progressive fibrosis in a scarred graft which functioned poorly from implantation).

5.4.1 Primary outcomes

5.4.1.1 Patient survival with functioning graft

The primary outcome of this trial was cumulative patient survival with a functioning graft; we were interested in cumulative survival/hazard in participants who either died or sustained graft loss, defined previously as the requirement of commencing dialysis. 12-month cumulative patients with a functional graft are best demonstrated in Figure 5-1 below.

1-year cumulative survival was 97.5% and 95.1% in the Campath and daclizumab groups. There were 2 grafts lost in each group in the first 12 months. In the Campath group, there was one graft with primary non-function, and a graft from elderly extended criteria was lost at 7 months after transplantation following recovery from prolonged delayed graft function. A repeat transplant biopsy on this allograft showed persistent tubulofibrosis with no evidence of rejection. There were 2 graft losses in the daclizumab group, there was one primary non-function and one patient died with severe CMV disease at 5 months which developed soon after completing the course of prophylaxis.

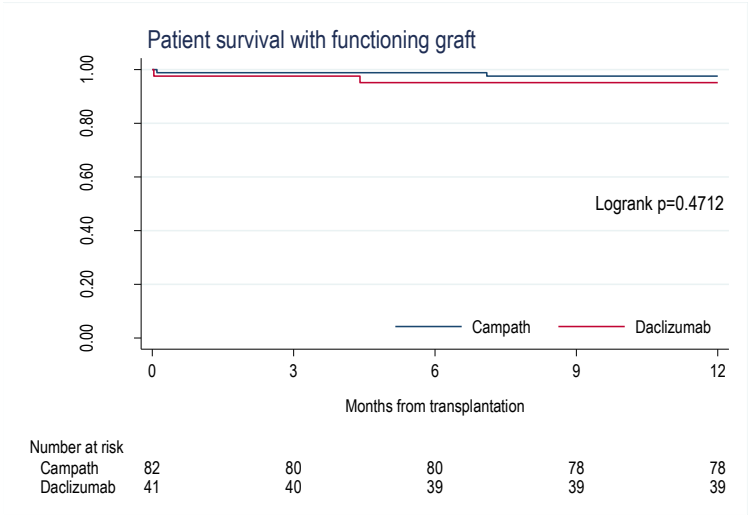

Figure 5-1 CamTac trial: Patient survival with functioning graft

Examination on primary outcome

This study was powered for a non-inferiority design with a 10% margin in patient survival with a functioning graft at 1 year in the Campath group. Analysis was based on FDA recommendation of a non-inferiority study using a fixed-margin approach.

1-year cumulative survival in the Campath group = 97.5%

1-year cumulative survival in the daclizumab group = 94.9%

Difference = Survival in Campath group – Survival in daclizumab group

= 97.5% - 94.9%

= 2.6%

Using the Greenwood formula, estimated standard error = 2.21% [Stata output].

Upper 95% CI of difference = 2.6 + 1.96 x 2.21%

= 6.9%

Lower 95% CI of difference = 2.6 - 1.96 x 2.21%

= -1.7%

Using the fixed-margin approach, estimated differences in the survival rate between the two groups were 2.6%, with a 95% confidence interval of -1.7%, and 6.9%. This does not cross the -10% margin

and thus there was no evidence to suggest inferior survival in the Campath group at a 5% significance level.

5.4.2 Secondary endpoints

5.4.2.1 Patient survival

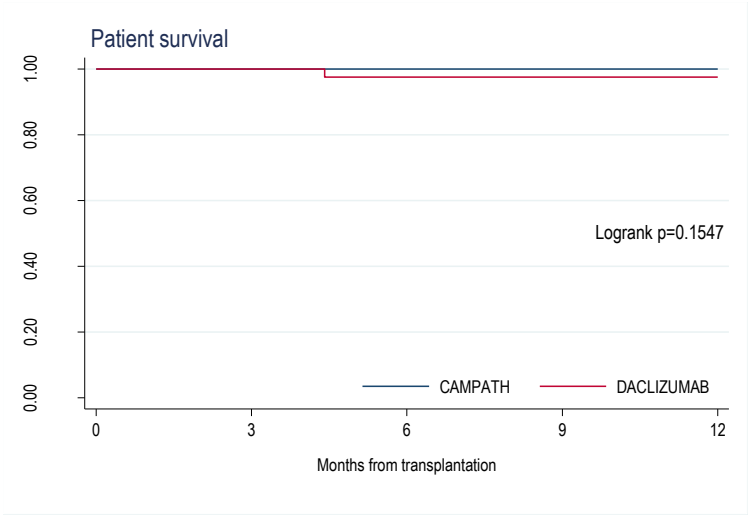

Figure 5-2 CamTac trial: Patient survival

Figure 5-2 (above) shows the Kaplan Meier plot of patient survival in this study. Cumulative 12-month patient survival was 100% and 97.5% in the Campath and daclizumab group [Logrank p=0.1547]. One patient in the daclizumab arm died of hemophagocytosis at 4.4 months associated with disseminated cytomegalovirus (CMV) disease after the cessation of CMV prophylaxis after a D+/R- live donor transplant.

5.4.2.2 Allograft survival after being censored for patient death with function

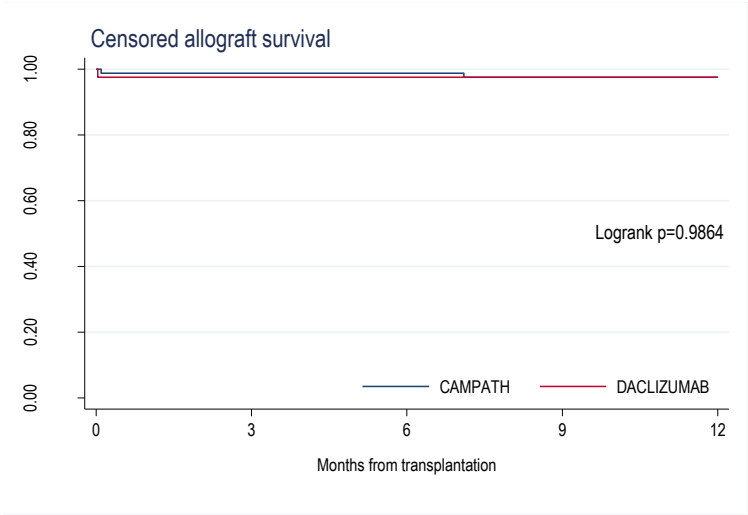

Figure 5-3 CamTac trial: Allograft survival [Censored for death with function]

Cumulative allograft survival after being censored for death with function was 97.6% in both groups at 1 year [Logrank test, p=0.9864].

At 1 year, two grafts were lost in the Campath group, and one was lost in the daclizumab group, with one of the Campath group graft losses being related to donor organ quality with significant interstitial fibrosis and tubular atrophy on early biopsies for poor initial graft function progressing to graft failure without any evidence of rejection on multiple biopsies, despite the recipient being sensitised. [The sister organ was randomised to the daclizumab group and provided significantly impaired function in a pre-emptively transplanted recipient with significant residual native renal function.] The second graft loss in the Campath group was a result of a primary non-function allograft from an extended criteria donor.

One early graft loss in the dadizumab group was due to technical failure.

5.4.2.3 Allograft function at 3, 6 and 12 months after transplantation

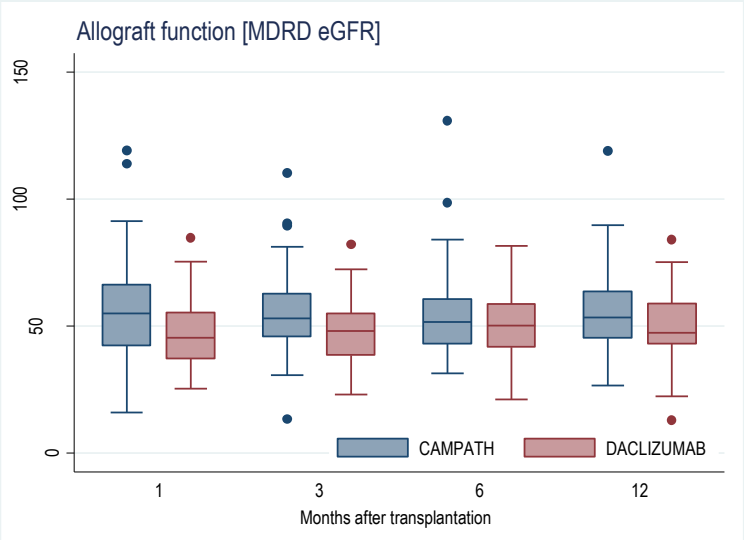

Figure 5-4 CamTac: Allograft function

Graft function did not differ significantly between the two groups [Figure 5-4], despite lower tacrolimus trough levels as per protocol in the Campath than daclizumab groups. Proteinuria [measured as spot urine protein/creatinine ratio P/Cr] did not differ at 6 or 12 months between the two groups [Campath arm 34.2/28.9 mg/mmol, daclizumab arm 31.4/59.7mg/mmol].

5.4.2.4 Rejection-free survival at 1 year

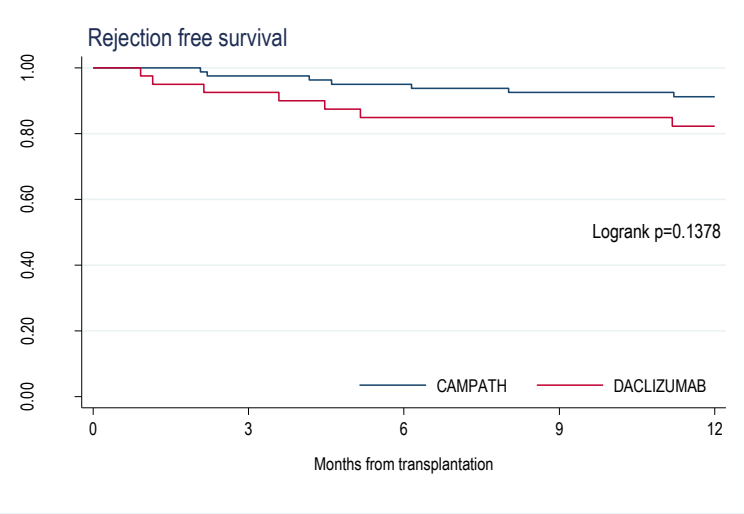

Figure 5-5 CamTac: Rejection-free survival

Although the results appeared to have a trend of a lower cumulative risk of rejection in the Campath group, the difference did not reach statistical significance at a 5% level [HR 2.2, 95%CI 0.76, 6.2; p=0.148]. 1-year rejection-free survival was 91.2% in the Campath group compared to 82.3% in the daclizumab group [Logrank test, p=0.1378].

Seven patients in the Campath arm suffered 12 rejection episodes in the first year, and seven patients in the daclizumab arm suffered 11 episodes. Although rejection was more frequent in the daclizumab arm, the difference did not reach statistical significance. One patient in each arm suffered acute antibody-mediated rejection [both in the presence of concomitant acute cellular rejection]. Both of these responded to treatment with a plasma exchange with 2g/kg human pooled intravenous immunoglobulin. Two patients in the Campath arm suffered repeated rejection episodes, responsive to steroids initially, but were treated as being steroid-resistant with intravenous immunoglobulin after the second recurrence, eventually leading to graft loss after 1 year.

5.4.2.5 Treatment survival

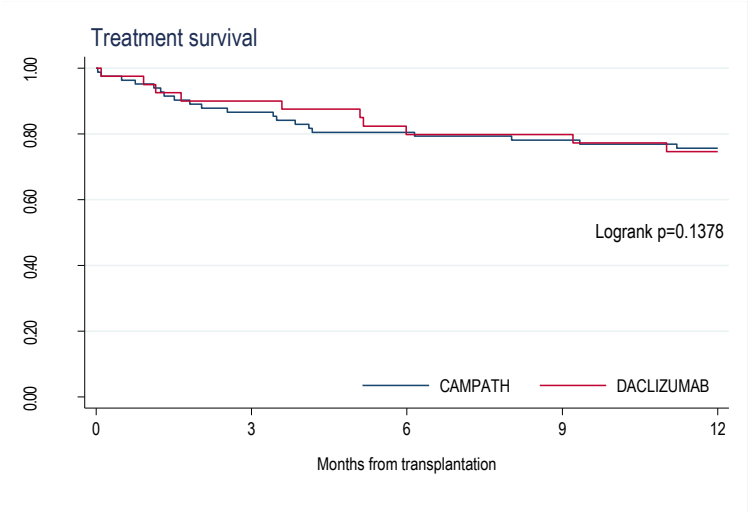

Figure 5-6 CamTac: Treatment survival

Analysis of the treatment survival on a per-protocol (which is heavily influenced by rejection-free survival because rejection episodes are the most important reason for failure of per-protocol persistence) showed no significant difference between the two arms (patient survival with a

functioning graft at 1 year in Campath arm patients remaining steroid-free on tacrolimus monotherapy at 1 year was 75.6%, and in the daclizumab arm, the comparable proportion of patients remaining steroid-free on tacrolimus/MMF therapy was 74.6%, p=0.1378 by log-rank test).

5.5 Summary

This Campath/tacrolimus monotherapy regimen has been shown to have good outcomes compared with an induction-free regimen using tacrolimus, MMF and steroids, with similar graft and patient survival, and a lower rejection rate at 6 months although not significantly so at 1 year.

We have now shown that this regimen produces equivalent, excellent graft and patient survival at 1 year compared with a more directly comparable regimen without long-term steroid exposure but with daclizumab induction therapy and MMF maintenance.

In common with most trials of Campath induction, we demonstrated a low incidence of acute rejection in the first 6 months associated with the use of Campath. Unlike some of the reported studies, our cohort did not suffer a significant level of "catch-up" acute rejection episodes beyond 6 months although the difference in rejection rates between the two arms did not reach statistical significance at 6 or 12 months. We did not observe the increased risk of CMV disease or antibody-mediated rejection that has been speculated to be associated with the use of Campath induction therapy.

Our trial was conservatively powered to detect a non-inferiority in efficacy, defined as a 10% non-inferiority margin in survival with a functioning graft at 1 year, so we were not able to exclude the possibility of smaller differences, and the single-centre nature of the study may limit its applicability although the similarity of our outcomes to those reported with similar regimens in other centres suggests that these good short-term results do not simply reflect local practice.

In the absence of stratification for sensitised or regrafted recipients, our study populations were not balanced for sensitisation against human leukocyte antigens, but this did not impact on the outcome measures reported here.

This RCT confirmed the short-term efficacy of Campath induction and tacrolimus monotherapy regimen and I will discuss the medium term outcomes of this regime in the next chapter, and the risk associated with this regimen in Chapter 11.

Note:

Part of the data from this chapter has been used in previously in my MSc Medical Statistics thesis titled "*3 year results of a randomized controlled trial of Campath and low dose tacrolimus monotherapy compared with daclizumab, tacrolimus and mycophenolate mofetil and best measures to model long term renal transplant function*" (124) with London School of Hygiene and Tropical Medicine, University of London. Complete data was published as a research paper *"Kidney transplantation with minimized maintenance: alemtuzumab induction with tacrolimus monotherapy--an open label, randomized trial* " in 2011 (111)

6 Medium-term outcome with Campath induction and tacrolimus monotherapy

6.1 Introduction

Short-term data from the literature and our study showing the results of using this minimalistic regimen with Campath induction and tacrolimus monotherapy maintenance were encouraging, showing similar allograft and patient survival at 1 year with a suggestion of a lower risk of rejection in the Campath group. However, there is a lack of long-term data regarding safety and efficacy of Campath induction, especially in combination with tacrolimus monotherapy.

There was only one three-year report by the Pittsburgh group found in the literature describing their experience with a similar regimen, but with tacrolimus weaning. Their group had attempted tacrolimus weaning following Campath induction at 100 days following transplantation, in hope of this allowing immune engagement to promote graft tolerance (71, 72) and avoiding early post-transplant immunosuppression and late toxicity as a result. Their study showed that weaning tacrolimus was associated with an increased risk and severity of rejection, as well as graft loss. This study confirmed the importance of long-term tacrolimus maintenance following Campath, although it did not address the question of late tacrolimus-related complications. Furthermore, there was concern that Campath induction only prevents rejection after early transplantation by inducing lymphopenia and that it may associate with an excess risk of late rejection (125), resulting in poorer long-term outcomes.

Our centre has the advantage that we actively follow up our patients after transplantation in our unit and this produces comprehensive data on long-term follow-up in our patients; in this chapter, I am going to examine the medium-term results on the group of patients who received our minimalist regimen. I will concentrate on examining the incidence and risk of late rejection as well as potential complications associated with long-term tacrolimus exposure.

6.2 Patients and methods

6.2.1 Patients

All patients receiving only a kidney transplant from a living donor or deceased donor between November 2002 and December 2006 were included in this retrospective cohort study, to provide a minimum of 5-year follow-up in all patients.

The study group consists of 146 patients who are receiving minimalistic Campath induction with a tacrolimus monotherapy maintenance protocol. A cohort of 148 patients who transplanted from November 2002 received the daclizumab induction; the tacrolimus and mycophenolate mofetil maintenance protocol acted as the control group.

All participants were followed up in the transplant clinics in the Imperial College Kidney and Transplant Institute.

Demographics

Table 6-1 Demographics

		Campath number [%]	daclizumab number [%]
Gender	F	58 [39.7%]	65 [43.9%]
	M	88 [60.3%]	83 [56.1%]
Ethnicity	Afro-Caribbean	20 [13.7%]	19 [12.8%]
	South Asian	42 [28.7%]	32 [21.6%]
	Caucasian	79 [54.1%]	91 [61.5%]
	Others	5 [3.4%]	6 [4.1%]
First transplant	Y	129 [88.4%]	133 [89.9%]
	N	17 [11.6%]	15 [10.1%]
Donor type	Deceased	63 [41.2%]	73 [49.3%]
	Living	83 [56.9%]	75 [50.7%]
Cold ischaemic time	Hours	20.4 + 11.9	16.9 + 12.8
Recipient age	Years	45.9 + 13.0	45.3 + 12.2
Donor age	Years	46.3 + 13.8	46.5 + 14.2
HLA mismatch		3.35 + 1.70	3.16 + 1.67
Follow-up	Months	71.7 + 8.6	86.7 + 16.4

Detailed demographics are listed in Table 6-1.

88/146 [60.3%] patients transplanted in the Campath group are male, and this was comparable to the control group of 56.1%. 42.2% of the cohort in this study is non-Caucasoid; this reflects the underlying ethnic diversity in the catchment area of the Imperial College Kidney and Transplant Centre. Proportions of non-Caucasoid transplanted in the two groups were similar. 13.7%, 28.7% and 3.4% of patients transplanted in the Campath group were Afro-Caribbean, South Asian and from other ethnic backgrounds, compared to 12.8%, 21.6% and 4.1% in the daclizumab group, respectively. 13.1% of transplants in this group are repeat grafts. The proportion of living donor transplantation recipients was higher in the Campath group [56.9% vs 50.7% in the daclizumab group]; this is likely reflecting a change of practice over the follow-up period as living donor transplantation, where feasible, has become a more favourable treatment for patients with end-stage renal failure. In the remaining patients who received a renal transplant from a deceased

donor, cold ischaemic time in the Campath group was 20.6 hours and was slightly higher, compared to the daclizumab-treated group at 16.9 hours. Average recipient and donor ages were indifferent between groups. Average recipient age was 45.8 and 45.3 years in the Campath and daclizumab group respectively; average donor age was 46.3 years in the Campath group and 46.5 years in the daclizumab group. On average, Campath has a 3.4 Human Leucocytes Antigen [HLA] mismatch between recipient and donor, compared to 3.2 in the daclizumab group.

140/146 [95.9%] patients in the Campath group and 141/148 [93.3%] patients in the daclizumab group have been under active follow-up in the Imperial College Kidney and Transplant Centre to date or until patient or graft loss.

6.3 Statistics

The purpose of this study is to examine the medium-term outcomes in the early cohort of patients treated with the minimalistic immunosuppressive protocol.

6.3.1 Primary endpoint:

- Patient survival

6.3.2 Secondary endpoints:

- Patient survival with functioning graft
- Allograft survival after being censored for patient death with function
- Allograft function at 3, 6 and 12 months and annually after transplantation
- Rejection-free survival
- Calcineurin inhibitor toxicity
- Transplant glomerulopathy
- Treatment survival

6.3.3 Outcome definition

Allograft failure was defined as the requirement of dialysis; the date of failure was the date dialysis commenced.

Allograft function was assessed using serum creatinine and MDRD eGFR.

Allograft rejections, calcineurin inhibitor toxicity and transplant glomerulopathy were all biopsy-proven and classified by the Banff 2007 criteria.

6.3.4 Statistics analysis plan

This study was examined as the intention to treat analysis.

The Logrank method was used in comparison, in survival, between the 2 groups, under an assumption that the underlying risk to failure and difference in hazard were constant. The Weibull parametric model was used in cases where this assumption is violated and where the underlying risk of failure changes with time. The Cox semi-parametric model was used if the assumption of constant difference in hazard was violated.

The Kaplan Meier method was used to estimate cumulative survival over time. Patient survival, allograft survival and freedom from rejection, CNI toxicity and transplant glomerulopathy were examined using the same method.

Censoring for loss of follow-up or death with a functioning graft was assumed to be non-informative.

A mixed-effect model was used to estimate the difference in function at the baseline and difference in trajectory between groups on allograft function, as well as accounting for repeat measurements from the same patient over time. The point estimate on numeric outcomes on allograft function at 6 months, 1, 2, 3, 4 and 5 years will be tested using a Student's t test.

All statistical tests were performed as a two-sided test and were deemed to be statistically significant if the p value was <0.05.

Statistics tests were performed using Stata 12.1 (StataCorp, Texas).

6.4 Results

6.4.1 Patient survival

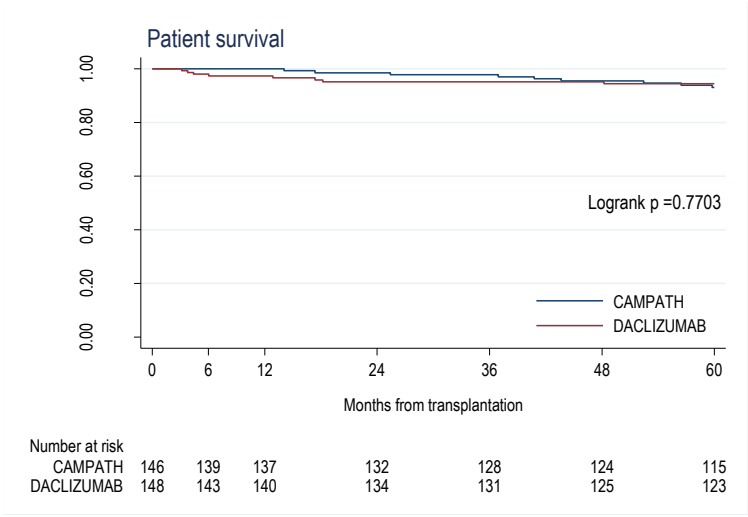

Figure 6-1 Five-year cumulative patient survival

Figure 6-1 (above) shows the cumulative patient survival between the two groups.

There were 9 deaths in the Campath group during the 5-year follow-up. Malignancy accounted for 4/9 deaths. There was a metastatic gastric carcinoma at 25.4 months, one patient died from metastatic pancreas carcinoma at 40.8 months, one patient died from angiosacroma at 36.9 months and one patient died from recurrent renal cell carcinoma at 56.4 months. There were 2 cardiac deaths at 14.1 and 44 months, 1 patient died as a result of pneumonia complicated with encapsulating sclerosing peritonitis at 17.4 months, 1 patient was found dead at home at 52.4 months (the cause of death was presumed to be cardiac-related) and 1 patient died from complications with idiopathic thrombocytopenic purpura at 59.7 months. The results at 1-, 3- and 5-year cumulative survival were 100%, 97.7% and 93.1% in the Campath group, respectively.

Medium-term patient survival was similar in the daclizumab group, and cumulative patient survival was 97.3%, 95.2% and 94.4% at 1, 3 and 5 years, respectively. There were 8 deaths recorded in the daclizumab group. 4 deaths were sepsis-related. 1 patient died from hemophagocytosis at 4.4 months, which was associated with disseminated cytomegalovirus (CMV) disease after the cessation of CMV prophylaxis after a D+/R- live donor transplant. 1 patient died from lung cancer at 12.9

months. 1 died from encapsulating sclerosing peritonitis at 17.4 months and one patient was found dead at home at 3.9 months. There was no statistically significant difference in survival between the groups [p=0.7703, Logrank].

6.4.2 Allograft survival

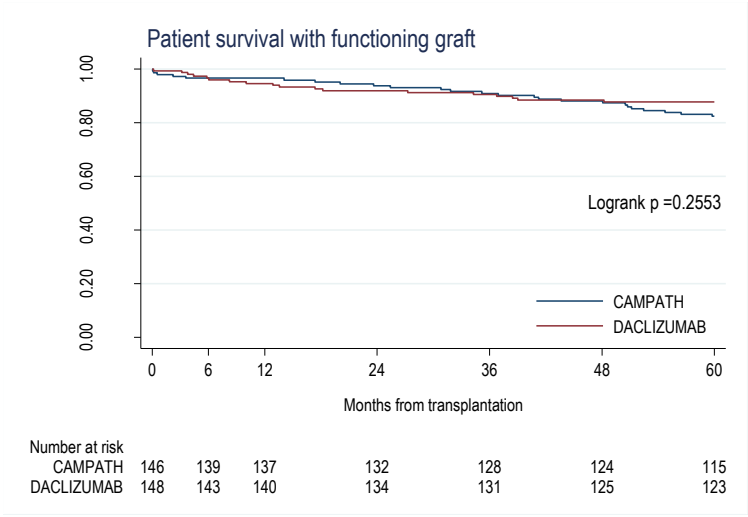

Figure 6-2 Five-year cumulative patient survival with functioning graft

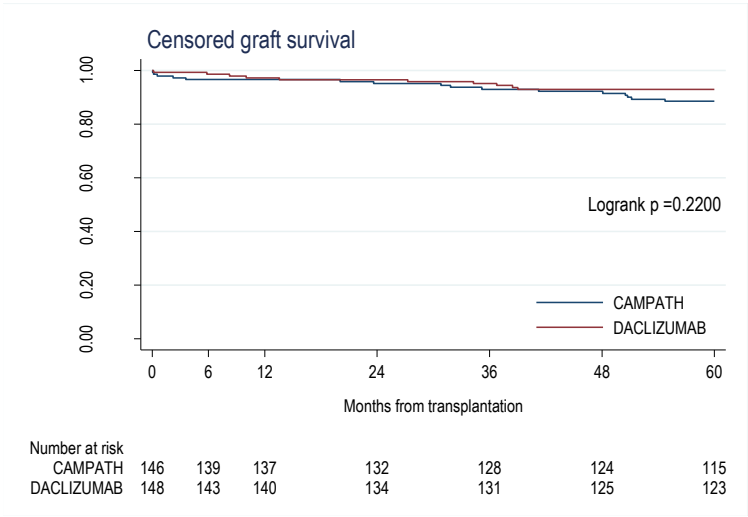

Figure 6-3 Five-year cumulative censored graft survival

Figure 6-2 and Figure 6-3 (above) show long-term patient survival with functioning graft and allograft survival after censoring with death with a functioning graft.

There were 25 graft losses in the Campath group during the 5-year follow-up. 9 grafts were lost as a result of patient death. One graft was lost due to renal vein thrombosis in a patient with positive pro-coagulant soon after transplantation, two were due to technical failure as a result of post-

operation haemorrhage, and one graft from an elderly donor with long cold ischaemia had never fully functioned – repeat biopsies showed scarring and absence of rejection. Rejection accounts for 11 cases of graft loss: 2/11 were due to transplant glomerulopathy, 4 were due to rejection resistance to treatment and 5 grafts were lost due to a rejection result of non-compliance.

Cumulative patient survivals with a function graft in the Campath group were 96.6%, 93.0% and 82.4% at 1, 3 and 5 years, respectively. Cumulative allograft survivals after being censored for patient death with a functioning graft were 96.6%, 93.0% and 88.5% at 1, 3 and 5 years, respectively.

17 graft losses were reported in the dadizumab group. Eight graft losses were a result of patient death in the dadizumab group. 6 grafts were lost as a result of rejection, one loss was due to transplant glomerulopathy, 1 was due to resistance rejection and 4 were related to non-compliance. One graft was lost as a result of transplant renal artery thrombosis soon after surgery in a patient with significant pro-coagulant. One graft was lost at 10 months after transplantation with late presented graft pyelonephritis associated with the ileal conduit and one graft was lost due to transplant renal artery stenosis.

Cumulative patient survivals with a function graft in this group were 94.6%, 90.5% and 87.7% at 1, 3 and 5 years, respectively. Cumulative allograft survivals after being censored for patient death with a functioning graft were 97.3%, 95.1% and 92.9% at 1, 3 and 5 years, respectively.

There were no statistically significant differences between the two groups in patient survival with functioning graft [p=0.2553, Logrank test] and allograft survivals after being censored for patient death with a functioning graft [p=0.2200, Logrank test].

6.4.3 Rejection

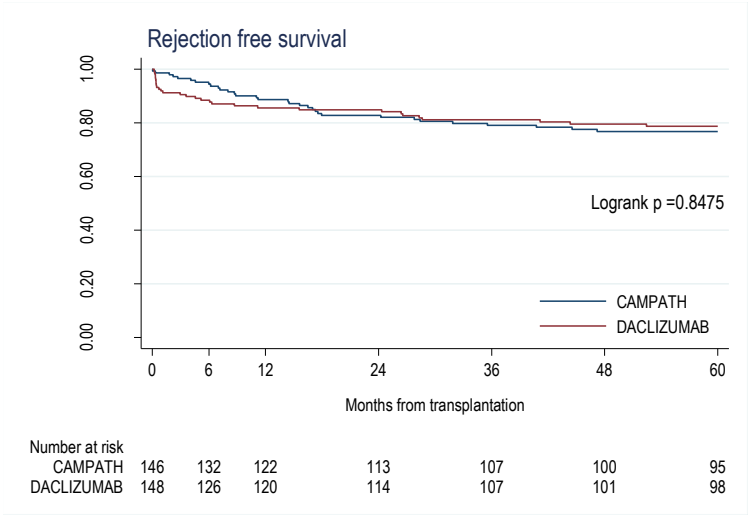

Figure 6-4 Five-year rejection-free survival

Cumulative rejection-free survival in the Campath group was 94.4%, 88.6%, 79.1% and 76.8% at 6 months, 1, 3 and 5 years after transplantation, respectively. 33 patients experienced rejection in this group, of which 25/33 [75.8%] were cell-mediated and the remaining 24% were antibody-mediated rejection.

Proportions of patients experiencing rejection were similar in the daclizumab group. 0.5-, 1-, 3- and 5-year cumulative rejection-free survival in this group was 88.4%, 85.6%, 81.1% and 78.7%, respectively. 23/31 rejections observed were cell-mediated and the remaining 25.8% were antibody-mediated rejection.

As demonstrated in Figure 6-4 above, the risk of rejection is highest in the first 12 months after transplantation. Therefore, assessment on difference of rejection between groups ought to be adjusted for time. The time-adjusted model did not show the Campath group experiencing a significant difference in rejection risk [HR 1.05; 95% CI 0.64, 1.73; p=0.837, Weibull regression model].

Furthermore, there was little evidence to support a difference in the risk of late rejection in the Campath group. After correcting for risk of rejection in the first year, adjusted 5-year cumulative rejection-free survival was 86.7% and 92.0% in the Campath and daclizumab groups. A time-adjusted

risk model did not suggest an increased risk of late rejection [HR 1.77, 95%CI 0.78, 4.01; p=0.169, Cox model].

6.4.4 Allograft function

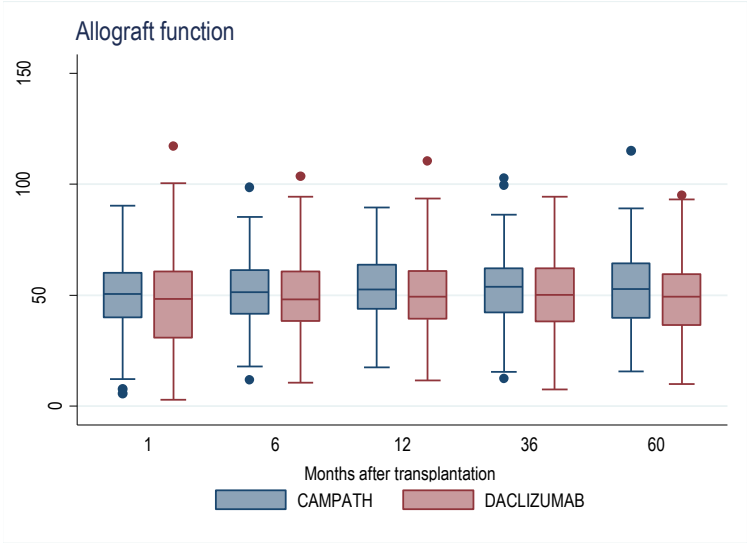

Figure 6-5 Long-term allograft function [MDRD eGFR]

Figure 6-5 (above) shows allograft function in the two groups over 5 years. MDRD eGFR in the Campath group was 51.7+15.0, 53.7+14.7, 52.7+15.7 and 52.8+17.6 ml/min/1.73m2 at 6 months, 1, 3 and 5 years after transplantation, respectively. Allograft function in the daclizumab group at a similar time point was 48.9+16.7, 50.5+16.7, 49.9+16.7 and 49.3+16.9 ml/min/1.73m2. There were no statistically significant differences in function at all time points.

A mixed-effect model was used to estimate the trend of function over time and to examine whether there would be a difference in trends of function after transplantation. The model confirmed that there was no overall statistically significant difference in function between groups [Difference -3.2ml/min/1.73m2, 95%CI -6.8, 0.4; p=0.080]. Data did not suggest any significant changes in function over time in either group. The model estimates non-significant changes of eGFR of -0.002ml/min/1.73m2 per month [95%CI -0.05, 0.05, p=0.925] in the Campath group and 0.002 ml/min/1.73m2 per month [95%CI -0.05, 0.05, p=0.929] in the daclizumab group. Thus, there was no significant difference in trajectories between the two groups [p=0.897].

6.4.5 Calcineuin inhibitor toxicity

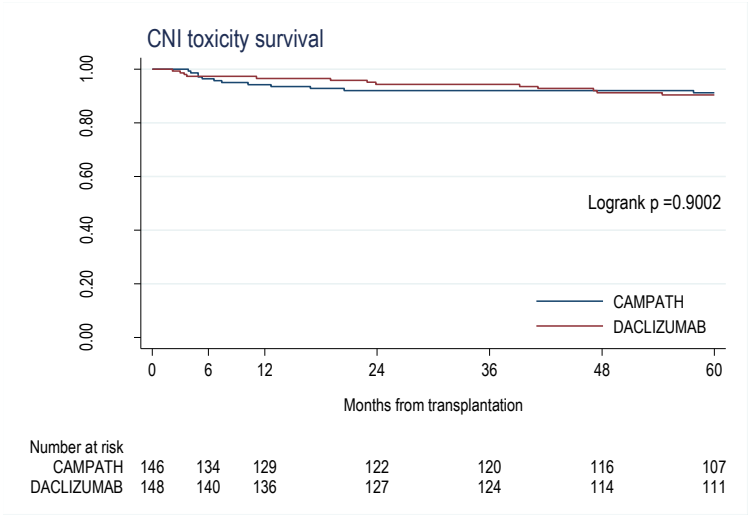

Figure 6-6 Five-year Calcineurin inhibitor toxicity-free survival

CNI toxicity-free survival between the two groups in the long term is shown in Figure 6-6 above. CNI toxicity remained rare in both groups. Although it was hoped that a lower, initial dose of tacrolimus in the Campath group might translate into a lower incidence of CNI toxicity in the long term, albeit the incidence of CNI toxicity remains low, the data did not demonstrate a significant difference between the groups [p=0.9002, Logrank].

Cumulative CNI toxicity-free survival in the Campath group was 94.3%, 92.0% and 91.2% at 1, 3 and 5 years, respectively, compared to 96.6%, 94.4% and 90.4% in the daclizumab group.

6.4.6 Transplant Glomerulopathy

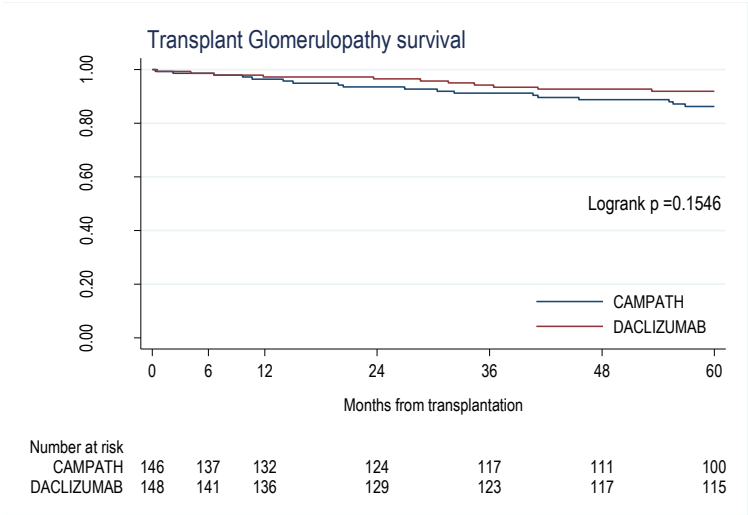

Figure 6-7 Five-year transplant glomerulopathy-free survival

Transplant glomerulopathy was diagnosed on a transplant biopsy. In total, 18 cases of transplant glomerulopathy were diagnosed in the Campath group, of which 8 cases had been diagnosed with rejection prior to biopsy evidence of transplant glomerulopathy. 11 cases of transplant glomerulopathy were diagnosed in the dadizumab group, and 5/11 cases had previous rejection.

Overall allograft survival was poor after diagnosed transplant glomerulopathy despite escalation of treatment. 3 [16.7%] of 18 cases of TG in the Campath group subsequently lost their graft, whereas 4 [36.4%] cases in the daclizumab-treated graft failed.

Overall cumulative freedom from TG in the Campath group was 96.4%, 91.2% and 86.3%, respectively, and was 97.2%, 94.2% and 91.9% in the daclizumab group over 1, 3 and 5 years. There was no evidence of significant difference between groups [Figure 6-7; p=0.1546, Logrank].

6.4.7 Treatment survival

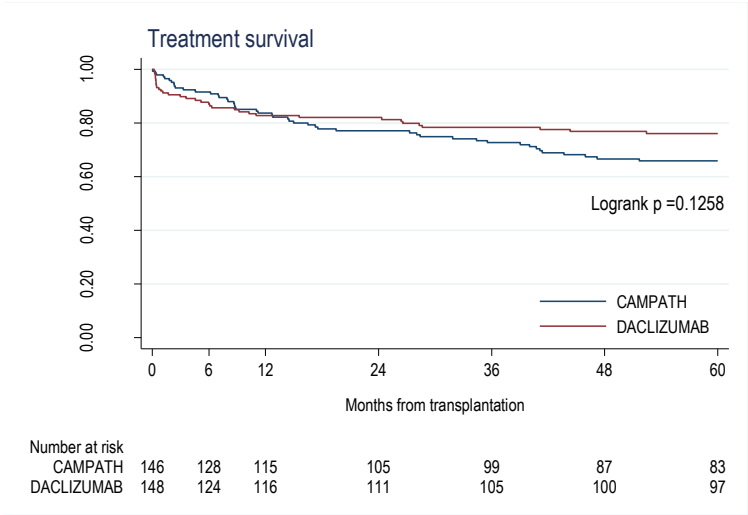

Figure 6-8 Five-year treatment survival

Treatment survival was defined as patients remaining on tacrolimus monotherapy in the Campath group and being steroid-free in the dadizumab group.

Data suggested that 83.6%, 72.6% and 65.8% of patients in the Campath group remained on tacrolimus monotherapy at 1, 3 and 5 years after transplantation, respectively.

Rejection was the commonest reason for treatment failure; 33 [70.2%] patients in the Campath group were started on steroids and MMF as a result. MMF was added in 5 [10.6%] patients because

of transplant glomerulopathy, and 3 [6.4%] treatment failures were a result of cases of CNI toxicity, in which MMF was added to facilitate a large reduction in tacrolimus. 5 [10.6%] cases had MMF added because of biopsy features of significant ischemia and interstitial fibrosis, and it was decided that the tacrolimus burden be reduced to improve allograft function. 1 [2.1%] case had MMF started for recurrent glomerulonephritis.

6.4.8 New Onset Diabetes after Transplantation [NODAT]

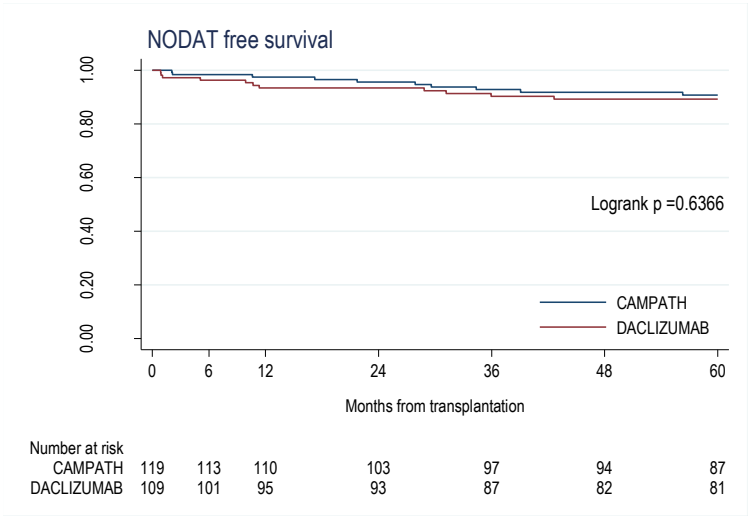

Figure 6-9 Five-year NODAT-free survival

The incidence of new onset diabetes after transplantation [NODAT], defined as the de novo need for oral hypoglycaemics or insulin, was shown in Figure 6-9 above. 119 patients in the Campath group were not diabetic before transplantation compared to 109 in the daclizumab group. Cumulative hazards of NODAT were comparable between the two groups [p=0.6366, Logrank].

Cumulative NODAT-free survival in the Campath was 97.4%, 92.8% and 90.8% at 1, 3 and 5 years, respectively, compared to 93.3%, 90.3% and 89.2% in the daclizumab group.

6.5 Summary

Short-term results using this minimalistic regimen with Campath induction and tacrolimus monotherapy maintenance were encouraging, showing similar allograft and patient survival at 1 year with a suggestion of a lower risk of rejection in the Campath group. I demonstrated this data in our pilot study [Chapter 3] and in our randomised controlled study [Chapter 5]. Data from the

literature also supports the use of Campath as an induction agent in renal transplantation (30); however, outcomes from these reports are limited to short-term results. There was a lack of medium or long-term data regarding safety and efficacy of Campath induction, especially in combination with tacrolimus monotherapy. Therefore, I studied the medium-term outcome in patients who reached at least 5-year follow-up in our centre to assess the safety and efficacy of Campath induction in the medium-long term.

My data showed 1-, 3- and 5-year cumulative survival of 100%, 97.7% and 93.1% in the Campath group, respectively, and the results were similar to the daclizumab group, wherein cumulative patient survival was 97.3%, 95.2% and 94.4% at 1, 3 and 5 years.

Cumulative patient survivals with a functioning graft in the Campath group were 96.6%, 93.0% and 82.4% at 1, 3 and 5 years, respectively, and were 96.6%, 93.0% and 88.5% at 1, 3 and 5 years after being censored for patient death. There was no statistically significant difference compared to the daclizumab cohort: the cumulative patient survivals with a functioning graft in the daclizumab cohort were 94.6%, 90.5% and 87.7% at 1, 3 and 5 years, respectively, with cumulative censored allograft survivals of 97.3%, 95.1% and 92.9% at 1, 3 and 5 years. The commonest cause of graft loss in the Campath group was rejection, accounting for 11 cases in total, and the causes of graft loss in the whole Campath cohort will be discussed in detail in Chapter 8.

The risk of rejection was lower in the first 6 months in the Campath group [5.6% vs 11.6%], and cumulative risk of rejection converges between the two groups by 12 months [11.4% vs 14.4%]. There was no evidence suggesting a higher risk of late rejection in the Campath group. The nature of rejection was also similar: antibody-mediated rejection accounted for 24% of cases of rejection in the Campath group and 25.8% in the daclizumab cohort. There was also no evidence of significant difference in the risk of developing transplant glomerulopathy between groups. Overall cumulative freedom from transplant glomerulopathy in the Campath group was 96.4%, 91.2% and 86.3% and was 97.2%, 94.2% and 91.9% in the daclizumab group over 1, 3 and 5 years, respectively.

Estimated GFR was stable in both groups over time. It was estimated that eGFR of 53.7+14.7 and 52.8+17.6 ml/min/1.73m2 at 1 and 5 years in the Campath group would be slightly higher compared to the daclizumab group – 50.5+16.7 and 49.3+16.9 ml/min/1.73m2 – although it did not demonstrate a statistically significant difference.

We hoped that the lower-dose tacrolimus exposure in the first year in the Campath group might translate into a substantial reduction in the already-low incidence of CNI toxicity. The incidence of Calcineurin inhibitor toxicity remains low in both groups in the long term in our unit [8.8% vs 9.6% at 5 years]; this is likely a result of meticulous monitoring and careful dose adjustment.

Finally, in the medium term, the risk of new onset diabetes after transplantation remained low with the spare use of steroids: my data showed that 90.8% and 89.2% of non-diabetic patients remained NODAT-free at 5 years.

This term study demonstrated that this minimalistic regimen with Campath induction and medium-dose tacrolimus is efficacious with a similar medium-term outcome compared to a conventional daclizumab regimen with dual maintenance. Furthermore, 65.8% of patients in the Campath group remained on tacrolimus monotherapy at 5 years after transplantation.

I will be describing the risk of infection and malignancy associated with this regimen in chapter 11.3 and chapter 11.4.

7 Weight-adjusted dose-finding study

7.1 Introduction

The use of Campath induction is becoming more popular, especially with those recipients who were immunologically sensitised (126). However, the optimal dosage of Campath used was not well studied (52). The current dosage of Campath varies between centres and these were described in detail later in this chapter.

It was also unclear as to whether the dosage should be adjusted for bodyweight of the recipients for optimal risk and immunosuppressive benefits. There were little data investigating the effect of dosage of Campath on the duration of lymphopenia and whether this temporal lymphopenia was associated with any significant risk to recipients. The impact of duration of lymphophenia and its impact on infective risk were studied in chapter 10 and chapter 11.3.

Calne's team administered a total dose of 40mg of Campath to 31 deceased donor renal transplant recipients who had had no previous transplant, at 20mg each for two consecutive days immediately after transplantation together with a cyclosporine monotherapy maintenance regimen [Target range 100-150ng/ml] in his early study (35, 127) in 1999. Both short and long term outcomes were encouraging.

The interest in using Campath in renal transplantation grew following Kirk et al. who published their findings in an attempt to use Campath to induce transplant tolerance in 2003 (46). Kirk's group attempted to induce transplant tolerance in seven patients using 3 to 4 doses of 0.3mg/kg Campath in transplantation without the use of any maintenance immunosuppressant; this was a substantially higher dose compared to previous reports. All patients experienced rejection within the first 3-4 weeks after rejection despite T cell depletion, and all rejections were reversed by reintroducing a maintenance immunosuppressant. Although this study failed to demonstrate a significant tolerogenic capacity with Campath, it did confirm that high-dose Campath does not prevent renal allograft rejection.

The Kirk study also laid the foundation that long-term maintenance in renal transplantation is necessary after Campath induction, and further study examined the effect of combining Campath with different immunosuppressants.

Knechtle et al. reported their experience of using Campath induction and Sirolimus induction in 2003 (52, 55). Knechtle's group treated 126 patients with a total of 40mg Campath induction in two divided doses over 2 days after operation and followed up with Sirolimus monotherapy maintenance. The dose of Campath was not corrected with weight; The short data from this study suggested that a similar efficacy compared to the historic control cohort. Although the absolute incidence was not reported explicitly, this report suggested that patients receiving 40mg of Campath have a similar risk of infection compared to other induction agents. Moreover, there were no data suggesting any difference in the risk of malignancy in the short term. This regimen is feasible for renal transplantation, despite concerns with the Sirolimus-resistant characteristics of residual lymphocytes after 40mg of Campath (77).

To date, there have been 7 randomised controlled studies investigating the use of Campath in renal transplantation. Each study used different dosages of Campath as well as different types of immunosuppressant maintenance.

A small, early randomised controlled study by Ciancio et al. published in 2007 (57) compared 30 patients receiving Campath induction with 30 patients receiving thymoglobulin and 30 receiving daclizumab induction. All patients received tacrolimus, mycophenolate mofetil and steroid maintenance. Patients in the Campath group received 0.6mg/kg of Campath in total in two divided doses; this is equivalent to approximately 40mg in a standard 70kg patient. The majority of patients recruited in this study are Caucasian, Hispanics and Afro-Americans. Clinical outcomes were similar between the three groups.

Vathsala et al. presented their results from Singapore which consisted of mainly oriental patients (64): 20 patients were given 40mg of Campath and cyclosporine monotherapy compared to 10 patients receiving conventional triple therapy – cyclosporine, azathioprine and prednisolone

maintenance. Intuitively, one would imagine the body habitus in oriental persons to be significantly smaller compared to the Western population. This suggested that this group of patients was exposed to a much higher dose of Campath compared to previous studies. The rate of rejection was similar to previous Western population studies, albeit this study is grossly underpowered.

A similar but larger study published by Margreiter et al. (65), using a similar steroid maintenance regimen that compared 65 patients receiving 40mg of Campath and tacrolimus monotherapy to 66 patients receiving conventional triple therapy, consisted of no monoclonal antibody induction and maintenance immunosuppressants with tacrolimus, mycophenolate mofetil and steroids. Short-term 1-year patient and allograft survival were similar between the groups. There was a suggestion that the incidence of rejection is lower in the Campath-treated group, likely because of the addition of induction therapy at transplantation.

Our group presented a lower dose of 30mg of Campath which is feasible and safe in renal transplantation (111). In our study, we compared a 30mg induction of Campath and tacrolimus monotherapy to daclizumab induction and tacrolimus with mycophenolate mofetil maintenance. Our study demonstrated that this lower dose of Campath delivers a similar outcome to conventional therapy and that a higher dose of Campath was not necessary.

The INTAC study group have presented the largest study to date, using 30mg of Campath in renal transplantation (123). 501 patients were included in this multicentre study; patients were randomised to receive either 30mg of Campath, basiliximab or rabbit antithymocyte globulin [rATG] depending on immunological risk. This study showed that Campath induction is significantly better in preventing rejection in 6, 12 and 36 months after transplantation in those with low immunological risk.

Results from these randomised controlled studies demonstrated that there is no consensus on what dosage of Campath should be given. It ranges from a body weight-adjusted dosage of up to 1.2mg/kg to a standard dosing of 30 to 60mg in total. There are no clear differences in transplant outcomes between these studies.

Infection is a major concern because of the strong lymphopenic power associated with Campath.

In transplantation literature, the emphases are often on efficacy outcomes and it is only recently that adverse events associated with immunosuppression started to be reported more explicitly. So far, the infection profile reported from the early cohort reports and randomised controlled study was encouraging. The 5-year report from the Calne team (63) suggested a slightly higher risk of CMV disease and herpes zoster infection in the Campath-treated group. There were no notable differences in other infections between groups. Recipient weights were not reported in this study and it was unclear as to whether these recipients carried a higher infection risk. Reports from Knechtle et al. did not detect a difference in infection risk with 40mg of Campath (52).

An early study by Ciancio et al. demonstrated that the incidence of severe infection requiring hospitalisation is similar in patients receiving 0.6mg/kg of Campath to those receiving daclizumab and Thymoglobulin. Their group reported that 27% of patients receiving Campath had at least one hospital admission with infective complications after transplantation, and data did not suggest an increased risk of infection with Campath. Despite the lymphopenia, viral infections were uncommon (57). This study is underpowered and did not describe the nature and incidence of infection in the groups.

In the Vathsala study (64), it mainly consisted of oriental persons receiving a large dose of Campath per body weight. The report suggested that 40% of Campath-treated patients experienced at least 1 moderate or severe infection, although the nature or severity of the infection was not described in detail. Data did not suggest an increased risk of viral infection.

The Margreiter study (65) presented some interesting data regarding infection. Despite the lower proportion of recipients with negative CMV status in the Campath group [34% vs 42%] and CMV prophylaxis to high-risk recipients, 18 cases of CMV infection were reported in the Campath group and were significantly higher compared to 8 cases in the control group [Rate Ratio 2.28]. Although the infection in the Campath group is less severe and there was no tissue-invasive CMV disease in

the Campath group, whereas 3/8 cases in the control group were invasive cases. Incidences of bacterial and fungal infection were similar between the two groups.

All these studies were based on a high dose of 40mg of Campath induction, showing transplant outcomes were satisfactory.

The effect of Campath in addition to concurrent immunosuppressants is best demonstrated by this large study reported from Pittsburgh aiming to address the incidence of opportunistic infection with Campath (99). The group reported an incidence of infection in 547 patients receiving Campath; this group consists of 65% of patients receiving Campath for organ transplant induction, and as maintenance or rescue therapy in the remaining group. Data suggested that patients receiving Campath on repeat dosing as recue therapy have a crude 3.5-fold increase in the risk of infection compared to those receiving Campath as induction agents. Although the data presented in this study did not measure the absolute changes in the rate of infection, it does highlight that patients who were already heavily immunosuppressed, particularly with concurrent use of steroids, were more likely to have an increased risk of infection.

The effect of repeat Campath dosing and infection in transplantation was best demonstrated by the report published by the Minnesota group, who previously used Campath as maintenance in their pancreas transplant programme wherein some patients received up to 30mg per month. Their group reported their experience with fungal infection with repeat Campath in 2005 (96). 121 pancreas transplant recipients were included in their study, 56 as part of transplant induction and 65 as part of conversion from calcineurin inhibitors. 6.6% developed an invasive fungal infection and the incidence of fungal infection was substantially higher in those receiving Campath as conversion therapy [9.2% vs 3.6%]. 3 patients died during ongoing treatment of their fungal infection. This raised the alarm that Campath doses should be limited to avoid the unacceptable risk of fatal fungal infection associated with higher total dosage.

Currently, most centres use either a single dose or two 30mg doses. Friend et al. argue that current 30mg doses were chosen mainly because Campath was made in 30mg vials. Therefore, it was more

convenient to use 30mg dosing (128). A recent study published by Margreiter et al. attempted to answer this question (129). In this small randomised study, 66 patients received two 20mg doses of Campath compared to 65 patients receiving 30mg of Campath. Patient and graft survival at 1 year after transplantation were similar between the groups, and the incidence of allograft rejection at 1 year was higher in the group receiving less Campath [20% vs 6%]; there was no difference in the incidence of infection between groups. This study is the first attempt to address the issue regarding dosage and complications with Campath; results did not suggest a marked difference in immunosuppressant-related complications. However, this study was substantially underpowered and the difference in Campath dosages is perhaps too little to judge the effect.

The aim of this study was to examine the risk and benefit of high and low doses of Campath in renal transplantation. We were fortunate enough that our centre covers a wide area of North West London with a large ethnic diversity, in which patients' sizes vary. In this chapter, we examined whether those receiving a lower dosage of Campath induction per body weight was associated with higher risk of rejection and allograft failure because of inadequate immunosuppression, as well as aiming to address the question of whether the immunosuppressant-related complications are related to a higher dose of Campath.

7.2 Patients and methods

7.2.1 Patients

This is a retrospective study based on data collected on subjects transplanted using the Campath Regime between Nov 2005 and May 2011 for the Imperial College Kidney and Transplant Centre transplant registry. The use of data was authorised by the Imperial College Kidney and Transplant Centre's transplant research group. Patient data were anonymised to secure patient confidentiality.

Demographics

608 patients were included in this study, and the mean weight was 76.1+17.3 kg. In order to study the effect of weight-adjusted Campath dosing, per bodyweight exposure was calculated by dividing the standard dosage of 30mg by a patient's body weight. It estimated that the median Campath

exposure was 0.40 mg/kg, and the cohort was divided into quartiles to maximise its statistical power [<0.35mg/kg, 0.35-0.40mg/kg, 0.40-0.48mg/kg and >0.48mg/kg]. Details of demographics are as follows.

Table 7-1 Demographics – Weight-adjusted dose-finding study

		Below 0.35mg/kg	0.35 to 0.40 mg/kg	0.40 to 0.48 mg/kg	Above 0.48 mg/kg
Gender	Female	35 [21.3%]	37 [27.0%]	63 [38.2%]	80 [56.3%]
	Male	129 [78.7%]	100 [73.0%]	102 [61.8%]	62 [43.7%]
Ethnicity	AFRO-CARIBBEAN	12 [7.3%]	21 [15.3%]	16 [9.7%]	12 [8.5%]
	ASIAN	37 [22.6%]	45 [32.9%]	59 [35.8%]	67 [47.2%]
	CAUCASIAN	108 [65.9%]	60 [43.8%]	80 [48.5%]	50 [35.2%]
	OTHER	7 [4.3%]	11 [8.0%]	10 [6.1%]	13 [9.2%]
Primary transplant	Yes	152 [92.7%]	126 [92.0%]	146 [88.5%]	127 [89.4%]
	No	12 [7.3%]	11 [8.0%]	19 [11.5%]	15 [10.6%]
Donor type	Deceased	82 [50.0%]	64 [46.7%]	91 [55.2%]	87 [61.3%]
	Living	82 [50.0%]	73 [53.3%]	74 [44.9%]	55 [38.7%]
Pre-Tx DSAb	No	148 [90.2%]	120 [87.6%]	160 [97.0%]	130 [91.6%]
	Yes	16 [9.8%]	17 [12.4%]	5 [3.0%]	12 [8.5%]
CIT	Hours	24.4 $\pm$ 8.3	22.9 $\pm$ 7.3	22.8 $\pm$ 7.0	23.7 $\pm$ 8.1
Age	Years	48.4 $\pm$ 11.8	49.7 $\pm$ 14.1	49.9 $\pm$ 14.6	45.1 $\pm$ 15.7
Donor age	Years	47.1 $\pm$ 13.5	47.3 $\pm$ 15.0	47.5 $\pm$ 15.6	46.4 $\pm$ 15.7
HLA MM		3.6 $\pm$ 1.8	3.5 $\pm$ 1.8	3.4 $\pm$ 1.8	3.5 $\pm$ 1.8
Mean follow-up	Months	29.1 $\pm$ 17.1	32.4 $\pm$ 18.1	33.5 $\pm$ 19.1	32.6 $\pm$ 17.8

Female patients are smaller in general and have contributed to a larger proportion in the group exposed to the biggest dose of Campath. Thus, the proportion of female patients falls as the Campath exposure falls and as patient weight increases.

In the lowest Campath-exposed group, there was a large portion of Caucasoid [65.9%], and vice versa, in the larger exposure group.

There were no substantial differences in the number of previous transplants and pre-transplant donor-specific antibody statuses between the 4 groups.

Furthermore, duration of cold ischaemic time, donor and recipient age, HLA mismatch and duration of follow-up were similar between the four groups.

7.2.2 Methods

The purpose of this analysis is to examine the relative benefit and risk on those who received a higher or lower dose of Campath by weight in our cohort.

Campath dosages per body weight were calculated by dividing the Campath dosage received [30mg] by a patient's dialysis dry weight or by the lowest recorded weight in the first 3 months after transplantation if information regarding dry weight was not available.

This cohort was divided into 4 groups following the interquartile ranges according to the Campath dosage received per body weight [<0.35mg/kg, 0.35-0.40mg/kg, 0.40-0.48mg/kg and >0.48mg/kg].

The primary outcome of this study is patient survival at 1, 3 and 5 years.

The secondary outcomes of this study include:

- Allograft survival at 1, 3 and 5 years
- Nature and incidence of acute rejection at 1 year
- Nature and incidence of late rejection after 1 year
- Allograft function at 1, 3, and 5 years
- Effect of lymphocyte repopulation between groups
- Incidence of infection between groups

Allograft failure is defined as the need of a patient requiring returning to long-term dialysis.

All rejections were based on histological diagnosis, and biopsies were performed on clinical grounds and were examined independently by a histolopathologist at the Imperial College and Kidney Transplant Centre.

Allograft functions were assessed using serum creatinine and MDRD eGFR. Data on follow-up creatinine were collected from the data collected via routine biochemistry blood work performed in regular clinical follow-up.

Data on lymphocyte profiles were collected from routine blood work performed in regular clinical follow-up.

Infections were diagnosed based on microbiological diagnosis. Positive urine, bronchiolar lavage, drain fluid, ascites and blood cultures constituted significant episodes of bacterial infection. Cytomegalovirus PCR positivity [>1000 copies/ml] and biopsy-proven BK virus nephropathy [intranuclear inclusion bodies with positive staining for SV40] were considered to be significant markers of viral infections.

7.3 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Patient, allograft and rejection-free survival were presented using the Kaplan Meier plot. Impact on survival from different variables was analysed using the Cox proportional hazard model or the Weibull parametric model to adjust for time scales if appropriate. Censoring was assumed to be non-informative.

Allograft function and lymphocyte data were analysed using a mixed-effect model to account for the nature of repeat measurements over time, as well as allowing analysis on the effects of various factors on trajectories over time. Continuous variables were log-transformed into normal distributed data to improve adherence to model assumptions if needed.

Incidences of infection were presented as the incidence per 100 patient years. The Poisson model was used to analyse rate and rate ratio; alternatively, a negative binomial model is used when the underlying assumption of equal mean and variance with the Poisson model is violated.

7.4 Results

7.4.1 Patient survival

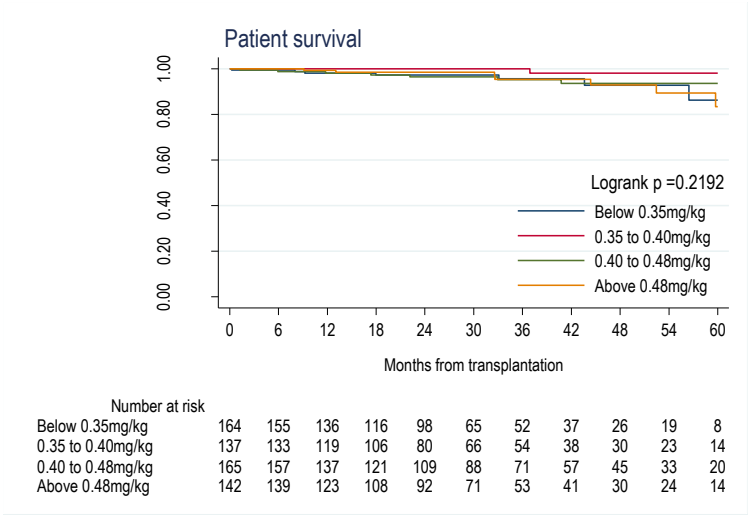

Figure 7-1 Patient survival between 4 dosage groups

Figure 7-1 (above) demonstrates overall patient survival between 4 groups. 1-, 3- and 5-year patient survivals in the 0.35mg/kg group below were 98.1%, 95.6% and 86.2%; 100%, 100% and 98.1% in the 0.35-0.40mg/kg group; 98.1%, 95.2% and 93.6% in the 0.40-0.48mg/kg group; and 99.3%, 95.4% and 83.4% in the 0.48mg/kg group above. There was no statistically significant difference in patient survival between the 4 groups [p=0.2192, Logrank].

22 patients died during follow-up within this cohort, and there was no significant difference in cumulative risk of death between the 4 groups, as discussed earlier. Cardiac death is the commonest cause of death in this cohort despite the proactive pre-transplant cardiac screening programme in the Imperial College Kidney and Transplant Centre; it accounts for 5/22 deaths. Unsurprisingly, a large proportion of cardiac deaths [4/5] were found in the patient group with the largest patients [those receiving <0.35mg/kg of Campath]. There were 3 patients that died as a result of complication of malignancy, 3 died with infectious causes and 3 died with complications of encapsulation sclerosing peritonitis. There was no clear association between cause of death and Campath dosage.

7.4.2 Allograft survival

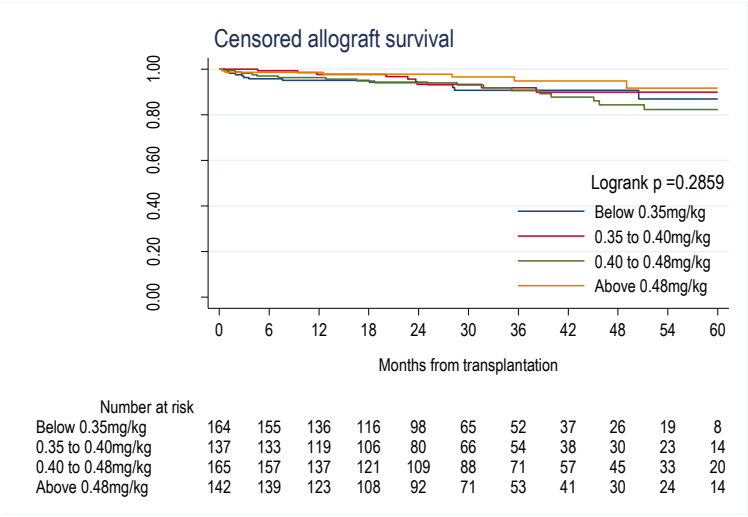

Figure 7-2 Censored allograft survival between 4 dosage groups

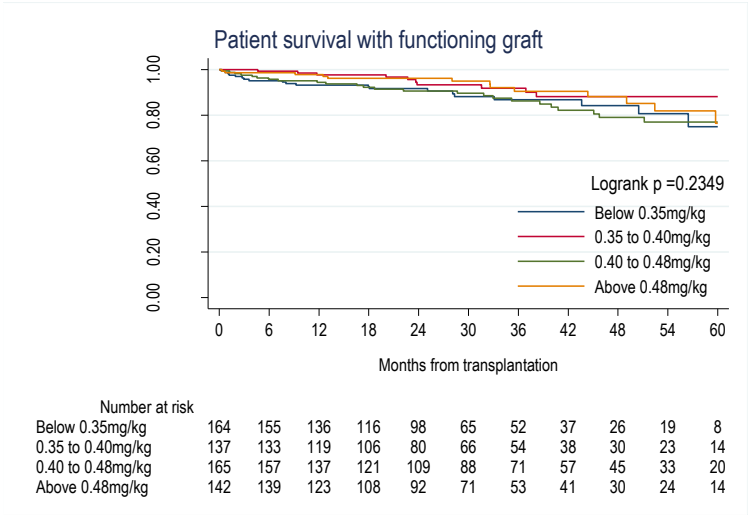

Figure 7-3 Patient survival with functioning graft between 4 dosage groups

Figure 7-2 and Figure 7-3 demonstrate the allograft survival after being censored for death with function and patient survival with a functioning graft over 5 years. 1-, 3- and 5-year patient survivals in the 0.35mg/kg group below were 85.1%, 90.7% and 86.9%; 97.7%, 91.8% and 89.9% in the 0.35-0.40mg/kg group; 94.8%, 90.6% and 82.3% in the 0.40-0.48mg/kg group; and 98.6%, 94.8% and 91.7% in the 0.48mg/kg group above. There was no statistically significant difference in patient survival between the 4 groups [p=0.2859, Logrank].

Patient death is the commonest cause of graft loss, accounting for 22/67 graft losses in this cohort. Despite the powerful immunosuppressive effect with Campath, rejection remains the commonest

cause of graft loss following a patient's death. 21 grafts were lost as a result of rejection, of which 3 were due to patient non-compliance with long-term immunosuppressive therapy. The proportion of graft losses as a result of rejection was between 3.5% and 4.5% between the 4 groups. There were 3 graft losses due to transplant glomerulopathy, one in each group apart from the 0.48mg/kg group above. 5 grafts were lost following withdrawing immunosuppression in cases with overwhelming infection. 2 grafts were lost because of recurrent disease; both cases were due to recurrent focal segmental glomeruloscloerosis. There were no associations between Campath doses and causes of graft loss.

7.4.3 Rejection

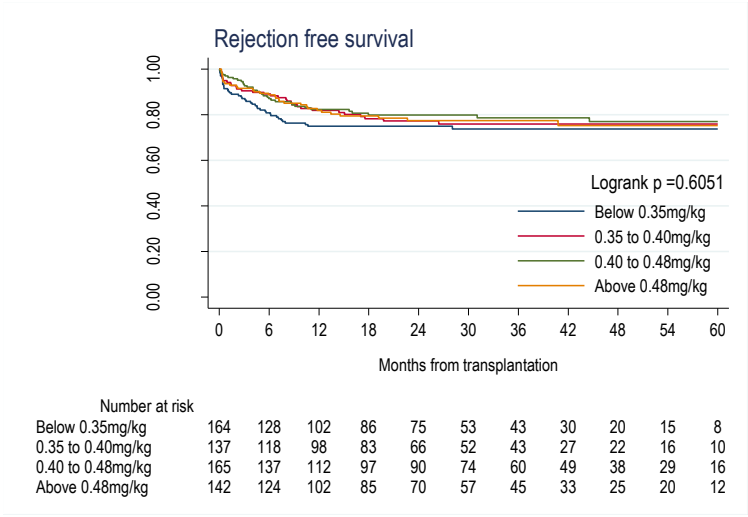

Figure 7-4 Rejection-free between 4 dosage groups

Figure 7-4 (above) shows rejection-free survival between the four groups in the long term. Although 5-year cumulative rejection-free survivals were similar in the 4 groups, it was 73.7%, 76.0%, 77.0% and 75.2%, respectively. The difference in rejection is best observed in the first 12 months post-transplantation: rejection risk appeared to be highest in those receiving less than 0.35mg/kg of Campath induction [28.7% vs 19.9%, 19.5% and 19.9% in the 0.35-0.40mg/kg, 0.40-0.48mg/kg and 0.48mg/kg groups above, respectively].

This is best demonstrated in Figure 7-5 below. It is estimated that those receiving less than 0.35mg/kg of Campath on induction have a 49.8% increase in the risk of rejection within the 12 months after transplantation [HR1.50, 95%CI 1.02, 2.20; p=0.038].

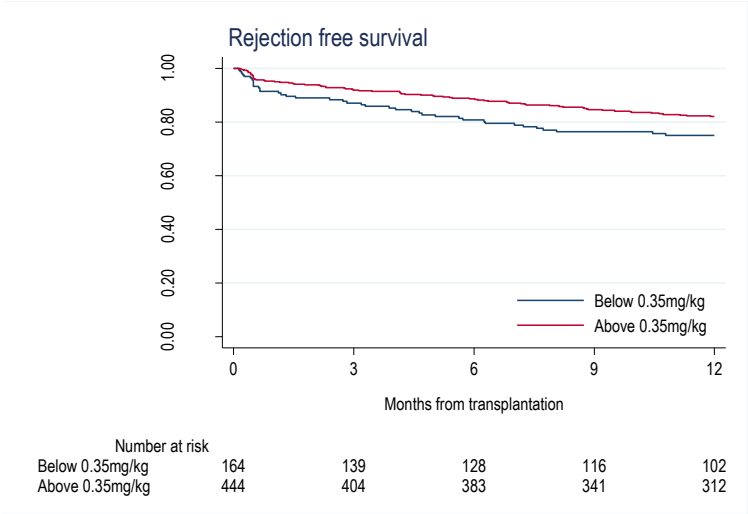

Figure 7-5 12-month rejection-free survival at 0.35mg/kg

There was no evidence suggesting an increased risk of late rejection after 1 year in any group [24 months post-transplantation vs 12-24 months post-transplantation: HR 0.30, 95%CI 0.11, 0.83; p=0.02, Exponential survival model with Lexis expansion] or a difference in the risk of late rejection between 4 groups [p=0.2617, Logrank].

Nature of rejections was similar between all weight groups, albeit those receiving less Campath experienced higher risk and earlier rejection, as demonstrated above. Table 7-2 (below) shows the nature of rejection between the groups. The actuarial rate of rejection was significantly higher in patients receiving less than 0.35mg/kg of Campath. The risk of rejection was increased in both antibody-mediated or T cell-mediated rejections. Antibody-mediated rejection accounted for 16 [40.0%] cases of acute rejection in those receiving less than 0.35mg/kg of Campath, and was similar to the patients receiving above 0.35mg/kg of Campath [37.7%].

Table 7-2 Nature of acute rejection

Group	Cellular	AMR	No rejection
<0.35mg/kg	24 [14.6%]	16 [9.8%]	124 [75.6%]
>0.35mg/kg	48 [10.8%]	29 [6.5%]	367 [82.7%]

7.4.4 Allograft function

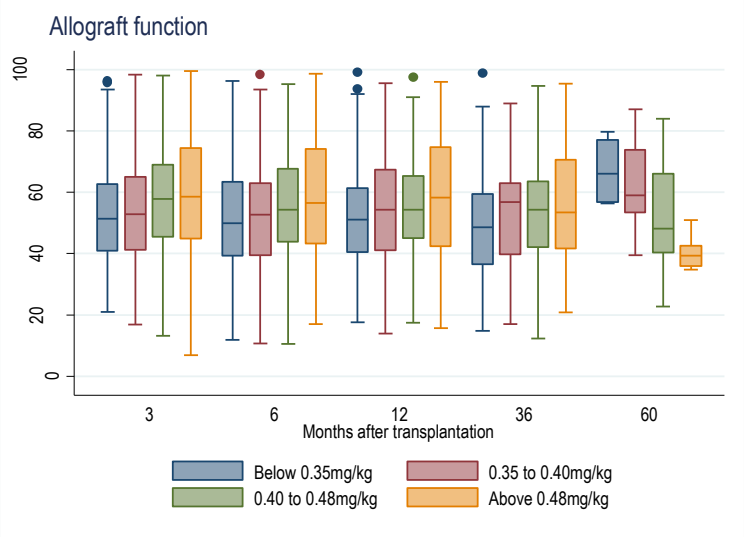

Figure 7-6 Long-term allograft function between 4 dosage groups

Figure 7-6 (above) shows allograft function between the four groups over 5 years of follow-up. Detailed allograft function is listed in Table 7-3 below. At first glance, allograft function in the 0.35mg/kg column appeared to be lowest in the first 6 months after transplantation; however, this result might be physiological as a result of higher creatinine with increased body mass.

Table 7-3 Allograft function between 4 groups [MDRD eGFR ml/min/1.73m2, mean ±SD]

Month	<0.35mg/kg	0.35-0.40mg/kg	0.40-0.48 mg/kg	>0.48mg/kg
6	51.0 ± 16.68	52.9 ± 18.08	56.3 ± 17.85	57.7 ± 18.93
12	51.7 ± 16.31	54.9 ± 18.34	55.6 ± 16.51	57.8 ± 19.54
24	49.8 ± 16.15	54.4 ± 16.34	54.2 ± 16.82	59.1 ± 18.02
36	48.7 ± 17.93	52.5 ± 16.86	53.2 ± 17.44	55.6 ± 18.65
48	47.2 ± 16.11	56.2 ± 20.86	52.3 ± 17.37	52.1 ± 20.47
60	67.0 ± 11.89	62.4 ± 16.07	52.0 ± 19.40	40.5 ± 5.88

Also, we ought to be aware that the estimation of average allograft function in this group is subject to a substantial influence of random variations caused by effects of correlations between repeat measurements from the same patients as well as a smaller number of subjects with a time lapse. Therefore, a mixed-effect model was used to estimate the difference in initial allograft function and in the effect of treatment on changes in function over time [Table 7-3].

Table 7-4 Effect of Campath dosage on allograft function [Mixed-effect model]

		eGFR	95%CI	p
Baseline function	<0.35mg/kg	51.24	48.28, 54.20	
ml/min/1.73m2 at month 0	0.35-0.40mg/kg[1]	2.81	-1.55, 7.17	0.207
	0.40-0.48 mg/kg[1]	5.86	1.69, 10.02	0.006
	>0.48mg/kg[1]	9.26	4.83, 13.69	0.000
Changes in eGFR per month	0.35mg/kg	-0.06	-0.16, 0.04	0.218
ml/min/1.73m2 per month	0.35-0.40mg/kg[1]	0.02	-0.12, 0.16	0.764
	0.40-0.48 mg/kg[1]	-0.01	-0.15, 0.12	0.828
	>0.48mg/kg[1]	-0.11	-0.26, 0.03	0.113

The mixed model suggested that patients who received higher dosages of Campath have a significantly higher eGFR, as predicted, resulting from a lower creatinine and body mass. More importantly, there was no evidence suggesting deterioration in function over time. The model estimated a non-statistically significant change of -0.06ml/min.1.72m2 of eGFR per month [95%CI -0.16, 0.04; p=0.218] in patients receiving 0.35mg/kg of Campath. There was no evidence suggesting that patients who received higher doses of Campath had different trajectories compared to the 0.35mg/kg group.

7.4.5 Transplant Glomerulopathy

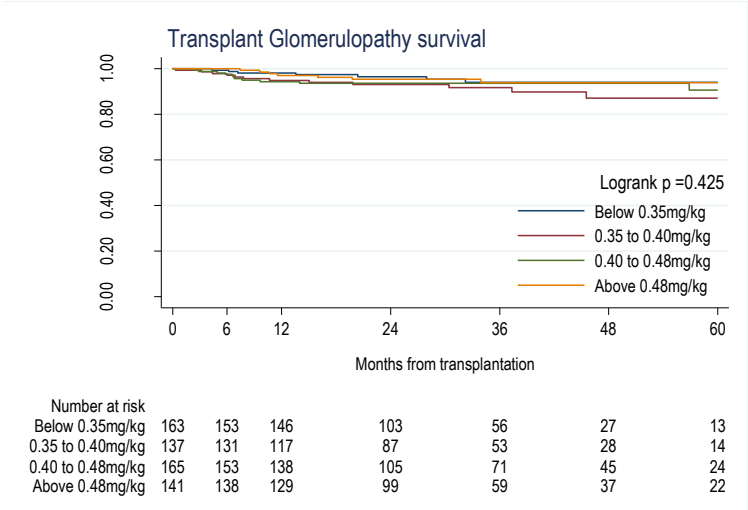

Figure 7-7 Transplant Glomerulopathy-free survival

[1] Difference compared to <0.35mg/kg group

Figure 7-7 (above) shows transplant glomerulopathy-free survival between the four groups. All transplant glomerulopathy was diagnosed with an allograft biopsy in cases with graft dysfunction. Transplant glomerulopathy-free survival was 94%, 87%, 90.6% and 93.9% in the below 0.35mg/kg, 0.35-0.40mg/kg, 0.40-0.48mg/kg and above 0.48mg/kg groups, respectively. There was no evidence suggesting a difference in the risk of developing transplant glomerulopathy between groups [p=0.425, Logrank].

7.4.6 Infection risk

All infections were diagnosed based on microbiological evidence. Positive urine, bronchiolar lavage, drain fluid, ascites and blood cultures constituted significant episodes of bacterial infection. Cytomegalovirus PCR positivity [>1000 copies/ml] and biopsy-proven BK virus nephropathy [intranuclear inclusion bodies with positive staining for SV40] were considered to be significant markers of viral infections.

Incidence of infection

The rate of infection over time is best presented in Figure 7-8 below, and is expressed as the incidence per 100 patient years. It is estimated that the rate of overall infection was 169.4$\pm$425.3 per 100 patient years in the first 6 months and that the rate was a reduced 49.7$\pm$164.5, 42.7$\pm$193.9 and 38.4$\pm$146.0 per 100 patient years at 6-12, 12-24 and 24-36 months after transplantation, respectively. The rate of infection in the first 6 months after transplantation was statistically significantly higher after adjusting for effects of repeat measurement using the Mixed-Effect Poisson Model. It was estimated that the rate of infection was 63% lower after the first 6 months [rate ratio 0.37 95%CI 0.30, 0.47; p<0.001], 71% lower between 12 and 24 months [rate ratio 0.29 95%CI 0.23, 0.36; p<0.001] and 75% lower 24-36 months after transplantation [rate ratio 0.25 95%CI 0.19, 0.15; p<0.001].

Infection rates were also differing between groups. Total infection was increased in the <0.35 mg/kg group in the first 6 months after transplantation, albeit it did not reach statistical significance; it was estimated that this group has a 31% increase in risk on overall infection compared to the 0.40-

0.48mg/kg group [95%CI 0.86, 1.99; p=0.201]. This was mainly due to an excess wound and surgical infection, which I will describe later in this report. As demonstrated in Figure 7-8, patients who received the highest dose of Campath >0.48mg/kg also appeared to have the highest risk of infection. Moreover, the increased infection risk appeared to have persisted after 6 months. Compared to the group who received an average dose of Campath, the group exposed to the highest dose of Campath have a 2.6-fold increased risk of infection [95%CI 1.35, 4.88; Neg binomial model p=0.004] during 6 months following the initial high-risk period, a 29% increased risk [95%CI 0.78, 2.13; Neg binomial model p=0.327] the second year after transplantation and a 2.6-fold increased risk of overall infection even 2 years after transplantation [95%CI 1.1, 5.9; Neg binomial model p=0.028].

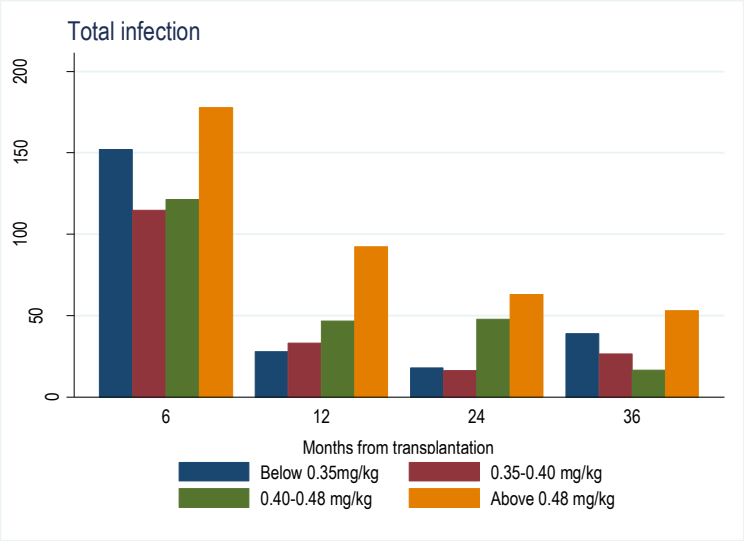

Figure 7-8 Total infection rate between 4 groups

Nature of infection

As a result of the changes in infection risk over time, the pattern and nature of infection between 4 groups were stratified into 4 timelines for analysis. As discussed earlier, patients receiving less than 0.35mg/kg of Campath have the highest risk of infection in the first 6 months; this was attributed to an increased rate of surgical infection. It was estimated that the incidence of surgical-related infection was 62.2 cases per 100 patient years [Table 7-5], and was 2.1-fold higher compared to

those receiving the average dosage [Table 7-6]. This increased risk of surgical infection is likely due to the large body habitus associated with the higher body weight.

Infection risk was highest in patients receiving above 0.48mg/kg of Campath throughout all timelines, particularly urinary tract infection and bacteraemia [Table 7-5]. There was a substantially higher proportion of females in this group who had a higher risk of urinary tract infection and who would confound the observed event rates; therefore, a multivariate approach was used to adjust the effect of gender difference. The adjusted analysis suggested that patients receiving a high dose of Campath have a 1.8-, 2.02-, 2.1- and 2.7-fold increased risk of urinary tract infection at 6, 12, 24 and 36 months, respectively, after transplantation after being adjusted for the gender effect. The incidence of bacteraemia was 12.5-fold higher in this group at 12 months after transplantation, compared to those receiving an average dose of Campath [Table 7-6].

Data from this study suggested that the difference in infection risk was limited to bacterial infection; there were no significant differences in infection risk in BK, CMV and fungal infections between 4 groups.

Table 7-5 Rate of infection between weight groups [Incidence /100 patient years]

		Below 0.35mg/kg	0.35 to 0.40mg/kg	0.40 to 0.48mg/kg	Above 0.48mg/kg
UTI	6 months	66.9±10.57	48.8±9.58	51.3±8.94	113.6±14.4
	6-12 months	13.0±4.91	20.8±6.58	30.0±7.08	55.0±10.39
	12-24 months	4.2±2.40	10.4±3.93	21.1±4.83	47.7±8.18
	24-36 months	7.8±4.48	12.1±5.41	11.6±4.40	41.5±9.80
Surgical	6 months	53.5±9.46	24.4±6.77	28.0±6.60	18.3±5.79
BK	6 months	-	7.5±3.76	-	5.5±3.17
	6-12 months	1.9±1.86	2.1±2.08	-	-
	12-24 months	-	1.5±1.49	-	1.4±1.40
	24-36 months	-	-	-	2.3±2.31
Fungal	6 months	-	-	3.1±2.20	-
	6-12 months	-	-	1.7±1.67	-
	12-24 months	-	-	1.1±1.11	1.4±1.40
	24-36 months	-	-	1.7±1.66	2.3±2.31
CMV	6 months	3.3±2.36	3.8±2.66	4.7±2.70	1.8±1.83
	6-12 months	7.4±3.72	2.1±2.08	1.7±1.67	2.0±1.96
	12-24 months	-	-	-	-
	24-36 months	-	-	-	2.3±2.31
Bacteraemia	6 months	16.7±5.29	16.9±5.63	28.0±6.60	25.6±6.85
	6-12 months	1.9±1.86	2.1±2.08	6.7±3.33	23.6±6.80
	12-24 months	-	-	12.2±3.68	7.0±3.14
	24-36 months	13.0±5.79	9.7±4.84	-	-
Other	6 months	11.7±4.42	13.1±4.97	6.2±3.11	12.8±4.84
	6-12 months	3.7±2.62	6.2±3.61	6.7±3.34	11.8±4.81
	12-24 months	13.9±4.38	4.5±2.57	13.3±3.84	5.6±2.81
	24-36 months	18.1±6.85	4.8±3.42	3.3±2.35	4.6±3.27
Total	6 months	152.1±15.95	114.6±14.67	121.4±13.75	177.7±18.1
	6-12 months	27.9±7.19	33.3±8.33	46.7±8.82	92.3±13.46
	12-24 months	18.0±5.00	16.3±4.93	47.7±7.27	63.1±9.41
	24-36 months	38.9±10.03	26.6±8.03	16.6±5.26	53.1±11.07

Table 7-6 Time-stratified infection rate ratio between groups [Adjusted for gender]

		Below 0.35mg/kg	0.35 to 0.40mg/kg	0.40 to 0.48mg/kg [Reference]	Above 0.48mg/kg
UTI	6 months	1.37 [0.78,2.40; p=0.278]	0.93 [0.52,1.66; p=0.801]	1	1.78 [1.03,3.09; p=0.038]
	6-12 months	0.67 [0.24,1.83; p=0.431]	1.34 [0.59,3.06; p=0.489]	1	2.02 [0.91,4.48; p=0.082]
	12-24 months	0.20 [0.06,0.69; p=0.011]	0.53 [0.21,1.32; p=0.174]	1	2.11 [1.13,3.97; p=0.020]
	24-36 months	0.77 [0.19,3.22; p=0.723]	1.27 [0.37,4.36; p=0.705]	1	2.73 [1.04,7.14; p=0.041]
Surgical	6 months	2.13 [1.06,4.27; p=0.033]	1.08 [0.51,2.31; p=0.840]	1	0.66 [0.27,1.60; p=0.361]
BK	6 months	-	-	1	0.87 [0.18,4.28; p=0.860]
	6-12 months	0.86 [0.05,13.93; p=0.914]	-	1	-
	12-24 months	-	-	1	-
	24-36 months	-	-	1	-
Fungal	6 months	0.78 [0.00,Inf; p=1.000]	-	1	4.65 [0.00,Inf; p=0.999]
	6-12 months	0.83 [0.00,Inf; p=1.000]	-	1	6.26 [0.00,Inf; p=0.999]
	12-24 months	-	-	1	
	24-36 months	-	-	1	
CMV	6 months	0.88 [0.12,6.34; p=0.895]	1.09 [0.17,6.74; p=0.929]	1	0.38 [0.03,4.46; p=0.440]
	6-12 months	3.51 [0.39,31.97; p=0.265]	0.95 [0.06,15.54; p=0.973]	1	1.31 [0.08,22.28; p=0.851]
	12-24 months	-	-	1	-
	24-36 months	-	-	1	-
Bacteraemia	6 months	0.94 [0.37,2.41; p=0.903]	1.62 [0.70,3.78; p=0.262]	1	1.50 [0.61,3.69; p=0.375]
	6-12 months	0.89 [0.06,14.44; p=0.936]	3.50 [0.38,31.95; p=0.267]	1	12.52 [1.56,100.25; p=0.017]
	12-24 months	-	-	1	0.52 [0.17,1.59; p=0.253]
	24-36 months	-	-	1	0.73 [0.00,Inf; p=1.000]
Other	6 months	0.89 [0.30,2.63; p=0.831]	0.44 [0.12,1.54; p=0.197]	1	0.83 [0.27,2.52; p=0.745]
	6-12 months	0.59 [0.10,3.58; p=0.562]	1.23 [0.27,5.64; p=0.793]	1	2.43 [0.57,10.38; p=0.230]
	12-24 months	0.86 [0.35,2.10; p=0.738]	0.31 [0.08,1.15; p=0.080]	1	0.46 [0.14,1.50; p=0.199]
	24-36 months	4.20 [0.82,21.46; p=0.084]	1.23 [0.17,9.16; p=0.839]	1	1.42 [0.19,10.61; p=0.730]
Total	6 months	1.31 [0.86,1.99; p=0.201]	0.99 [0.65,1.51; p=0.964]	1	1.34 [0.88,2.05; p=0.178]
	6-12 months	0.85 [0.40,1.82; p=0.683]	1.41 [0.72,2.75; p=0.317]	1	2.57 [1.35,4.88; p=0.004]
	12-24 months	0.35 [0.18,0.69; p=0.003]	0.35 [0.17,0.72; p=0.004]	1	1.29 [0.78,2.13; p=0.327]
	24-36 months	2.43 [0.99,5.98; p=0.053]	1.81 [0.70,4.69; p=0.219]	1	2.55 [1.10,5.87; p=0.028]

7.5 Summary

The use of Campath is becoming more popular as an induction agent in transplantation; however, the dosage of Campath used in an immunosuppressive protocol varies depending on the transplant centre protocol. Results from these randomised controlled studies have demonstrated that there is no consensus on what dosage of Campath should be given. It ranges from a body weight-adjusted dosage of up to 1.2mg/kg to a standard dosing of 30 to 60mg in total (42, 46, 57-59, 64, 65, 111, 130, 131). There are no clear differences in transplant outcomes between these studies.

We observed that the risk of systemic fungal and mycobacterial infections was high in patients who received high and repeated doses of Campath as maintenance therapy (97). Therefore, an optimal dosage of Campath induction should be sought to balance the infective risk associated with Campath whilst providing maximum immunosuppressive benefits.

There were no data in the literature defining the optimal dosage of Campath, and the purpose of this retrospective study is to examine the effect of a body weight-adjusted dosage of Campath on transplant outcomes.

608 patients were included in this study and were divided into 4 groups based on the quartile of Campath exposure per weight [<0.35mg/kg, 0.35-0.40mg/kg, 0.40-0.48mg/kg and >0.48mg/kg].

1-, 3- and 5-year patient survival was similar between the 4 groups. There were 22 deaths in this cohort and cardiovascular death remained the commonest cause of death despite our proactive approach to cardiac screening. Unsurprisingly, 80% of cardiac deaths were found in the group with the largest patients [<0.35mg/kg group]. There were 3 cases of sepsis-related death and these were distributed evenly between groups.

My data showed that allograft survival was similar between the 4 groups and that the results were not different after being censored for death with function. Rejection was the commonest cause of graft loss. The proportion of graft loss as a result of rejection was between 3.5% and 4.5% between the 4 groups.

The incidence of rejection was low in the Campath-treated group, which was consistent with our experience with the randomised controlled study [Chapter 5]. However, I noted that there was an approximate 49.8% increase in the risk of rejection in the first 12 months with larger patients who had received less than 0.35mg/kg of Campath. There was no evidence to suggest a difference in the risk of late rejection between groups, albeit uncommon.

The natures of rejection were similar between all weight groups, and the risk of rejection was increased in both antibody-mediated or T cell-mediated rejections.

My mathematical model on allograft function using MDRD eGFR suggested that patients who received a higher dosage of Campath have a significantly higher eGFR, as predicted, resulting from a lower creatinine and body mass. There was no evidence suggesting deterioration in function over time. There was no evidence suggesting that patients who received different doses of Campath have different trajectories compared to the 0.35mg/kg group.

I used the incidence of infections as a marker of immunosuppressive risk in this cohort. All infections were diagnosed based on microbiological evidence. It is estimated that the rate of overall infection was 169.4±425.3 per 100 patient months in the first 6 months and that the rate was a reduced 49.7±164.5, 42.7±193.9 and 38.4±146.0 per 100 patient years at 6-12, 12-24 and 24-36 months after transplantation.

Total infection was increased in the <0.35 mg/kg group in the first 6 months after transplantation, albeit it did not reach statistical significance. It was estimated that this group have a 31% increase in risk on overall infection compared to the 0.40-0.48mg/kg group and that this was mainly due to an excess wound and surgical infections.

Patients who received the highest dose of Campath >0.48mg/kg also appeared to have the highest risk of infection. Compared to the group who received an average dose of Campath, this group exposed to the highest dose of Campath have a 2.6-fold increased risk of infection in the first 6 months and this increased infective risk persisted after 2 years. Urinary tract infection and bacteraemia were most common in this group compared to patients exposed to less Campath. The

gender-adjusted analysis suggested that patients receiving a high dose of Campath have a 1.8-, 2.02-, 2.1- and 2.7-fold increased risk of urinary tract infection at 6, 12, 24 and 36 months, respectively, after transplantation after being adjusted for the effect of the higher proportion of females. The incidence of bacteraemia was 12.5-fold higher in this group at 12 months after transplantation, compared to those receiving an average dose of Campath.

My study is the first of its kind in the literature, which has aimed to examine the ideal body weight-adjusted Campath dosage to provide maximum immunosuppressive benefits with the least infective risk. I showed that patients who were exposed to a high dose – above 0.48mg/kg – did not result in a lower risk of rejection, whereas those receiving less than 0.35mg/kg of Campath experienced a 48% increased risk of rejection. Furthermore, the higher weight-adjusted Campath dosage is associated with an increased risk of urinary tract infection and bacteraemia. The effects of this increased risk of infection appeared to persist for years after transplantation.

As a result of this study, we changed our immunosuppressive protocol to a weight-adjusted Campath dose at 0.40mg/kg induction followed by targeted tacrolimus monotherapy in January 2012.

8 Causes of graft loss

8.1 Introduction

Short-term renal allograft survival has improved over the last decades, but it is yet to translate into improvement in long-term survival (132).

Howard et al. have examined the causes of graft loss in their centre over the last three decades, showing allograft rejection as the commonest cause of graft loss despite the proportion of graft loss due to rejection falling from 66% in the 1970s to 45% in the 1990s with the improvement in immunosuppression (119).

Patient death with a functioning graft persisted to be an important cause of long-term graft loss (119, 133). Howard et al. reported that a patient's death accounted for an increased incidence of allograft failure; this is likely due to the changes in transplant recipient population and that more patients with complex medical histories have been transplanted over the last three decades.

Cardiovascular disease is the commonest cause of transplant death, accounting for approximately 36% of cases. Infection was the second commonest cause of death and this accounted for 17.6% of death recorded in the UNOS registry. Albeit less common, malignancy after long-term immunosuppressant exposure is associated with 9.2% of total mortality.

Long-term allograft dysfunction due to medication toxicity is another important cause of graft loss and long-term allograft dysfunction. It was reported that a high incidence of tubular fibrosis followed 10 years of cyclosporine exposure (16). The effect of long-term tacrolimus exposure on renal transplantation has remained unclear, although it is believed that tacrolimus is less toxic compared to cyclosporine (17).

Other factors such as recurrent glomerular disease (134) and transplant glomerulapathy (135) are both important causes of graft failure in the long term.

The number of patients receiving Campath induction in renal transplantation has been rising in the last decade following the report by Kirk et al. (46). Furthermore, there are plenty of clinical trials and cohort studies describing effects of Campath induction in the short term (52-57, 62, 65, 73, 136), but

a few are describing the long-term effects and causes of allograft loss following this powerful induction.

The purpose of this chapter is to examine the cause of graft loss and death in our centre and to examine whether and how much this differs from published literature in patients receiving this simple immunosuppressive regimen.

8.2 Methods

This is a retrospective study based on data collected on subjects transplanted between Aug 2004 and May 2011 for the Imperial College Kidney and Transplant Centre transplant registry. The use of data was authorised by the Imperial College Kidney and Transplant Centre transplant research group. Patient data were anonymised to guarantee patient confidentiality.

All patients receiving induction therapy consisted of 30mg of Campath intravenously and long-term tacrolimus monotherapy maintenance.

Demographics

Table 8-1 Demographics

		N [%], Mean ± SD
Gender	Female	261 [35.2%]
	Male	481 [64.8%]
Ethnicity	Afro-Caribbean	75 [10.1%]
	Asian	242 [32.6%]
	Caucasian	370 [49.9%]
	Others	55 [7.4%]
First transplant	Yes	682 [91.9%]
	No	60 [8.1%]
Donor type	Deceased	389 [52.4%]
	Living	353 [47.6%]
Cold ischaemic time [hrs.]		21.9±9.0
Age at transplant [yrs.]		47.8±14.3
Donor age [yrs.]		47.2±15.0
HLA mismatch		3.5±1.8
Follow-up [months]		37.2±23.1

Table 8-1 (above) shows the detailed demographics on the patient cohort included in this study. The majority of patients transplanted in our centre are male. 50% of patients transplanted in our unit are non-Caucasoid; this reflects the ethnicity mix of our catchment population. Furthermore, the majority of patients received the renal allograft as their first transplant. 52.4% of renal allografts were from deceased donors with an average cold ischaemic time of 21.9 hours. The mean HLA mismatches were 3.5. 98.1% of patients were under active follow-up in our centre until allograft loss or death. Mean follow-up was 37.2 months, with median and longest follow-up of 33.6 and 92 months, respectively.

All patients who had only undergone kidney transplantation without a prior desensitisation procedure were included in this report.

Primary outcomes of this study include:

- Allograft survival in 1, 3, 5 and 7 years
- Causes of graft loss

Allograft failure is defined as the need of a patient requiring returning to long-term dialysis or death. All causes of graft loss were confirmed following reviewing clinical notes.

8.3 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Allograft survivals were presented using the Kaplan Meier plot, and cumulative survival was presented to adjust for loss of follow-up and variation in transplant vintage.

8.4 Results

8.4.1 Overall allograft survival

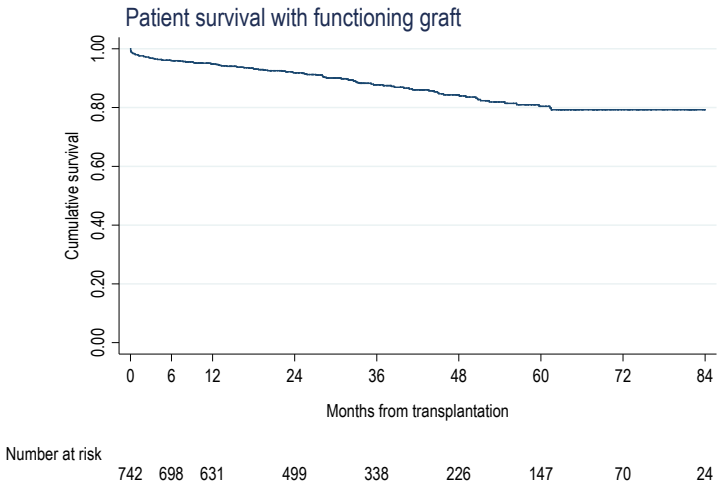

Figure 8-1 Overall patient survival

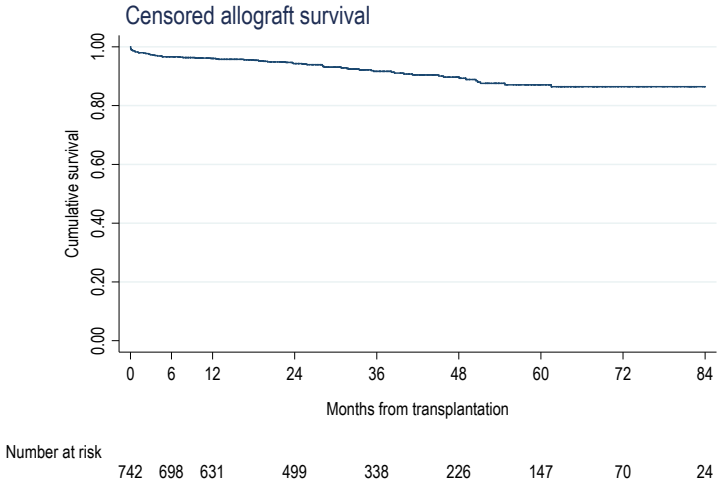

Figure 8-2 Overall graft survival [Censored for death with function]

Figure 8-1 and Figure 8-2 (above) show the cumulative patient survival with functioning graft and censored allograft survival in the whole cohort. There were 97 graft losses in total, of which 33/97 [34.0%] were a result of a recipient's death.

The estimated cumulative patient survivals with a functioning graft were 94.8%, 87.7%, 80.4% and 79.3% at 1, 3, 5 and 7 years following transplantation, respectively.

Moreover, cumulative allograft survivals were 96.0%, 91.6%, 87.1% and 86.4% at 1, 3, 5 and 7 years after censoring for patients having died with a functioning graft.

8.4.2 Causes of graft loss

Table 8-2 (below) lists details of causes of allograft loss in our unit.

Patient death with a functioning graft remained the commonest cause of allograft failure under this simple minimalistic regimen: patient deaths account for 34% of total graft losses, which is equivalent to 4.4% of the study cohort. 13/33 deaths were due to overwhelming sepsis, and 8 graft losses were due to cardiac deaths. 5 patients died as a result of underlying malignancies, of which 3/5 cases originated from the gastrointestinal system, 1 was recurrent renal cell carcinoma and 1 case was metastatic angiosacroma originating from a haemodialysis fistula. 2 patients died as a result of complications associated with encapsulating peritoneal sclerosis [EPS] related to previous peritoneal dialysis. 1 patient died following a haemorrhagic stroke, 1 patient died following dissection of a thoracic aneurysm and 1 patient with significant vascular disease died from an ischaemic bowel. Causes of death in 2 patients were unclear.

Despite the powerful immunosuppressive effects of Campath, rejection remains an important cause of long-term graft losses. 31.9% of graft losses were caused by rejection; this is equivalent to 4.2% of the cohort. There were 12 cases of cellular rejection leading to graft losses despite the reintroduction of oral prednisolone and mycophenolate mofetil. 8 grafts were lost following diagnosis and treatment of antibody-mediated rejection; all 8 cases were treated with intravenous immunoglobulin and plasma exchange parallel to the reintroduction of steroids and mycophenolate mofetil. 3 allografts were lost soon after diagnosis of transplant glomerulopathy.

8 allografts were lost as a result of non-compliance and 7 allografts were lost following the medical decision to stop immunosuppression in the face of life-threatening sepsis.

6 allografts from extended-criteria deceased donors failed soon after transplantation despite producing initial marginal function post-transplantation. Repeat histological examination did not show evidence of acute rejection in these cases.

Technical failure and primary non-function accounted for 6 and 2 cases of graft losses respectively. There were 2 cases of severe transplant renal artery stenosis and resulting graft loss.

The incidence of calcineurin inhibitor toxicity in our group was reportedly low with the low dose of maintenance tacrolimus (16), and there was only 1 [0.1%] case of CNI toxicity-related graft loss in the cohort.

2 graft losses were following renal artery and venous thrombosis and 1 graft underwent graft nephrectomy because of an intractable ureteric leak.

Table 8-2 Causes of graft loss

Cause	**n [%]**
Death	33 [34.0%]
Sepsis	*13 [13.4%]*
Cardiac	*8 [8.2%]*
Malignancy	*5 [5.2%]*
EPS	*2 [2.1%]*
Unknown	*2 [2.1%]*
CVA	*1 [1.0%]*
Dissection of thoracic aneurysm	*1 [1.0%]*
Ischaemic bowel	*1 [1.0%]*
Rejection	31 [31.9%]
Acute cellular rejection	*12 [12.4%]*
Non-compliance	*8 [8.2%]*
Antibody-mediated rejection	*8 [8.2%]*
Immunosuppressant withdrawal	*7 [7.2%]*
Transplant Glomerulopathy	*3 [3.1%]*
Donor factors	6 [6.2%]
Technical failure	6 [6.2%]
Primary non-function	2 [2.1%]
Pyelonephritis	2 [2.1%]
Recurrent disease	2 [2.1%]
TRAS	2 [2.1%]
Cardiac failure	1 [1.0%]
CNI toxicity	1 [1.0%]
Renal vein thrombosis	1 [1.0%]
Arterial thrombosis	1 [1.0%]
Unknown	1 [1.0%]
Ureteric leak	1 [1.0%]

8.5 Summary

Published reports from the literature have showed a significant improvement in short-term allograft survival over the last three decades, but it has yet to translate into long-term survival improvement. Although there is a large quantity of literature showing a favourable short-term use of Campath induction, the use of Campath induction has shown to improve short-term allograft survival in a meta-analysis (29). The long-term outcome of using Campath and its associated causes of graft loss was not well documented.

In the chapter, I examined the overall graft and patient survival in our unit and explored the causes of graft failure under this simple regimen.

My study showed that the estimated cumulative patient survivals with a functioning graft were 94.8%, 87.7%, 80.4% and 79.3% at 1, 3, 5 and 7 years following transplantation, respectively. And cumulative allograft survivals were 96.0%, 91.6%, 87.1% and 86.4% at 1, 3, 5 and 7 years, respectively, after censoring for patients having died with a functioning graft.

Patient death with a functioning graft remained the commonest cause of graft loss in our unit, accounting for 34% of graft losses. Sepsis was the commonest cause of patient death in our unit, corresponding to 13/742 [1.8%] patients in this study cohort. On the contrary to previous UNOS reports, cardiovascular death only accounted for a moderate portion of total patient deaths [8/33, 24.2%]. The reduction of cardiovascular death is likely because of a proactive cardiac screening prior to transplantation in our transplant recipients, and this is reported in detail in the thesis submitted by my colleague, Dr. Nichola Kumar, from Imperial College.

Although our data in the RCT showed that acute rejection was lower in the Campath-treated group at 1 year and little evidence suggested an increased risk of late rejection in the long term, rejection remained an important cause of graft losses. 12/31 cases were cellular rejection, 8/31 were antibody-mediated rejection and 3/31 cases were lost as a result of transplant glomerulopathy.

Despite this simple monotherapy regimen, 8 grafts were lost as a result of rejection caused by medication non-compliance.

9 Long-term allograft pathology

9.1 Introduction

Data in the literature suggested a lack of improvement in long-term allograft survival despite a substantial improvement in short-term results in terms of survival and lower rejection risk (132). It was reported that patient death and rejection were the main causes of long-term graft loss (119, 133) and this is consistent with our experience [Chapter 8].

Long-term allograft dysfunction due to medication toxicity is another important cause of graft loss and long-term allograft dysfunction. It was reported that a high incidence of tubular fibrosis followed 10 years of cyclosporine exposure (16). Other factors such as recurrent glomerular disease (134) and transplant glomerulopathy (135) are both important causes of graft failure in the long term.

Calcineurin inhibitor toxicity

It is well established that calcineurin inhibitors are crucial in long-term allograft survival, but they are associated with a significant risk of nephrotoxicity after long exposure with either cyclosporine or tacrolimus. CNI toxicity can manifest in acute settings with a transient fall in allograft function due to an excess dosage or, more commonly, in a chronic setting following a long exposure to CNI over years.

A previous report looking at 888 surveillance biopsies on 99 patients over 10 years by the Nankivell group suggested that 100% patients who were treated with cyclosporine will manifest at least one of the elements of CNI toxicity in their allograft histology. And 53.9% of patients will have some features of CNI toxicity on a protocol biopsy after 1 year (137).

It is unclear whether tacrolimus is associated with a similar risk of long-term allograft toxicity to cyclosporine. A large US transplant registry report examined almost 70,000 non-renal solid organ transplants and showed that liver transplant patients treated with tacrolimus have a lower risk of developing CNI toxicity and have a better renal function in the long term (67). A smaller study in renal transplant recipients showed data supporting a better CNI toxicity profile with tacrolimus (138).

The effect of long-term tacrolimus exposure on renal transplants has remained unclear, although it is believed that tacrolimus is less toxic compared to cyclosporine (17). We were hoping that by further reducing the maintenance dosage of tacrolimus following Campath induction, it might translate into a reduced incidence of CNI toxicity.

Transplant Glomerulopathy

Transplant glomerulopathy [TG] in a renal allograft was first described in 1970 by Hood (139) and has been a major cause of graft loss and dysfunction in the long term. TG is a histological diagnosis based on features described by the Banff Working Group (140), defined as duplication of glomerular basement membranes in 10%, or more, of glomerular capillary loops in glomerulus. It is thought that development of TG is associated with the presence of anti-HLA antibodies and results from chronic rejection (141).

Previous reports estimated that TG is rare during the first year after transplantation; however, the incidence of transplant glomerulopathy could rise to as high as 20% in 5 years in a steroid- and cyclosporine-treated era (116, 135). Furthermore, development of TG is associated with worse clinical outcomes despite augmentation of immunosuppressants (142).

The incidence and nature of TG is unclear following a powerful lymphopenic Campath induction and tacrolimus monotherapy. I will try to address the incidence of TG in our programme.

Recurrent glomerular disease

Recurrent glomerular disease is a well-recognised complication in renal transplantation (134, 143-146) and it is thought that recurrent glomerular disease is the third commonest cause of graft loss in the first 10 years following a patient death with functioning graft and allograft rejection (134). The author estimated that the risk of graft loss from recurrence increased with the years of follow-up, from 0.6% in the first year to 8.4% in the tenth year.

A recent review paper by Choy et al. examined the risk of recurrent disease in renal transplant recipients, suggesting that 80-100% with Type 2 membranoproliferative glomerulopathy experience

recurrent disease in the first 5 years, and 15-30% experience allograft loss in renal recipients with Type II MPGN as a result of recurrent disease (143).

Focal segmental glomerulosclerosis [FSGS] is an important cause of end-stage disease in our centre and other units. Recurrence of FSGS after transplantation was thought to be rapid and common (143, 146). It was estimated that 20-50% of patients with underlying primary FSGS would experience recurrent disease following transplantation (143) and that 13-20% of patients with primary FSGS will experience graft loss as a result of recurrent disease in the long term.

IgA nephropathy is the commonest glomerular disease leading to end-stage renal failure. Recurrent IgA nephropathy is reported in 13-46% of patients, and 2-16% of cases of allograft loss result in the long term.

Lupus nephritis, ANCA-associated glomerulonephritis and anti-GBM disease are other common causes of recurrent glomerular disease after transplantation. It is estimated that up to 2-9% of recipients with lupus nephritis and up to 17% of patients with ANCA vasculitis experience recurrent disease after transplantation.

The aim of this chapter is to examine the pattern of long-term allograft pathology in the whole of Campath-treated cohort transplants between Aug 2004 and May 2011, to investigate whether our medium-dose tacrolimus monotherapy maintenance translates to a further reduction in CNI toxicity as well as a lower risk of TG with a low risk of acute rejection early after transplantation. Moreover, I am going to report the incidence of recurrent glomerular disease in this group of patients after this lymphocyte-depleting treatment.

9.2 Methods

This is a retrospective study based on data collected on subjects transplanted between Aug 2004 and May 2011 for the Imperial College Kidney and Transplant Centre transplant registry. The use of data was authorised by the Imperial College Kidney and Transplant Centre transplant research group. Patient data were anonymised to guarantee patient confidentiality.

All patients receiving induction therapy consisted of 30mg of Campath intravenously and long-term tacrolimus monotherapy maintenance..

Demographics

Table 9-1 Demographics: Long-term allograft pathology

		N [%], Mean ± SD
Gender	Female	261 [35.2%]
	Male	481 [64.8%]
Ethnicity	Afro-Caribbean	75 [10.1%]
	Asian	242 [32.6%]
	Caucasian	370 [49.9%]
	Others	55 [7.4%]
First transplant	Yes	682 [91.9%]
	No	60 [8.1%]
Donor type	Deceased	389 [52.4%]
	Living	353 [47.6%]
Cold ischaemic time [hrs.]		21.9±9.0
Age at transplant [yrs.]		47.8±14.3
Donor age [yrs.]		47.2±15.0
HLA mismatch		3.5±1.8
Follow-up [months]		37.2±23.1

Table 9-1 (above) shows the detailed demographics on the patient cohort included in this study. The majority of patients transplanted in our centre are male. 50% of patients transplanted in our unit are non-Caucasoid; this reflects the ethnicity mix of our catchment population. Furthermore, the majority of patients received the renal allograft as their first transplant. 52.4% of renal allografts were from deceased donors with an average cold ischaemic time of 21.9 hours. The mean HLA mismatches were 3.5 in 98.1% of patients under active follow-up in our centre until allograft loss or death. Mean follow-up was 37.2 months, with median and longest follow-up of 33.6 and 92 months respectively.

The purpose of this analysis is to examine the cause of graft loss in our Campath-treated cohort since we started using this minimalistic regimen.

All patients who had only undergone kidney transplantation without a prior desensitisation procedure were included in this report.

Primary outcomes of this study include:

- Incidence and timing of development of CNI toxicity
- Incidence and timing of development of transplant glomerulopathy
- Incidence and timing of development of recurrent disease

Diagnosis of these long-term allograft pathologies is based on histological examination on indicative biopsies performed when there is a clinical need.

9.3 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Times to event survivals were presented using the Kaplan Meier plot, and cumulative survivals were presented to adjust for loss of follow-up and difference in transplant vintage. Censoring was assumed to be non-informative.

9.4 Results

9.4.1 Calcineurin inhibitor toxicity

Incidence

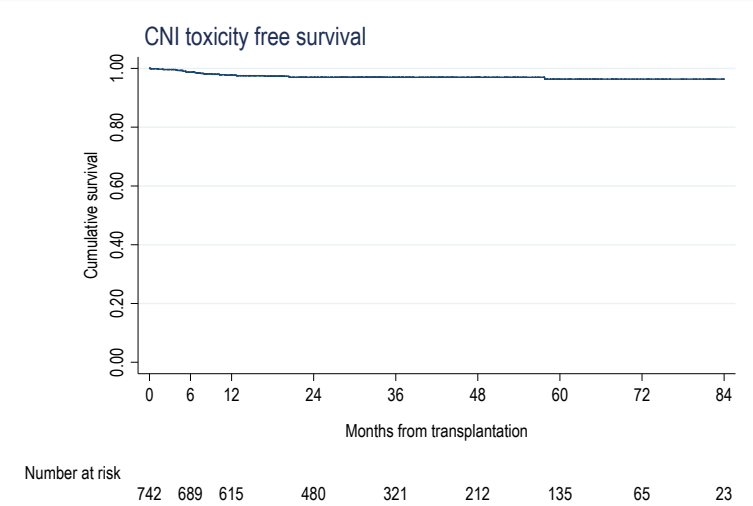

Figure 9-1 CNI toxicity-free survival

Figure 9-1 (above) shows the cumulative survival of calcineurin inhibitor toxicity in this study cohort. In total, 21 patients were diagnosed with CNI toxicity on clinical indicative biopsies, of which 3/21 cases were diagnosed following dose increase after allograft rejection.

1-, 3-, 5- and 7-year cumulative incidences of CNI toxicity were 2.3%, 3.0%, 3.7% and 3.7%, respectively.

There was only one case of graft loss as a result of CNI toxicity and this accounted for 1% of graft loss in our large cohort [Chapter 8].

These results were encouraging and markedly different from previous literature based on a Cyclosporine regimen. Furthermore, this study provides insightful data that supports our regimen with low-dose tacrolimus being associated with a minimal risk of CNI toxicity in the long term.

9.4.2 Transplant Glomerulopathy

Transplant glomerulopathy is diagnosed on histological features associated with thickening and duplication of glomerular basement membranes in the absence of active immune reactivity or immune complex deposits. It was thought to be associated with the presence of an antibody-

mediated response, either in the form of donor-specific antibodies or acute or chronic antibody-mediated rejection (140).

Incidence

Transplant glomerulopathy is uncommon in our experience using this minimalistic regimen. Figure 9-2 (below) shows the cumulative transplant glomerulopathy-free survival in our cohort. All TG cases were diagnosed on indicative biopsies in cases of transplant allograft dysfunction or increasing proteinuria. Results include all biopsies which demonstrated features of transplant glomerulopathy diagnosed by histopathologists.

Our results showed cumulative TG-free survival of 96.8%, 94.4% and 91.1% at 1, 3 and 5 years, respectively, substantially lower than published data.

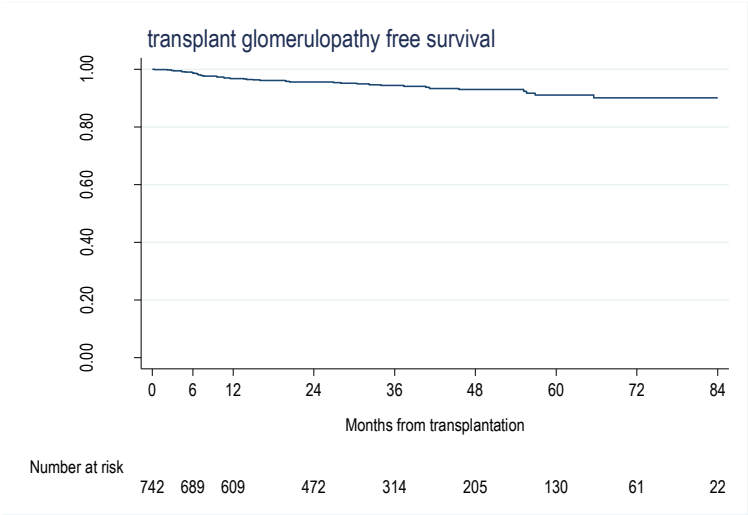

Figure 9-2 Transplant Glomerulopathy

There were 3 graft losses as a result of transplant glomerulopathy despite optimisation of immunosuppressants; TG accounts for 3% of graft losses in our cohort [Chapter 8].

9.4.3 Recurrent disease

In this study, I examined the incidence of recurrent disease in patients who were thought to have a glomerular disease causing end-stage renal failure and who underwent renal transplantation in the Imperial College Kidney and Transplant Centre. Patients with reported glomerular disease prior to

transplantation were included in this analysis. Recurrent glomerular disease was diagnosed based on indicative transplant biopsies at the time of allograft dysfunction or other clinical indications.

181 [24.7%] patients were thought to have a glomerular disease prior to renal transplantation. 9/181 [4.97%] cases of recurrent glomerular disease were observed. The incidence of glomerular disease in this cohort and risk of recurrence are detailed in Table 9-2.

IgA nephropathy is the commonest glomerular disease that leads to renal transplantation in our unit. 4/62 [6.5%] patients experienced recurrent disease within 7-year follow-up at 8, 11, 23 and 37 months post-transplantation. 2/10 [20%] patients with membranous glomerulopathy recurred at 22 and 42 months following transplantation. Recurrent MCGN is the second commonest cause of recurrent disease in this cohort; 1 [8.3%] patient developed recurrent MCGN at 13 months. 1 patient developed recurrent Anti GBM disease at 24 months.

Of all patients with underlying FSGS, 1 [2.3%] developed recurrent disease at 1 month following transplantation and disease progression was aggressive despite plasmapheresis and increased immunosuppressive therapy. Thus, it resulted in graft loss at 16 months.

Table 9-2 Recurrent Glomerular disease

	No recurrent disease	Recurrent disease
Anti GBM disease	7 [87.5%]	1 [12.5%]
Amyloid	1 [100.0%]	0 [0.0%]
Chronic Glomerulonephritis	15 [100.0%]	0 [0.0%]
FSGS	42 [97.7%]	1 [2.3%]
IgA Nephropathy	58 [93.5%]	4 [6.5%]
Lupus Nephritis	18 [100.0%]	0 [0.0%]
MCGN	11 [91.7%]	1 [8.3%]
Membranous Glomerulopathy	8 [80.0%]	2 [20.0%]
ANCA Vasculitis	12 [100.0%]	0 [0.0%]

Figure 9-3 Glomerular disease recurrence-free survival (below) depicts the cumulative recurrence disease-free survival. The median time to recurrence was 22 months. Cumulative survivals were 98.2%, 94.9% and 97.8% at 1, 3 and 5 years, respectively.

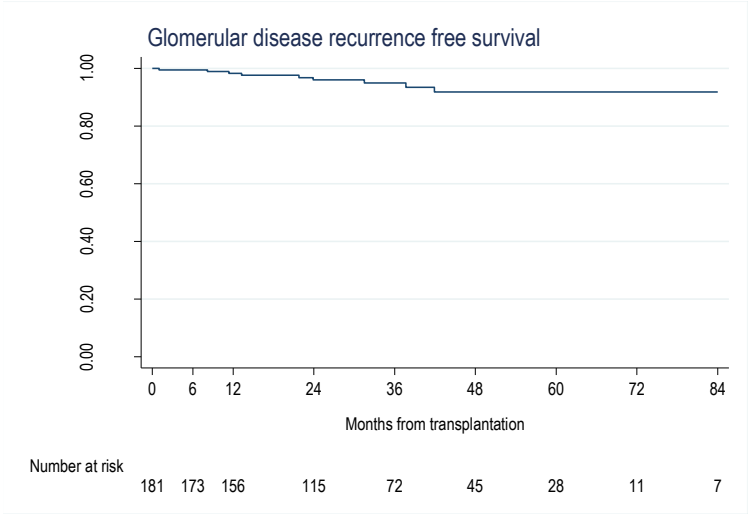

Figure 9-3 Glomerular disease recurrence-free survival

9.5 Summary

Long-term allograft dysfunction due to medication toxicity, recurrent glomerular disease (134) and transplant glomerulopathy (135) are important causes of graft failure in the long term. In this chapter, I examined the incidence of CNI toxicity, transplant glomerulopathy and recurrent glomerular disease in our cohort following Campath induction.

Nankivell et al. reported results from 888 surveillance biopsies on 99 patients over 10 years, suggesting that 100% of patients who were treated with cyclosporine will manifest at least one of the elements of CNI toxicity in their allograft histology at 10 years, and 53.9% of patients will have some features of CNI toxicity on a protocol biopsy after 1 year (137). This is very different from our experience: our results are certainly very similar to previous reports suggesting that tacrolimus is less toxic compared to cyclosporine (17). Only 21/742 [2.8%] patients in this study experienced allograft dysfunction caused by CNI toxicity, of which 3/21 cases were diagnosed after the tacrolimus dose was increased after rejection. Thus, 1-, 3-, 5- and 7-year cumulative incidence of CNI toxicity

was 2.3%, 3.0%, 3.7% and 3.7%, respectively. There was only one case of graft loss as a result of CNI toxicity and it accounted for 1% of graft loss in our large cohort. My data showed that the incidence of CNI toxicity was substantially lower compared to previous reports using this minimalistic medium-dose tacrolimus regimen.

Development of transplant glomerulopathy is associated with worse clinical outcomes despite augmentation of immunosuppressants (142). Previous reports have estimated that the incidence of transplant glomerulopathy has been as high as 20% in 5 years in a steroid- and cyclosporine-treated era (116, 135). The incidence of transplant glomerulopathy is low in our centre. My results showed cumulative TG-free survival of 96.8%, 94.4% and 91.1% at 1, 3 and 5 years, respectively, substantially lower than published data. There were 3 graft losses as a result of transplant glomerulopathy despite optimisation of immunosuppressants; TG accounts for 3% of graft loss in our cohort [Chapter 8].

Recurrent glomerular disease is a well-recognised complication in renal transplants (134, 143-146) and it is thought that recurrent glomerular disease is the third commonest cause of graft loss in the first 10 years following patient death with functioning graft and allograft rejection (134). Furthermore, it is estimated that the risk of graft loss from recurrence increased with the years of follow-up from 0.6% in the first year to 8.4% in the tenth year, and the risk of recurrent disease varies depending on the underlying primary disease.

In this study, I identified 181 [24.7%] patients who were thought to have a glomerular disease prior to renal transplantation, of which 9/181 [4.97%] cases of recurrent glomerular disease were observed. The median time to recurrence was 22 months. Cumulative survivals were 98.2%, 94.9% and 97.8% at 1, 3 and 5 years, respectively. Patients with membranous glomerulopathy have the highest risk of recurrent disease, whereas none of the patients who were thought to have underlying lupus or ANCA vasculitis experience recurrences following Campath induction.

10 Haematological profile

10.1 Introduction

Results from the clinical studies demonstrated that Campath is a powerful lymphopenic agent and repopulation of lymphocytes varies depending on the subsets (46, 49, 52, 54, 62, 76). Campath itself does not induce transplant tolerance, and maintenance immunosuppression is required to avoid acute rejection despite lymphopenia (46, 49). We also observed that the choice of maintenance immunosuppression has a huge impact on a transplant outcome. The incidence of acute rejection was low in patients treated with a calcineurin inhibitor-based immunosuppression protocol following Campath, regardless of whether maintenance mycophenolate mofetil or steroids were used (52, 56, 57, 61, 63). On the contrary, the incidences of acute rejection in the rapamycin maintenance regimes were unacceptably high, even with the concurrent use of mycophenolate mofetil (54, 55). The difference in efficacies between different types of immunosuppression maintenance may be due to the functional nature of a residual lymphocyte following depletion.

Pearl et al. underwent a study to investigate the phenotype and functioning characteristics of residual lymphocytes following lymphodepletion of Campath (77). Blood samples were drawn and analysed using flow cytometry prior to depletion and this was done weekly for 4 weeks in 5 patients during the original tolerance induction study (49); samples from patients who received rabbit anti-thymocyte globulin or daclizumab acted as controls. This study showed almost complete lymphodepletion with naive T cells and regulatory T cells after Campath, but with a single phenotype (CD3$\underline{+}$ CD4$\underline{+}$ CD45RA- CD62L CCR7-). This depletion-resistant subset was thought to be a memory-effect T cell. These cells expand quickly following depletion and were prevalent during rejection. Interestingly, these memory T cells were also resistant to steroids, rapamycin and deoxysepergualin in vitro, but were uniquely sensitive to calcineurin inhibitors. These data might explain the reason why Campath is not pro-tolerant and why calcineurin inhibitors are crucial for transplant immunosuppression maintenance following Campath induction.

The effects of the recovery of lymphocytes and risk of infection following Campath are not clear; there is no literature describing whether lymphocyte repopulation is associated with an increased risk of rejection. University of Minnesota adopted a trial of using repeat doses of Campath following lymphocyte repopulation to avoid rejection; with little success, the trial was terminated early for safety reasons.

Furthermore, there were concerns that high-dose Campath is associated with an increased incidence of autoimmune disease as well as autoimmune haemolytic anaemia (100, 101, 147). The incidence of haematological disease is not well described in the literature, and together with my colleague, Dr. Rawya Charif, we examined the trend of the haematological profile in patients treated with this simple immunosuppressive regimen.

I examined the relationship between rejection and residual lymphocyte/monocyte counts, investigating whether the lymphocyte compartment in patients who experienced acute rejection in the first 12 months recovers at a different rate.

I collaborated with Dr. Charif in this study design, we collected data together and I performed the necessary statistical analysis and developed the mathematical models used to analyse the relationship between the lymphocyte profile and risk of rejection.

10.2 Methods

The purpose of this study was to examine the changes of the haematological profile following Campath induction in our transplant patient cohort.

Primary objectives of this study were:

1. Investigate the pattern of the haematological profile and its recovery following Campath
2. Investigate whether there was an association between the rate of lymphocyte repopulation and risk of rejection

96,695 results reported from a routine haemological laboratory on 483 patients transplanted between 1st November 2005 and 1st April 2010 were used in this analysis.

Lymphopenia and neutropenia were defined as lymphocyte counts being below 1 $x10^9$/L.

Thrombocytopenia was defined as total platelet counts being below 50 x10^9/L.

All rejections were diagnosed on allograft biopsies and were performed on clinical indications. All lymphocyte data before rejection were included in the rejection model as lymphocyte counts might be affected by the addition of immunosuppressants and might influence the accuracy of the model.

10.3 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Haematological profiles were analysed using a mixed-effect model to account for the nature of repeat measurements over time, as well as allowing analysis of the effects of various factors on trajectories. Continuous variables were log-transformed into normal distributed data to improve adherence to model assumptions if needed.

10.4 Results

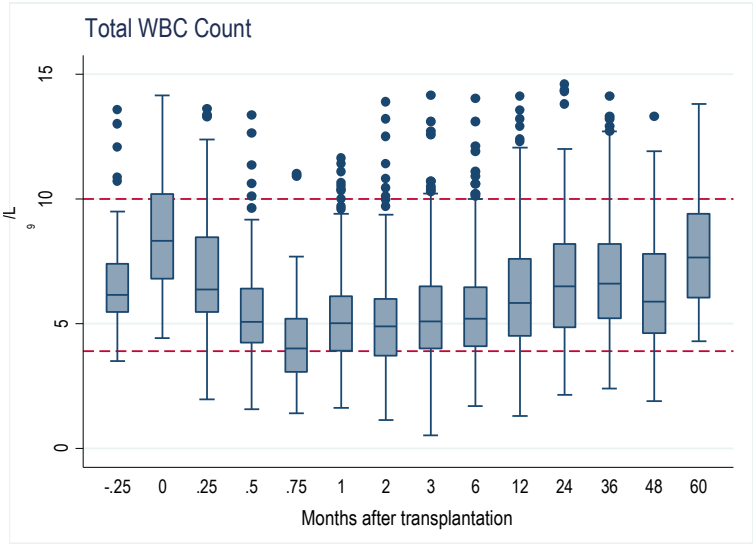

Figure 10-1 Long-term total white blood cell counts following Campath induction

Figure 10-1 (above) shows the average total white blood cell [WBC] profile in our cohort over time. There was a transient rise in the total WBC count immediately after transplantation, which was likely due to the steroids administered during the induction phase. Following the first week, the total white blood cell started to fall and appears lower than pre-transplant levels, which was likely caused by the transient lymphopenia induced by Campath. The total white blood cell count started to

recover after 6 months and reached the pre-transplant level at 12 months, following lymphocyte repopulation.

Lymphocyte, Neutrophils, Monocytes and Basinophils were described in more detail in this chapter.

10.4.1 Lymphocyte

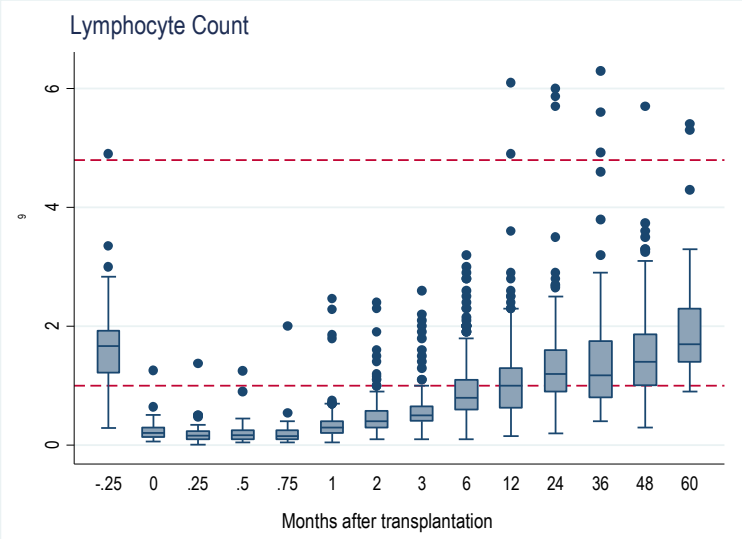

Figure 10-2 Long-term lymphocyte counts following Campath induction

Campath is a power agent-inducing transient lymphopenia following transplantation; the effect of Campath on lymphocytes is best demonstrated in Figure 10-2 above. The recipient lymphocyte compartment depleted instantly following administration and the lymphocyte remained suppressed in the first 12 months after transplantation. Lymphocyte counts dropped from $1.67 \pm 0.75 \times 10^9$/ml on average prior to transplantation to $0.19 \pm 0.17 \times 10^9$/ml in the first week following administration.

99.8% of patients remain significantly lymphopenic in the first month [95%CI: 98.3, 100%], and as demonstrated in the figure above, lymphocytes started to repopulate after 3 months. Average lymphocyte counts were 0.55 ± 0.32 at 3 months and continued to improve, whereby reaching an average of 1.03 ± 0.55 at 12 months after transplantation.

Although the average lymphocyte counts appeared to have reached the lower limit of reference, there was a concern that the duration of lymphopenia varies between patients following treatment. Using a survival model to adjust for difference in timelines, I demonstrated that the proportion of patients in the cohort with lymphopenia decreases gradually from 96.8% at 3 months to 52.3% at 6

months [Table 10-1]. By 2 years, 75.6% of patients in this cohort had recovered their lymphocyte compartment. Interestingly, my data show a very small proportion [1.4%] of the cohort remaining lymphopenic at 5 years. I demonstrated in an earlier chapter that there was no association between lymphopenia and infection risk.

Table 10-1 Percentage of patients with lymphopenia following Campath induction

Months from transplantation	Percentage patient with lymphocyte <1 x 10^9	
0	99.8%	[98.3%, 100.0%]
3	96.8%	[94.5%, 98.1%]
6	76.5%	[72.1%, 80.4%]
12	52.3%	[47.3%, 57.0%]
24	24.6%	[20.5%, 28.9%]
36	7.5%	[5.1%, 10.5%]
48	1.4%	[0.5%, 3.4%]
60	1.4%	[0.5%, 3.4%]

10.4.2 Neutrophil

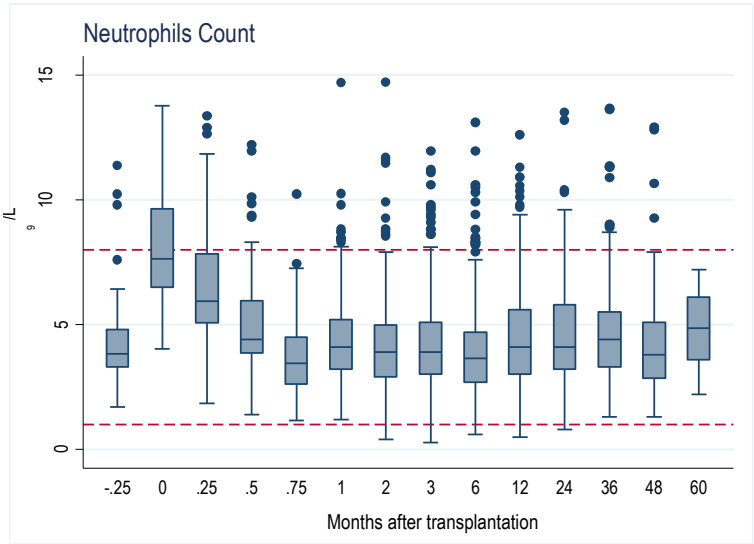

Figure 10-3 Long-term neutrophil following Campath induction

There was a transient neutrophilia following administration of induction steroids in the first week following transplantation [Figure 10-3]. The overall neutrophil profile appeared to be stable over time.

There were 8 cases of neutropenia in this cohort [Figure 10-4].

3/8 cases developed neutropenia after reintroduction of mycophenolate mofetil following treatment of allograft rejection.

2/8 cases developed neutropenia during prophylactic treatment of PCP with co–trimoxazole, and neutrophil counts recovered following treatment change to nebulise pentamidine.

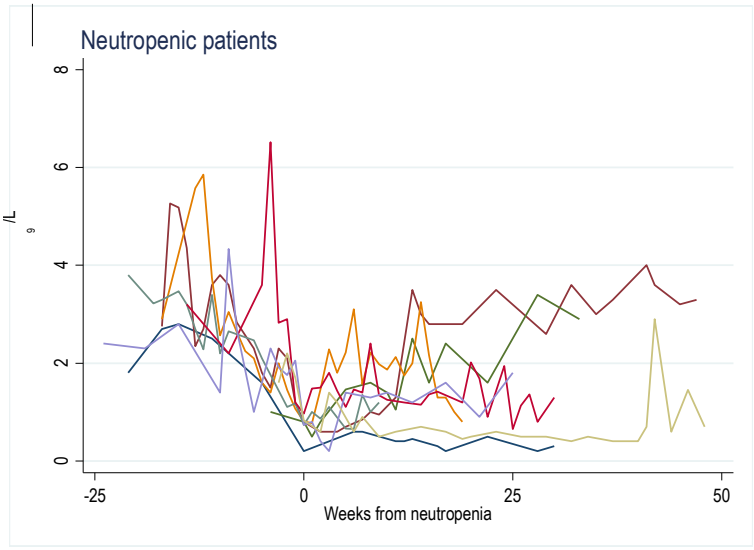

Figure 10-4 Neutrophil profiles in patients with reported persistent neutropenia

10.4.3 Monocytes

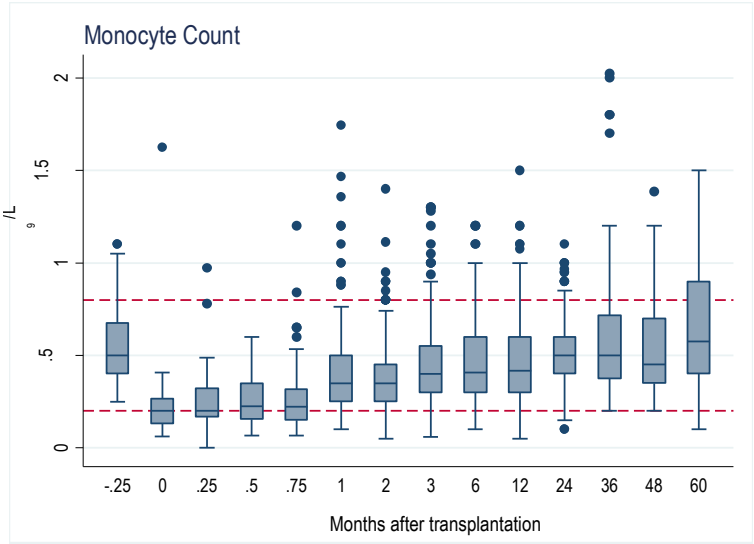

Figure 10-5 Long-term monocyte counts following Campath induction

It had been previously demonstrated that Campath has a different degree of effects on lymphocyte subsets. In particular monocytes, it was noted that monocytes are more resilient to Campath and

the residual monocyte populations are resistant to a list of conventional immunosuppression types apart from calcineurin inhibitors (77).

My study confirmed previous findings that monocytes depleted immediately following Campath and their level remained suppressed for the first 4 weeks after transplantation [Figure 10-5]. Monocytes repopulated substantially faster compared to the lymphocyte compartment. Monocyte counts in 45% of cohort patients reached a normal level within the first 6 months and 65% of patients' routine blood tests demonstrated normalised monocyte levels by 1 year.

10.4.4 Platelets and haemoglobin

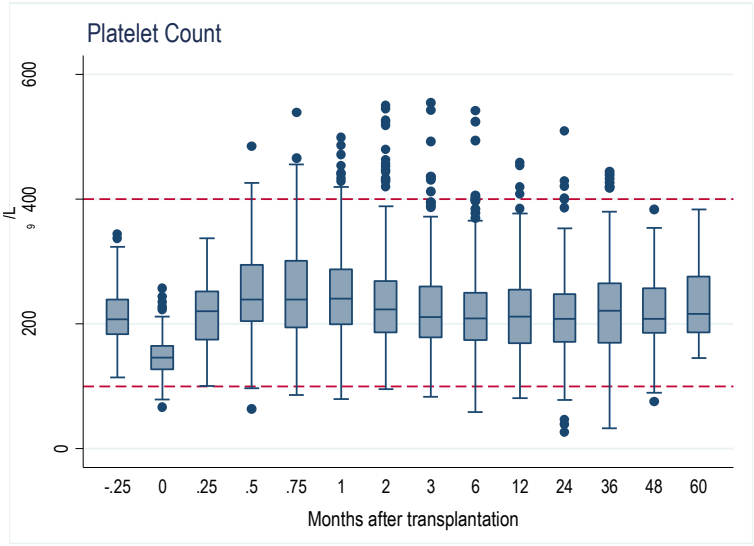

Figure 10-6 Long-term platelet counts following Campath induction

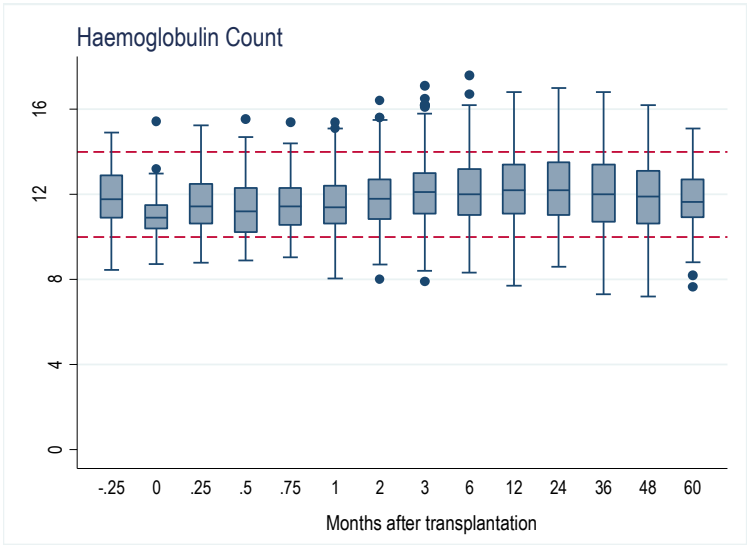

Figure 10-7 Long-term haemoglobin following Campath induction

Figure 10-6 and Figure 10-7 (above) showed the long-term trend of platelet counts and haemoglobin of this cohort. With courtesy to data from Dr. Rawya Charif, we observed 11 cases of haematological autoimmune disorder. 7 cases of idiopathic thrombocytopenic purpura [ITP] alone, 1 case of autoimmune haemolytic anaemia [AHA] and 3 patients experienced both ITP and AHA.

The majority of patients presented with a sudden onset of anaemia and/or thrombocytopenia. All patients received a combination of intravenous immunoglobulin, steroids and rituximab. 1 patient underwent splenectomy. 3 patients required Romiplostim. 2 patients died [1 sepsis, 1 myocardial infarction], and 1 graft was lost following the withdrawal of immunosuppression in the context of sepsis. 7 of the 9 surviving patients achieved complete remission with a normal platelet count and haemoglobin. The remaining 2 patients with ITP are in partial remission, with a platelet count >50x10^9/L, having been treated for less than 3 months.

10.5 Rejection and lymphocyte repopulation

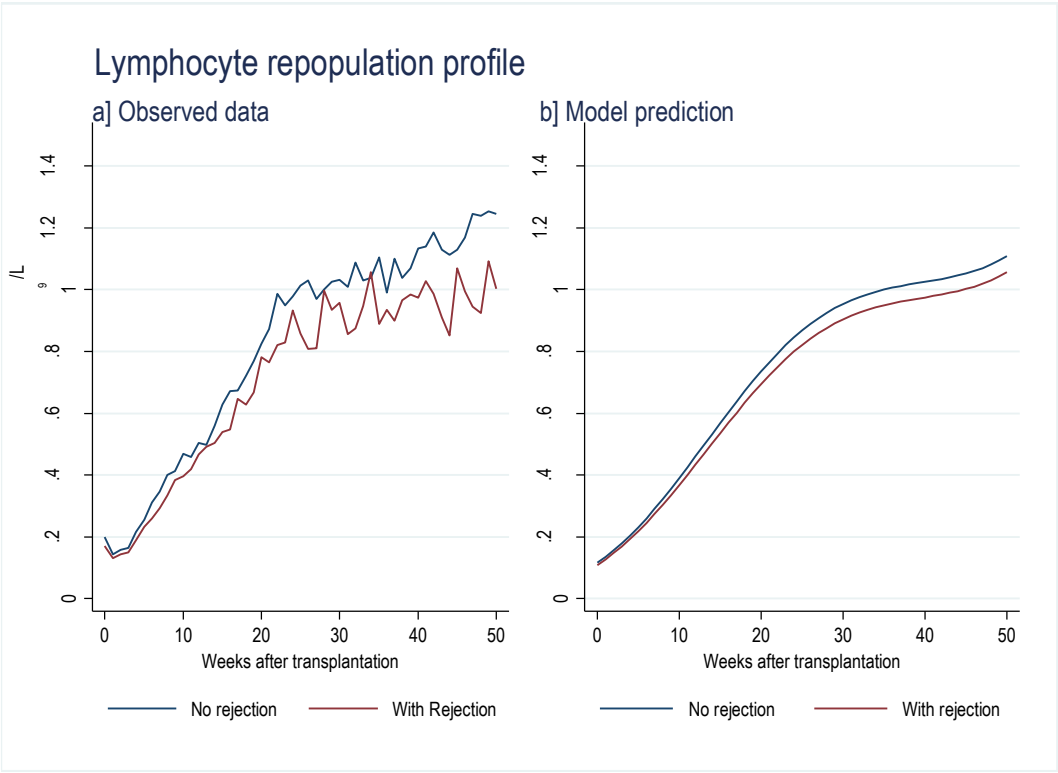

Figure 10-8 Lymphocyte repopulation profiles between rejecters and non-rejecters

Figure 10-8a (above) showed the lymphocyte proliferation profiles in patients who experienced rejection and those who did not have rejection. I was interested in examining whether the

individuals subsequently experienced rejection repopulating their circulatory lymphocyte at a different rate compared to those who did not reject. In order to study this question, the mathematical model used must be adjusted for repeat measurement in an individual so as to examine the underlying trend over all groups. Therefore, I fitted a mixed-effect model to examine the trajectory of lymphocyte proliferation between the two groups of patients over time. Furthermore, lymphocyte counts measured in routine blood work are highly skewed and the model was fitted in a natural log scale as a result. As a result of the estimation, the model is fitted in Figure 10-8b and is displayed in the table below.

Repopulation of lymphocytes in Campath-treated patients follows a cubic mathematical function manner. Therefore, there was an early, sharp rise in lymphocyte counts following time 0 and the rate of repopulation changes over time and reached the plateau at the end of the observation. In this case, we observed the fastest rise in lymphocyte counts in the first 20 weeks following Campath and reached the plateau stage at around 30 weeks after transplantation.

It was estimated that residual lymphocyte counts in the rejecter group of 0.110 $x10^9$/ml compare to 0.117 $x10^9$/ml in those who did not experience rejection subsequently. The difference between the two groups did not reach statistical significance [Relative difference 0.94, 95%CI 0.85, 1.03; p=0.197 Mixed-effect model]. The model also estimated the average difference in the rate of lymphocyte repopulation between the rejecter and non-rejecter of 0.03% per week and this did not reach statistically significant difference [Mean 0.03%, 95%CI -0.34%, 0.40%; p=0.875].

Table 10-2 Mixed-effect model showing differences in lymphocyte repopulations

Factor		Mean	95% CI	p value
Time	Week	16.79%	[16.33, 17.25]	<0.001
	$Week^2$	-0.38%	[-0.40, -0.36]	<0.001
	$Week^3$	0.00%	[0.00, 0.00]	<0.001
Difference in rate of repopulation		0.03%	[-0.34, 0.40]	0.875
Lymphocyte count at day 0	Non-rejecter	0.117 $x10^9$/ml	[0.111, 0.123]	
	Rejecter	0.110 $x10^9$/ml	[0.101, 0.119]	0.197

This study showed repopulation of lymphocytes following Campath induction following a cubic mathematical pattern. There was no statistically significant difference in baseline residual

lymphocyte counts between rejecters and non-rejecters. Furthermore, the rate and pattern of lymphocyte repopulation between the two groups were similar and not associated with acute rejection.

10.6 Rejection and monocyte repopulation

Monocyte repopulation followed a very similar pattern to lymphocyte repopulation, but was slightly more complex with a quartic pattern. The majority of monocytes depleted following Campath induction, but they recovered quickly and reached the plateau stage by around 10 weeks [Figure 10-9].

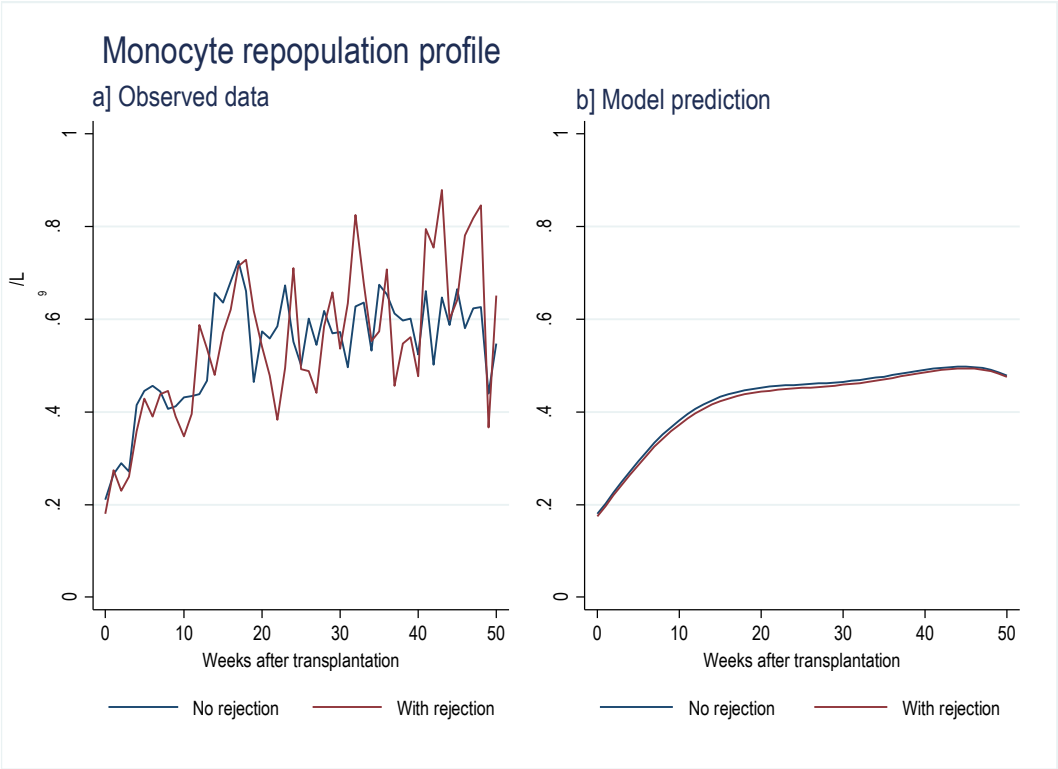

Figure 10-9 Monocyte repopulation profiles between rejecters and non-rejecters

Previous reports suggested that effect of Campath on leucodepletion were variable depending on the sub-type of leucocytes, of which monocytes were most resilient to the effect of Campath and residual monocytes are likely to be resistant to most immunosuppressants apart from calcineurin inhibitors.

In this part of the study, I examined the effects of residual monocytes and the relationship between monocyte repopulation and later rejection. Similar to the lymphocyte repopulation model, I used a mixed-effect model to account for the nature of data.

As with the lymphocyte repopulation, monocytes recover following a cubic manner and recovery was much quicker compared to lymphocytes. The rate of recovery was highest in the first 15 weeks and reached the plateau stage soon after.

This model estimated that monocytes recover at 13.4% per week. My model did not detect a significant difference in the monocyte recovery rate between the groups. It estimated a 0.05% difference [95%CI -0.32%, 0.42%; p=0.801] in the rate between rejecter and non-rejecter.

Residual monocyte counts were 0.180 $x10^9$/ml in the non-rejecter compared to 0.175 $x10^9$/ml in the rejecter and there was no statistically significant difference between the two groups [p=0.611].

Table 10-3 Mixed-effect model showing differences in monocyte repopulations

		Mean	95% CI	p value
Time	week	13.40%	[12.63, 14.16]	<0.001
	$week^2$	-0.63%	[-0.6786, -0.5882]	<0.001
	$week^3$	0.0141%	[0.0126, 0.0157]	<0.001
	$week^4$	0.0001%		<0.001
Difference in rate of repopulation		0.05%	[-0.32, 0.42]	0.801
Monocyte count at day 0	Non-rejecter	0.180 $x10^9$/ml	[0.170, 0.191]	<0.001
	Rejecter	0.175 $x10^9$/ml	[0.158, 0.193]	

10.7 Summary

Campath is a powerful lymphopenic agent and the rates of repopulation of lymphocytes are variable depending on the lymphocyte subset. In this chapter, I examined 96,695 results reported from a routine haemological laboratory on 483 patients from transplantation to investigate the effect of Campath on long-term haematological profiles.

My analysis showed that total white cell counts immediately increase following induction with methylprednisolone and they remained high during the 7-day course of oral prednisolone. Total white cell counts fall after withdrawal of steroids and they appeared to be lower than pre-transplant levels. Total white cell counts start to recover after 6 months and they reach the pre-transplant level at 12 months.

Within the leucocyte compartments, lymphocytes were most strongly affected by Campath. Effects of Campath on lymphocytes were immediate and I demonstrated immediate lymphopenia following induction. Lymphocytes remained low: 99.8% of patients remain significantly lymphopenic in the first month, and they only started to recover after 3 months. I demonstrated that the proportion of patients in the cohort with lymphopenia decreases gradually from 96.8% at 3 months to 52.3% at 12 months, and 75.6% of patients in this cohort had recovered their lymphocyte compartment by 2 years.

Although there was a suggestion that CD52 expresses on neutrophils and that activated CD52 results in neutrophil cell lysis in vitro (148), data obtained from a routine clinical haematological study did not demonstrate Campath incurring significant effects on neutrophils. There was a transient neutrophilia following administration of induction steroids in the first week following transplantation. The overall neutrophil profile appeared to be stable over time. There were 8 cases of neutropenia in this cohort and 5/8 cases were due to other medications, with the remaining 3 cases recovering spontaneously, and there was no evidence to suggest that this transient neutropenia was associated with an increased risk of infection [Chapter 11.3].

It had been previously demonstrated that Campath has a different degree of effects on leucocyte subsets. In particular monocytes, it was noted that monocytes are more resilient to Campath and the residual monocyte populations are resistant to a list of conventional types of immunosuppression apart from calcineurin inhibitors. Results from my data were similar to previous reports in literature: monocytes remained low in the first 4 weeks; however, monocytes recover earlier and faster compared to lymphocytes. Monocyte compartments recovered in 45% of patients in the first 6 months and 65% at 1 year.

My data also demonstrated that Campath has little impact on haemoglobin and platelet counts, at large, although there were 11 cases of haematological disorder reported in this cohort: 7 idiopathic thrombocytopenic purpura, 1 autoimmune haemolytic anaemia and 3 cases of both AHA and ITP. Most patients responded to treatment and the majority of patients achieved total remission with treatment.

Pearl et al. report that lymphopenia following Campath is incomplete and residual cells are mainly memory-effect T cells, which are only sensitive to calcineurin inhibitors (77). Therefore, there was a concern that repopulation of lymphocytes might trigger allograft rejection.

In order to examine the effect of lymphocyte profiles accurately, I used a mixed-effect model to examine the rate of lymphocyte and monocytes change over time between rejecters and non-rejecters. My study showed that neither the trajectories of lymphocyte nor monocyte recovery were different in patients who experienced rejection and in those who did not.

11 Risk

11.1 Introduction

Infection

It has long been established that immunosuppression increases the risk of infection (107, 108) and that the pattern and nature of infection change over time after transplantation (108) and differ with immunotherapy used (109, 110).

It was thought that the powerful lymphodepleting ability of Campath might result in a high risk of infection (42), in particularly CMV infection (65) although this was not supported by subsequent papers with short follow-up (52, 54-57, 98). There were previous reports suggested poor patient survival associated with its use in patients with multiply relapsed vasculitis exposed to Campath 1-H during the 1990s (94), as well as reports of fatal fungal infections in kidney/pancreas transplant recipients exposed to multiple doses (95-97).

Infective risk in the long term following Campath use is unclear, mainly related to the lack of long-term follow-up data in the literature, although the 5-year long-term follow-up pioneer study in Calne's group did not show a substantial difference in the risk of infection in the Campath group (63).

The effect of Campath on the risk of infection appears to be dose-related in both transplantation and rheumatological literatures, which used up to 400mg of Campath per treatment (79, 96, 99). The incidence of infection was higher in patients given a high dose of Campath 1M in the pioneer study in the 1980s (42). Nath et al. (96) reported a 5.6 percentage point increased risk of system fungal infection in patients receiving Campath as maintenance therapy in their pancreas programme, and systemic fungal infection in these patients was associated with high mortality – 3/8 [38%] (97, 98). A further report from Pittsburgh suggested that patients who received Campath as a supplement with concurrent treatment of rejection are associated with a 3.5-fold increase in the risk of opportunistic infection (99).

These data strongly suggest that Campath 1-H should not be used in repeated, multiple-dosing regimens or in individuals already carrying a heavy burden of immunosuppression. However, an extensive body of reports, both cohort studies and randomised prospective trials, is now available to show this when used at total cumulative doses of between 30 and 60 mg.

Malignancy

Chronic immunosuppression not only reduces the immune response of recipients to an allograft, but also reduces a recipient's immune reaction to infection as well as the ability of immune surveillance that prevents growth and development of malignancies. It was also suggested that long-term immunosuppression reduces DNA repair at a molecular level and further increases risk in developing malignancy (102). A recent report analysing data from the UNOS registry suggested a cumulative incidence of malignancy of up to 7.5% at 3 years, of which skin cancer was the most prevalent (103). It was estimated that the rates of skin cancer and lymphoma in transplant recipients were 90 and 37 times higher, respectively, compared to the general population after being adjusted for age. Furthermore, these findings were consistent throughout other registry data from Australia, New Zealand, Denmark and Norway. It was an interesting finding that long-term immunosuppression has a selective effect on skin cancer and lymphoma, which were both found to be linked to a viral pathology with human papilloma virus (104) and Epstein-Barr virus, respectively (105).

An immunosuppression regimen also has a substantial impact on the risk of malignancies: recipients on a tacrolimus-based immunosuppressant regimen have a 35% reduced risk of non-melanoma skin malignancy, whereas those who received azathioprine maintenance increased the risk by 17%.

The risk of skin malignancy is also 17% lower in recipients receiving induction antibodies; this might be confounded by the reduction in Azathioprine use following induction therapy (106).

Post-transplant malignancy is related to an increased morbidity and mortality (149) and the risk of developing malignancy is correlated with intensity and duration of immunosuppression (150). Understanding the nature and risk factors of developing malignancy will add development of

screening and medical management to reduce the impact. However, there are no published data studying the risk of malignancy in Campath-treated patients.

New Onset Diabetes After Transplantation [NODAT]

New Onset Diabetes After Transplantation [NODAT] is a common complication following renal transplantation. It was reported that patients with NODAT have a higher cardiovascular risk and, therefore, a poorer long-term survival, as well as inferior allograft function (151-153). A single-centre study from the Norwegian group reported a long-term follow-up longitudinal study that examined the cardiovascular risk in patients with NODAT, suggesting that cardiovascular mortality and risk of myocardial infarction increase by 3.27 times from 7% to 20% at 8 years. Furthermore, overall all-cause mortality increased from 20% to 37% in patients with NODAT (152).

The true incidence of NODAT is unclear. It was reported that the NODAT rate was as high as 46% in the first year under a high-dose steroid regimen in 1979 (154), and the rate of NODAT falls as the steroid-sparing protocol becomes more popular with more powerful immunosuppression. It was reported that the incidence of NODAT ranged between 2% and 50% in the first 3 months after transplantation and that variables depended on the immunosuppressive protocol used (155).

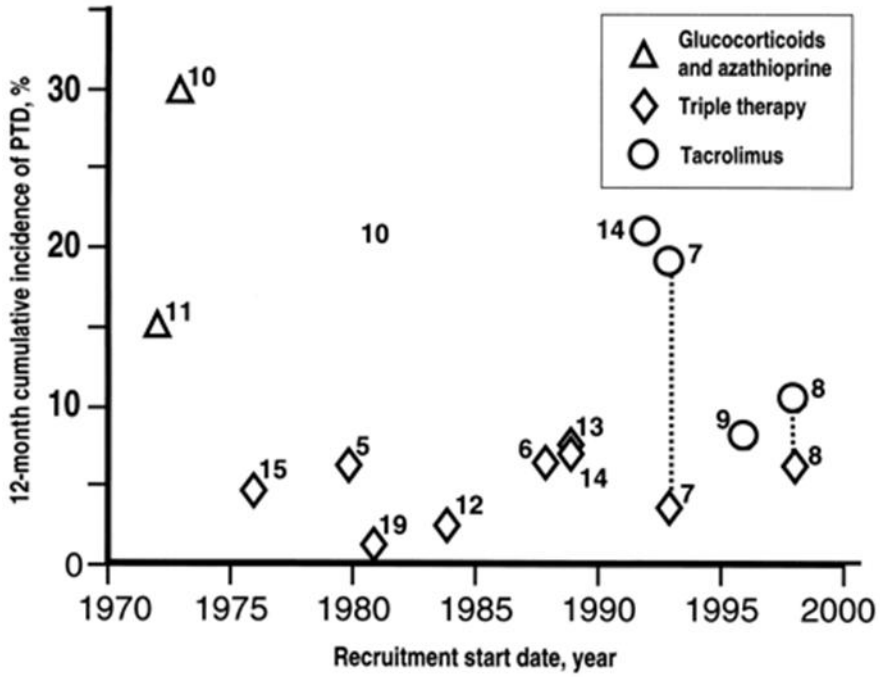

Figure 11-1 Incidence of NODAT over time and immunosuppressant (155)

Many risk factors had been identified as being associated with the increased risk of NODAT.

Recipient Age. It was noted that NODAT patients were generally older recipients compared to the non-NODAT group. It estimated that recipients older than 45 years have a 2.2-fold increase in the risk of developing NODAT (156), as well as there being a further 2.5-fold increase in the risk of NODAT in recipients older than 65 (157).

Obesity. It has long been established that obesity is associated with increased risk of type II diabetes mellitus and its effects appeared to be more pronounced in transplant recipients. In a large US study, it was reported that patients who have a BMI>30kg/m2 at transplantation have a 1.73 increased risk of NODAT post-transplantation (151, 157, 158). However, the effect of weight gain post-transplantation is not well studied.

Ethnicity. There is a substantial risk in developing NODAT between ethnicities. It had been reported consistently that Afro-Americans and Hispanics have a significantly increased risk of NODAT compared to Orientals and Caucasians (153, 157-159). Most of these studies are based on the UNOS US database, and the risk of NODAT in South Asians is not well documented in the literature.

Medications. It is well known that long-term use of corticosteroids is associated with a high risk of diabetes, even with a small maintenance dose. Its effects on NODAT were first reported in 1949 and showed 46% of patients developing NODAT within the first year after 1 year of steroid exposure (154). Subsequent large epidemiological studies confirm the association of NODAT and steroid use in transplantation (153, 158, 159).

There are consistent data in the literature suggesting that calcineurin inhibitors are also associated with an increased risk of NODAT (153, 158, 159) and that there is a significantly higher risk of NODAT with tacrolimus compared to cyclosporine. A 2004 meta-analysis reported a 7.1% increase in the risk of insulin-dependent diabetes in tacrolimus-treated patients compared to those treated with Cyclosporine (160). Furthermore, a large randomised controlled study reported a higher rate of NODAT in tacrolimus-treated patients compared to those treated with cyclosporine or Sirolimus (161).

In terms of effects of induction agents, UNOS registry data showed that patients who received Campath induction have a 48% reduction in the risk of NODAT (21) which is most likely related to a spare use of steroids in immunosuppressant maintenance following Campath induction.

There is little in the literature describing the precise nature and rate of infection, type and timing of malignancy and the risk of NODAT in recipients receiving this MMF-free, steroid-free immunosuppressive regimen following Campath induction.

And in this chapter, I am going to address this issue with experience from the Imperial College Kidney and Transplant Centre.

11.2 Patients and methods

Patients

This is a retrospective study based on data collected on subjects transplanted betwee Nov 2005 and March 2011 for the Imperial College Kidney and Transplant Centre transplant registry. The reason for the data range being up until 1st March 2011 is so all patients will have at least 12 months follow-up prior to data analysis.

The use of data was authorised by the Imperial College Kidney and Transplant Centre transplant research group. Patient data were anonymised to guarantee patient confidentiality.

11.3 Infection

11.3.1 Methods

The purpose of this study is to examine the nature and trend of infection in our transplant patient cohort, who were treated with this simple minimalistic protocol consisting of Campath induction and tacrolimus monotherapy.

Primary objectives of this study were:

1. Incidence of infection over time following Campath induction
2. Nature of infection over time following Campath induction
3. Investigate the concern of Campath-induced lymphopenia and risk of infection
4. Examine the effect of female gender, diabetes, reflux nephropathy and treatment of rejection on infection risk

For the purpose of this study, all infections recorded with microbiological evidence over time in this cohort were included up until patient loss to follow-up or when a patient returns to dialysis. All infections were diagnosed based on microbiological evidence. Positive urine, bronchiolar lavage, drain fluid, ascites and blood cultures constituted significant episodes of bacterial infection. Cytomegalovirus PCR positivity [>1000 copies/ml] and biopsy-proven BK virus nephropathy [intranuclear inclusion bodies with positive staining for SV40] were considered to be significant markers of viral infections. [2]

In order to examine the effects of Campath-induced haematogical anomalies on the risk of infection, a subject-specific detailed profile on full haematological reports and infection incidences over transplant follow-up on all subjects were created. A random-effect longitudinal statistical model was then developed to analyse the effects of risk factors on infection risk.

[2] Adapted from Horan et al., Surveillance of nosocomial infections in Mayhall CG. Hospital epidemiology and infection control 2004:1659.

Furthermore, this analysis on the associations with infection risk and factors was stratified into a timeline following transplantation for the following reasons:

- During the initial examination of data, it became clear that the risk of infection changes over time [Table 11-1].
- The follow-up in the cohort differs depending on the transplant vintage. This might cause bias with the potential missing data and this would be best handled with stratification.
- Lastly, one would argue that the effects of risk factors on infection risk differ over time.

This piece of work was done in collaboration with Dr. Rawya Charif and I would like to acknowledge the help from Dr. Charif – she collected and permitted the use of data regarding the incidence of infection in this cohort for my work. My involvement comprised study development, collection of clinical data and haematology profiles on all transplant recipients, as well as data analysis and statistics modelling.

11.3.2 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Allograft function and lymphocyte data were analysed using a mixed-effect model to account for the nature of repeat measurements over time, as well as allowing adjusting the effects of lymphocyte changes over time. Continuous variables were log-transformed into normal distributed data to improve adherence to model assumptions if needed.

Incidences of infection were presented as the incidence per 100 patient years. Poisson was used to analyse rate and rate ratio; a Negative binomial model is used in cases when the underlying assumption of equal mean and variance with the Poisson model is violated.

Association of risk factors [e.g. gender, lymphopenia] and infection was examined using a random-effect logistic regression model to adjust for repeat measurements over time. Results were presented as odds ratios, which can be interpreted as the relative odds of developing the infection type of interest in the presence of the risk factors.

11.3.3 Results

11.3.3.1 Overall infection risk

Figure 11-2 (below) shows the overall rate of infections following Campath induction. Infection rates were shown in the incidence per 100 patient years to adjust for time of follow-up. The overall incidence of infection is highest in the first 6 months after transplantation. It was estimated that the rate of infection reached 141 incidences per 100 patient years [Table 11-1], gradually falling in the subsequent months, as expected: 50.1, 37.4, 32.2 and 19.4 incidences per 100 patient years at 6-12 months, 12-24 months, 24-36 months and 36-48 months following transplantation, respectively.

The nature of infections did not change substantially over time, and urinary tract infection was the commonest cause of infection throughout all timelines. It accounts for approximately 50% of all cases reported throughout follow-up. Despite the profound lymphopenia, the incidence of viral infection remains low late after transplantation. Details of the nature of infection will be examined in more detail in the latter part of this chapter.

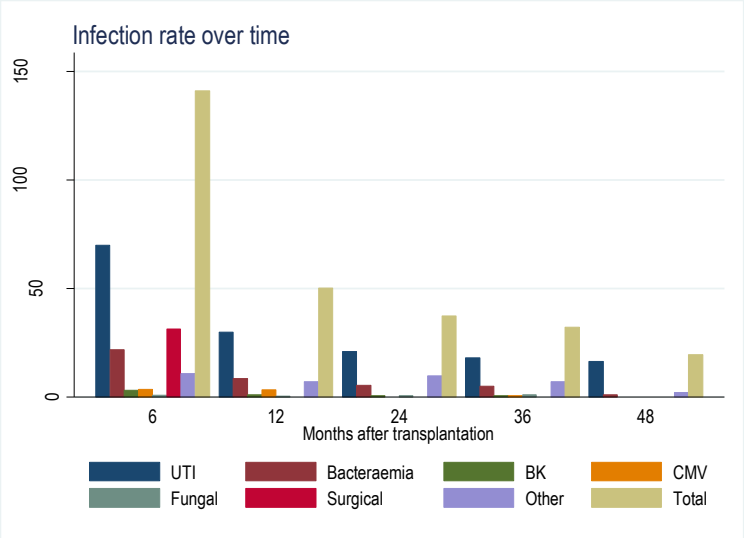

Figure 11-2 Incidence of infection after Campath induction

Table 11-1 Rate [Incidences per 100 patient years] and nature of infection after Campath induction

	6 months	6-12 months	12-24 months	24-36 months	36-48 months
Surgical	31.3 [24.81,39.37]				
UTI	69.9 [59.88,81.56]	29.8 [23.27,38.12]	21.0 [16.42,26.91]	18.0 [12.80,25.33]	16.4 [10.03,26.73]
Bacteraemia	21.7 [16.45,28.64]	8.5 [5.36,13.51]	5.3 [3.27,8.72]	4.9 [2.56,9.44]	1.0 [0.14,7.27]
BK	3.0 [1.45,6.37]	0.9 [0.24,3.78]	0.7 [0.17,2.67]	0.5 [0.08,3.87]	0.0 [0.00,0.00]

CMV	3.5 [1.74,6.94]	3.3 [1.58,6.94]	0.0 [0.00,0.00]	0.5 [0.08,3.87]	0.0 [0.00,0.00]
Fungal	0.9 [0.22,3.47]	0.5 [0.07,3.36]	0.7 [0.17,2.67]	1.1 [0.27,4.36]	0.0 [0.00,0.00]
Other	10.9 [7.33,16.06]	7.1 [4.27,11.76]	9.7 [6.73,13.93]	7.1 [4.12,12.22]	2.0 [0.51,8.19]
Total	141.1 [126.54,157.27]	50.1 [41.42,60.62]	37.4 [31.06,44.98]	32.2 [24.94,41.55]	19.4 [12.41,30.49]

Risk factors

First 6 months

The overall infection rate was highest in the first 6 months following transplantation [Figure 11-2]. Univariate analysis demonstrated that the risk of overall infection is time-dependent, with background diabetes, reflux nephropathy and female gender being positively associated with increased risk of infection [Appendix 14.1.1, Table 14-1].

A multivariable model was used to examine the combined effects of all these factors [Appendix 14.1.1, Table 14-1]. Table 11-2, which consists of factors that have a significant impact on infection risk, confirms that infection risk is time-dependent. It estimated a reduction of 7% in risk in every week following transplantation. Patients with background diabetes have a 2.1-fold increase in the risk of infection [OR 2.12, 95%CI 1.31, 3.44; p=0.002], those with reflux nephropathy experienced a 3.8-fold increased risk of infection [OR 3.8, 95%CI 1.81, 7.95; p<0.001], and women have an 80% increased risk of infection in the first 6 months after transplantation [OR1.80 95%CI 1.22, 2.68; p=0.003]. Interestingly, there is a suggestion that patients who had a change of immunosuppressant as a result of rejection experienced a 71% increased risk of infection, although it did not reach statistical significance [OR1.71, 95%CI 0.99, 2.96; p=0.054].

Data did not suggest early lymphopenia or neutropenia being associated with an increased risk of infection.

After 6 months

After the initial 6 months after transplantation, patients with reflux nephropathy are persistently associated with an increased risk of infection. It was estimated that these patients have a 5.1-, 4.0-, 3.0- and 3.3-fold increase in the risk of infection 6 months and annually afterwards [Table 11-2]. This is likely due to the higher incidence of urinary tract infection in this group [Table 14-3].

Treatment changes after late rejection are associated with an increased risk of infection. Data suggest that patients treated for rejection 24 months after transplantation have a 4-fold increased risk of infection [OR 4.04, 95%CI 1.66, 9.87; p=0.002].

Despite the profound lymphopenia, defined as total lymphocyte counts less than 1 x 10^9 per 1mm, data did not show an increased risk of infection in the first 12 months after transplantation [0-6 months: OR 0.65, 95%CI 0.38, 1.13; p=0.127, 6-12 months: OR 1.55, 95%CI 0.85, 2.83; p=0.150]. Although there is some evidence suggesting a 2.22-fold increased infection risk in the second year of post-transplantation with lymphopenia, data from the later years did not support this finding. Neutropenia is rare; it was not feasible to accurately estimate the effects of neutropenia on overall infection risk. However, it did not appear to have a clear association with neutropenia and infection in this cohort.

Table 11-2 Final multivariable analysis on overall infection risk

		Final model	
	Risk Factors	Odds Ratio	p value
0-6 months	Week	0.93 [0.91,0.94]	0.000
	Lymphopenia		
	Neutropenia		
	Diabetes	2.12 [1.31,3.44]	0.002
	Reflux nephropathy	3.80 [1.81,7.95]	0.000
	Female gender	1.80 [1.22,2.68]	0.003
	Post-rejection	1.71 [0.99,2.96]	0.054
6-12 months	Week	1.02 [0.99,1.05]	0.216
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy	5.13 [1.67,15.77]	0.004
	Female gender		
	Post-rejection		
12-24 months	Week	1.00 [0.99,1.02]	0.743
	Lymphopenia	2.22 [1.24,3.96]	0.007
	Neutropenia		
	Diabetes		
	Reflux nephropathy	3.96 [1.56,10.03]	0.004
	Female gender		
	Post-rejection		
24-36 months	Week	0.98 [0.96,1.00]	0.095
	Lymphopenia		

	Neutropenia		
	Diabetes		
	Reflux nephropathy	6.16 [1.99,19.10]	0.002
	Female gender	3.01 [1.23,7.35]	0.016
	Post-rejection	4.04 [1.66,9.87]	0.002
36-48 months	Week	1.00 [0.97,1.04]	0.884
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy	3.25 [0.95,11.03]	0.059
	Female gender	3.19 [1.03,9.93]	0.045
	Post-rejection		

11.3.3.2 Urinary tract infection

Urinary tract infection was the commonest cause of infection following transplantation. It was estimated that there is an incidence of 69.9/100 patient years in the first 6 months and that accounts for approximately 50% of cases of infection observed throughout the timeline [Table 11-1]. Coliforms are the commonest pathogen; they account for 52.6% of all cases of urinary tract infection and the data did not suggest a change of pathogen over time. The rate of urinary tract infection being highest in the first 6 months might be related to the use of ureteric stents following transplantation in our centre.

The incidence of urinary tract infection fell to 29.8 cases/100 patient years at the second 6 months post-transplantation and between 16.4 and 210 cases/100 patient years between 12 and 48 months after transplantation [Table 11-1].

Risk factors

Reflux nephropathy and female gender were associated with a high risk of urinary tract infection independently [Section 14.1.3, Table 14-3]. The final adjust model suggested that patients with background reflux nephropathy are associated with a 3.7-fold increased risk of urinary tract infection in the first 6 months and that they remained raised throughout the subsequent 42 months thereafter.

Female patients also have a higher risk of urinary tract infection, as described in the literatures; it was estimated that female patients have a 2.7-3.95 times increased risk of urinary tract infection throughout the first 4 years after transplantation.

Lymphopenia and neutropenia following Campath induction have a little effect on the risk of urinary tract infection [Table 11-3]

Table 11-3 Final multivariable analysis on urinary tract infection

		Final model	
	Risk Factors	Odds Ratio	p value
6 months	Week	0.97 [0.95,1.00]	0.042
	Lymphopenia	0.52 [0.28,0.98]	0.044
	Neutropenia		
	Diabetes	1.90 [1.00,3.60]	0.049
	Reflux nephropathy	3.65 [1.46,9.09]	0.006
	Female gender	2.71 [1.61,4.58]	0.000
	Post-rejection		
6-12 months	Week	1.01 [0.97,1.04]	0.720
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy	4.94 [1.42,17.27]	0.012
	Female gender	3.23 [1.46,7.14]	0.004
	Post-rejection	3.26 [1.47,7.23]	0.004
12-24 months	Week	1.00 [0.98,1.02]	0.877
	Lymphopenia		
	Neutropenia	10.57 [0.92,121.63]	0.059
	Diabetes		
	Reflux nephropathy	5.82 [1.76,19.29]	0.004
	Female gender	2.41 [1.01,5.75]	0.047
	Post-rejection		
24-36 months	Week	0.99 [0.96,1.03]	0.692
	Lymphopenia	2.63 [0.99,7.04]	0.053
	Neutropenia		
	Diabetes		
	Reflux nephropathy	13.08 [4.00,42.80]	0.000
	Female gender	3.49 [1.16,10.52]	0.027
	Post-rejection		
36-48 months	Week	1.00 [0.97,1.04]	0.928
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy	3.44 [0.97,12.17]	0.055
	Female gender	3.95 [1.16,13.43]	0.028
	Post-rejection		

11.3.3.3 Surgical-related infections

In the first 6 months, surgical wound-related infection also contributed to a substantial amount of infection incidences observed. Surgical wound-related infection accounts for approximately a quarter of infection observed. It was estimated that there is a rate of 31.3 incidences per 100 patient years [Table 11-1].

Risk Factors

Surgical infection is highest in early post-transplantation; data confirms that surgical infection is strongly time-dependent and that risk falls by 34% per week following transplantation in Surgical infection [Section 14.1.2, Table 14-2]. After adjusting for time, the final model estimated that surgical infection was highest in those with diabetes – these patients experienced a 2.5-fold increase in the risk of wound infection [OR 2.49, 95%CI 1.43, 4.34; p=0.001] – as well as those who were treated for rejection [OR 2.89, 95%CI 1.29, 6.48; 0.010] [Table 11-4].

Apart from risk factors examined in this model, body habitus was also a significant risk factor for wound infection. We have demonstrated that patients in the highest body mass quadrant experienced a 2.1-fold increased risk of wound infection [Section 7.4.6].

Table 11-4 Final model on risk of surgical infection

		Final model	
	Risk Factors	Odds Ratio	p value
6 months	Week	0.63 [0.56,0.71]	0.000
	Lymphopenia		
	Neutropenia		
	Diabetes	2.49 [1.43,4.34]	0.001
	Reflux nephropathy		
	Female gender		
	Post-rejection	2.89 [1.29,6.48]	0.010

11.3.3.4 BK nephropathy

There were 12 cases of biopsy-proven BK nephropathy in the cohort, with the highest rate of BK nephropathy being in the first 6 months after transplantation [Table 11-1]; the median time from transplantation to diagnosis of BK nephropathy was 24 weeks. Interestingly, 7/12 [58%] cases happened soon after treatment of rejection. This reflected in the final model that rejection is associated with a 4.2-fold increased risk of BK nephropathy in the first 6 months [OR4.15, 95%CI 0.98, 17.56; p=0.053]. However, because of the little number of cases of late BK, it was difficult to accurately estimate the impact of rejection on the risk of BK nephropathy later after transplantation. This model did not show an association between lymphopenia, neutropenia, diabetes, reflux nephropathy and female gender with BK nephropathy at any time point.[Table 11-5]

Table 11-5 Final model on risk of BK nephropathy

		Final model	
	Risk Factors	Odds Ratio	p value
6 months	Week	1.12 [0.99,1.25]	0.062
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Female gender		
	Post-rejection	4.15 [0.98,17.56]	0.053
6-12 months	Week	1.12 [0.99,1.25]	0.062
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection	4.15 [0.98,17.56]	0.053
12-24 months	Week	1.04 [0.94,1.15]	0.417
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
24-36 months	Week	0.97 [0.84,1.12]	0.685
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
36-48 months	Week		
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		

11.3.3.5 CMV disease

All patients in our centre received 3 months of oral valgancidovir as CMV prophylaxis – a total of 14 cases of CMV infection were noted in the cohort. The median time to CMV disease was 26 weeks after transplantation. There was 1 case of CMV infection noted within the 3-month prophylaxis period; this patient had the prophylactic antiviral stopped because of lymphopenia previously. All cases were successfully treated with intravenous gancidovir and an antiviral-resistant strain was not detected.

Almost all (but 1) cases were detected within the first year and the last case was detected at 105 weeks after transplantation [Table 11-1].

Risk factors

The overall risk of CMV disease remains low despite the profound lymphopenia, and the statistical model confirms the lack of correlation between lymphopenia and CMV disease.

Our data showed that the risk of developing CMV disease was not increased following treatment of rejection, despite the increased immunosuppression burden following treatment of rejection. This might be related to the liberal use of CMV prophylaxis following immunosuppressant escalation in our unit.

Furthermore, the model confirms that neutropenia, diabetes mellitus, reflux nephropathy and female were not associated with CMV disease [Table 11-6].

Table 11-6 Final model on risk of CMV disease

	Risk Factors	Final model Odds Ratio	p value
6 months	Week	1.14 [1.02,1.27]	0.021
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
6-12 months	Week	1.00 [0.90,1.11]	0.984
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
12-24 months	Week	No cases	
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
24-36 months	Week	NA	
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
36-48 months	Week	No cases	
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		

11.3.3.6 Bacteraemia

The risk of developing bacteraemia was highest in the first 6 months following transplantation. It was estimated that there is an average of 21.7 episodes per 100 patient years in the first 6 months and that it falls to 8.5, 5.3, 4.9 and 1.0 episodes per 100 patient years at 6-12 months and annually afterwards [Table 11-1].

The high rate of bacteraemia is likely secondary to the high incidences of wound infection [Section 11.3.3.3] and urinary tract infection [Section 11.3.3.2] in the first 6 months.

A total of 133 episodes of bacteraemia were recorded. Coagulase Negative Staphylococcus and Coliforms were the commonest pathogens, and the nature of the common pathogens did not change over time.

Risk Factors

In the first 6 months after transplantation, initial multivariate analysis suggested that patients with diabetes mellitus and reflux nephropathy have an increased risk of bacteraemia [Section 14.1.6, Table 14-6]. However, the final model did not show these factors having any significant impact on bacteraemia after adjustment [Table 11-7].

Treatment of late rejection is associated with a 7.89-fold increase in the risk of bacteraemia at 2 years after transplantation.

The model didn't show either lymphopenia or neutropenia following Campath induction increasing the risk of bacteraemia. There was no evidence suggesting diabetes, reflux nephropathy or female gender increasing the risk of bacteraemia in the long term.

Table 11-7 Final model on risk of bacteraemia

		Final model	
	Risk Factors	Odds Ratio	p value
6 months	Week	0.94 [0.90,0.98]	0.005
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
6-12 months	Week	1.02 [0.95,1.09]	0.623
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
12-24 months	Week	1.01 [0.97,1.06]	0.488
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		
24-36 months	Week	0.99 [0.94,1.05]	0.841
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection	7.89 [1.44,43.07]	0.017
24-36 months	Week		
	Lymphopenia		
	Neutropenia		
	Diabetes		
	Reflux nephropathy		
	Female gender		
	Post-rejection		

11.3.3.7 Fungal infection

There are reports from the literature suggesting that the use of Campath is associated with an increased risk of fungal infection, particularly when it is used as maintenance immunosuppression. In our study, fungal infections are rare.

We observed 0.9 cases per 100 patient years in the first 6 months and 0.5 cases per 100 patient years between 6 and 12 months post-transplantation. Incidences of fungal infections were largely unchanged annually thereafter [Table 11-1].

20 cases of fungal infection were documented in this cohort. There were 4 cases of aspergillus pneumonia and 2 cases of Pneumocystis pneumonia, all of which were diagnosed by bronchial lavage or sputum. There had been 7 cases of candidaemia in 6 patients, and 2 cases of subcutaneous fungal infections with dematiaceous fungi and pyrenchaeta romoroi.

The risk factor analysis did not show any association of fungal infection with lymphopenia, neutropenia, diabetes, reflux nephropathy, female gender or rejection [Section 14.1.7, Table 14-7].

11.3.3.8 Other infections

For the purpose of this study, all infection that was not classified in the above category was pooled and classed as “other infection”. Chest pathogen was the commonest cause of infection in this category; it accounts for 27/109 [24.8%] of the cases observed. 22/109 [20.2%] infections were related to non-surgically related wound infection.

Over the follow-up period, we observed 3 cases of H1N1 infection and 3 cases of Parovirus B19 infection.

Overall, we were not able to demonstrate any significant association between risk of development of other infection and lymphopenia or neutropenia [Section 14.1.8, Table 14-8].

11.4 Malignancy

11.4.1 Methods

Primary objectives of this study were:

1. Incidence and nature of malignancies over time following Campath induction
2. Examine the effect of gender, ethnicity, previous immunosuppressive therapy and age on risk of developing malignancies.

All malignancies were identified clinically and confirmed with histological diagnosis when possible.

11.4.2 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Times to malignancies were analysed using an age-adjusted survival model to account for effects of age on malignancy risk.

Effects of transplant vintage on malignancy risk were analysed using Nexus expansion to allow adjustment on patient levels. Risk factors were analysed using a Cox proportional hazard model, under an assumption that risk between groups within a factor was proportional throughout time. A Weilbull parametric model was used in cases where this assumption is violated.

11.4.3 Results

11.4.3.1 Incidence

13 [2.2%] cases of malignancy were identified in the cohort of 602 patients [Table 11-8]. Figure 11-3 (below) shows cumulative freedom from malignancy in the cohort from transplantation. Cumulative risk of developing malignancy in this cohort was 0.5%, 3.1% and 3.5% at 1, 3 and 5 years, respectively.

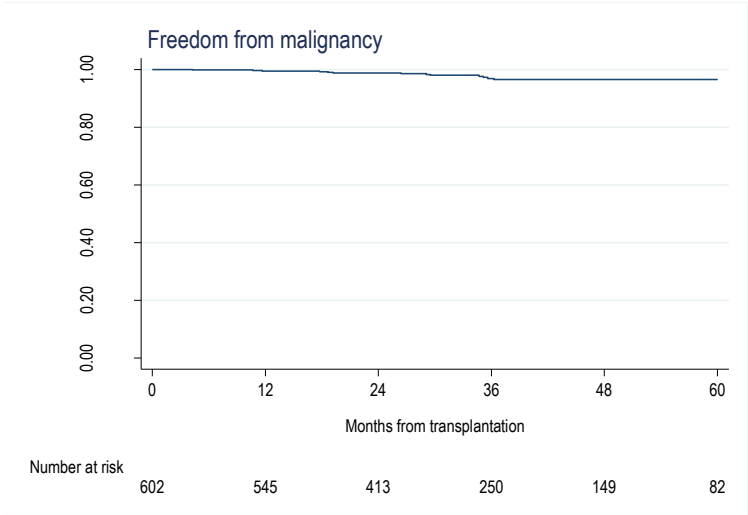

Figure 11-3 Malignancy-free survival

11.4.3.2 Type of malignancies

The commonest malignancy identified was skin in origin and accounted for 4/13 [30.8%] cases; there were 3 [23.0%] cases of malignancy related to the gastrointestinal system and 2 [15.4%] cases of renal cell carcinoma. There was one case of angiosacroma, one case of breast carcinoma, one case of post-transplant lymphoproliferative disorder and one case of prostate cancer.

Table 11-8 Malignancy type

Type	Frequency
Angiosacroma	1
Breast	1
PTLD	1
Prostate	1
Renal	2
Gastrointestinal	3
Skin	4

Skin

There was a total of 4 skin malignancies identified. All 4 recipients were Caucasians and were diagnosed at 4.3, 17.8, 18.7 and 34.7 months following successful living donor transplantation, respectively. Histological examination from 3 cases demonstrated squamous cell carcinoma and one case of basal cell carcinoma. All 4 cases were successfully treated, but one patient died with complication of encapsulating sclerosing peritonitis 26 months following diagnosis.

GI

3 cases of gastrointestinal malignancies were identified. 1 patient was diagnosed with spindle cell carcinoma in the stomach at 29.5 months following transplantation and was successfully treated. 1 case of metastatic pancreatic cancer and 1 case of metastatic adenocarcinoma, possibly in the lower bowel origin, was diagnosed at 35.1 and 26.4 months following transplantation, respectively. Unfortunately, both patients with metastatic diseases died within 6 months of diagnosis.

Renal

2 cases of renal tumours were identified in this cohort. 1 case of multifocal papillary renal cell carcinoma in the native kidney at 11.7 months after transplantation was successfully treated with native nephrectomy. There was no clinical suggestion of recurrence at the last follow-up at 4 years after treatment. The second patient underwent bilateral nephrectomy for renal cell carcinoma and was presented late at 35.6 months after transplantation with metastatic disease, and died 2 years following diagnosis.

Others

A case of prostate carcinoma and a case of breast carcinoma was diagnosed at 10.7 months and 29.1 months respectively. One case of EBV pos post-transplantation lymphoproliferative disorder was diagnosed at 19 months and was successfully treated with a course of intravenous rituximab. Lastly, one patient developed angiosacroma over a functional fistula at 36.3 months after transplantation and died with metastatic disease.

11.4.3.3 Risk factors

Table 11-9 (below) summarised the result of an age- and time lapse-adjusted univariable analysis on effects of ethnicity, previous immunosuppression with cyclophosphamide for vasculitis and gender on the risk of developing malignancy. All three models confirm the risk of malignancy increasing with time [$p<0.05$, not listed].

The model did not show a statistically significant relationship between ethnicity, previous immunosuppressant exposure and gender to the risk of developing malignancy under this regimen.

Table 11-9 Age and time-adjusted univariable analysis on risk factors for development of malignancy

Risk factors		HR	95%CI	p
Ethnicity	Caucasian	1		
	Afro-Caribbean	2.6	0.75,8.73	0.135
	South Asian	0.38	0.07,1.83	0.227
	Others	NA		
Vasculitis	N	1		
	Y	3.1	0.39,25.32	0.282
Gender	F	1		
	M	0.51	0.14,1.87	0.312

11.5 New Onset Diabetes After Transplantation

In this part of the thesis, I am going to report the incidence and timing of NODAT in this cohort of patients receiving a steroid-sparing maintenance regimen, as well as examine risk factors of developing NODAT, including the effect of South Asians and effects of weights after transplantation.

11.5.1 Methods

Primary objectives of this study were:

1. Incidence of NODAT over time following Campath induction
2. Examine the effect of gender, ethnicity, previous immunosuppressive therapy and age on risk of developing NODAT.

NODAT is defined as the requirement of starting antiglycaemic medications post-transplantation.

11.5.2 Statistics

All statistical analyses were performed using Stata 12 [StataCorp, Texas].

Pre-transplant risk factors were analysed using a Cox proportional hazard model, under an assumption that risk between groups within a factor was proportional throughout time. A Weilbull parametric model was used in cases where this assumption is violated.

Post-transplant risk factors, e.g. weight changes, were analysed using a time-adjusted logistic regression model in the same patient and risk on NODAT.

11.5.3 Results

433/602 [71.9%] non-diabetic patients in the whole study cohort were included in this analysis. 56 patients developed NODAT during the study period. Cumulative NODAT survival is best depicted in Figure 11-4 below; 1-, 3- and 6-year NODAT survival was 92.2%, 86.2% and 84.2%, respectively.

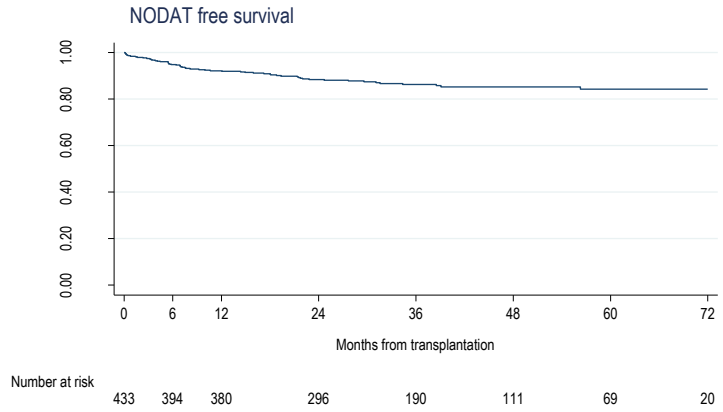

Figure 11-4 NODAT-free survival

There is a substantial difference in the incidence of NODAT between ethnicity origins. Caucasoid [6.1%] has a much lower incidence compared to Afro-Caribbean [27.7%] and South Asians [19.2%]. The groups developing NODAT have a higher mean age at transplantation compared to the group without NODAT [51.6 vs 46.0 yrs.].

It did not appear to have a substantial difference between gender, donor group, donor age and HLA mismatch between the two groups. Interestingly, there is no substantial difference between pre-transplant weights between the two groups [Table 11-10].

Table 11-10 Demographics - NODAT patients

		NODAT -ve	**NODAT ±ve**
Donor type	Deceased	196 [86.7%]	30 [13.3%]
	Living Donor	181 [87.4%]	26 [12.6%]
Ethnicity	Afro-Caribbean	34 [72.3%]	13 [27.7%]
	Asian	101 [80.8%]	24 [19.2%]
	Caucasian	216 [93.9%]	14 [6.1%]
	Other	26 [83.9%]	5 [16.1%]
Gender	Female	143 [87.2%]	21 [12.8%]
	Male	234 [87%]	35 [13%]
Recipient age		46.0±14.71	51.6±11.68
Donor age		38.5±19.11	42.7±16.96
HLA MM		3.5±1.78	3.7±1.91
Pre-transplant weight		75.3±17.24	75.9±17.74

Risk factors

Univariant analysis showed that ethnicity and transplant age are significant factors associated with development of NODAT. Patients who are Afro-Caribbean and South Asian in origin have a 5.0- and 3.4-fold increase in the risk of developing NODAT compared to Caucasoid. Moreover, there is a 3% increased risk of NODAT for every year increase in age at transplantation. Both factors appeared to be independent, and effect size did not change within a multivariant analysis.

On the contrary to data reported in the literature, neither donor type nor pre-transplant weight have a significant impact on subsequent risk of NODAT. There is no significant association between gender, donor age and HLA mismatch to the risk of NODAT [Table 11-11].

Table 11-11 NODAT: Pre-transplant risk factors

	Univariant model			**Multivariant model**		
Factors	**HR**	**95%CI**	**P value**	**HR**	**95%CI**	**P value**
Donor type						
DD	1					
LD	0.9	0.55,1.57	0.773			
Ethnicity						
Caucasian	1					
Afro-Caribbean	5.0	2.36,10.69	0.000	5.3	2.49,11.33	0.000
South Asian	3.4	1.77,6.61	0.000	3.4	1.77,6.63	0.000
Other	3.3	1.17,9.07	0.023	4.0	1.42,11.16	0.009
Gender						
Female	1					
Male	1.1	0.62,1.83	0.827			
Age [per year]	1.03	1.01,1.05	0.004	1.03	1.01,1.05	0.002
Donor age [per year]	1.0	0.97,1.01	0.337			
HLA MM	1.1	0.92,1.25	0.381			
Pre-transplant weight						
Under 62.3kg	1					
62.3-73.3kg	1.2	0.57,2.50	0.645			
73.3kg-86.0kg	1.0	0.49,2.26	0.903			
Above 86.0kg	1.2	0.59,2.62	0.560			

The model above only examined the risk factors before transplantation. A patient's body habitus changes significantly following transplantation, mainly due to improvement in appetite because of the non-uremic states and lifting of dietary restrictions. Post-transplant factors incurred a substantial risk on development of NODAT; however, conventional medical literature did not examine these issues in a systemic and mathematically sound analysis. Therefore, I am going to examine the effects of the post-transplantation risk factors in this time-adjusted model. We have established that age at transplantation is a significant risk factor of developing NODAT and we examined the risk factor using an age-adjusted model.

Univariant analysis confirmed age, ethnicity, post-transplant weight and steroids after rejection having had a significant impact on the risk of developing NODAT. Gender has little impact on the risk. In an age-adjusted multivariant model, we showed patients from an Afro-Caribbean background having an 8.1-fold increase in the risk of NODAT [95%CI 6.41, 10.19; p<0.001] compared to Caucasoid. South Asians persons and other patients from other ethnical backgrounds have a 6.9- [95%CI 5.56, 8.48; p<0.001] and 8.1- [5.91, 11.1; p<0.001] fold increased risk of NODAT.

Although pre-transplant weight has a little effect on subsequent NODAT, obesity post-transplantation posts a significant risk on NODAT. Patients who reached a weight of 90kg have a 50 percent [HR 1.5, 95%CI 1.09, 1.94; p=0.011] increased risk and this is even higher in those reaching above 100kg – estimated at a 2.3-fold increase in risk of NODAT [HR 2.3, 95%CI 1.73, 3.06; p<0.001].

Apart from weight and ethnicity, the recommencement of steroids following rejection increased risk of NODAT 3.5-fold [95%CI 2.97, 4.12; p<0.001].

Table 11-12 Risk factors of NODAT: Post-transplantation

	Univariant model			Multivariant model		
Factors	**HR**	**95%CI**	**P value**	**HR**	**95%CI**	**P value**
Age	1.03	1.03,1.04	0.000	1.04	1.03,1.04	0.000
Ethnicity						
Caucasian	1					
Afro-Caribbean	6.7	5.36,8.34	0.000	8.1	6.41,10.19	0.000
South Asian	5.5	4.51,6.65	0.000	6.9	5.56,8.48	0.000
Others	5.2	3.86,6.96	0.000	8.1	5.91,11.10	0.000
Current weight						
Below 65kg	1			1		
65-75kg	1.0	0.78,1.22	0.829	1.2	0.92,1.49	0.203
75-90kg	0.9	0.74,1.10	0.320	1.0	0.78,1.24	0.895
90-100kg	1.1	0.87,1.43	0.392	1.5	1.09,1.94	0.011
Above 100kg	1.3	1.03,1.65	0.029	2.3	1.73,3.06	0.000
Post-rejection						
No	1					
Yes	3.1	2.65,3.58	0.000	3.5	2.97,4.12	0.000
Gender						
Female	1			1		
Male	1.2	1.02,1.36	0.030	1.1	0.96,1.36	0.125

11.6 Summary

In this chapter, I examined the risk related to long-term immunosuppression exposure related to the use of this powerful immunosuppressive regimen.

Campath induction is a powerful lymphopenia and its effects persist for months following transplantation; I have demonstrated the effects of Campath on leucocytes in the previous chapter [Section 10]. Lymphopenia recovers within the 12 months in 50% of patients.

There were concerns about the increased risk of viral infection in Campath-treated patients. Our data showed the overall rate of infection being highest in the first 6 months, estimating it reached 141 incidences per 100 patient years and fell gradually and plateaued after 12 months. Urinary tract infection was the highest cause of infection over time, although the risk of surgical-related infection was highest in the first 6 months.

The time of post-transplantation is an important risk factor on infection; the risk of infection falls as time lapses following transplantation. The model estimated a 7% reduction in risk every week following transplantation.

The backgrounds of diabetes mellitus, end-stage renal disease with reflux uropathy and female gender are all significant factors associated with a 2.1-, 3.8- and 1.8-fold increase in infection risk, respectively, using this simple Campath induction and tacrolimus monotherapy regimen.

The incidence of infection remained low despite lymphopenia and I was not able to elicit a relationship between lymphopenia and infection risk in our centre. This is likely a result of the spare use of MMF; our data showed treatment of rejection by restarting corticosteroids, and MMF increased the risk of infection by 71%, albeit it just reached statistical significance.

Despite the lymphopenia, the incidence of viral infection remained low late after transplantation. A total of 14 cases of CMV disease were reported in this cohort, with a median time to infection of 26 weeks. 3.5 episodes per 100 patient years of CMV infection in the first 12 months and cases remained rare after 12 months. All cases occurred following stopping the 3-month valganciclovir

prophylaxis. All cases were successfully treated with intravenous ganciclovir and antiviral resistance was not detected.

There were 12 cases of BK nephropathy overall and the risk of BK nephropathy is highest in the first 6 months, with the median time to event of 24 weeks. Approximately 60% of cases occurred after the increment of immunosuppressants following rejection and this is equivalent to a 4.2-fold increased risk.

Previous reports examining the risk of fungal infection in the Campath maintenance protocol in pancreas transplants suggested an increased risk of fungal infection in repeated and high-dose Campath-treated patients. My data from this large cohort showed 20 cases of fungal infection in total, equivalent to 0.9 cases per 100 patient years in the first 6 months and 0.5 cases per 100 patient years between 6 and 12 months post-transplantation. Incidences of fungal infections were largely unchanged annually thereafter. There were 4 cases of aspergillus pneumonia and 2 cases of Pneumocystis pneumonia, of which all cases were diagnosed by bronchial lavarge or sputum. There had been 7 cases of candidaemia in 6 patients, and 2 cases of subcutaneous fungal infections with dematiaceous fungi and pyrenchaeta romoroi.

My results showed that the incidence of infection in this simple regimen is similar to data published previously; the incidence of bacteraemia in the first 6 months was 21.7 episodes per 100 patient years and this fell to 8.5 episodes per 100 patient years after the first 6 months in our unit. This is similar to the report published by the Spanish RESITRA registry, showing incidences of bacteraemia of 24.8 and 7.31 episodes per 100 patient years in the first 6 months and between 6 and 12 months after transplantation, respectively (162).

Chronic immunosuppression not only reduces the immune response of recipients to an allograft, but also reduces a recipient's immune reaction to infection as well as the ability of immune surveillance, which prevents growth and development of malignancies. Therefore, the risk of developing malignancies with long-term immunosuppression should also be examined for a risk benefit balance.

13 [2.2%] cases of malignancy were identified in the cohort of 602 patients, and the cumulative risk of developing malignancy in this cohort was 0.5%, 3.1% and 3.5% at 1, 3 and 5 years, respectively. Skin cancer is the commonest malignancy identified and it accounted for 4/13 [30.8%] cases; there were 3 [23.0%] cases of malignancy related to the gastrointestinal system and 2 [15.4%] cases of renal cell carcinoma. There was one case of angiosacroma, one case of breast carcinoma, one case of post-transplant lymphoproliferative disorder and one case of prostate cancer.

I examined the effects of ethnicity, previous use of immunosuppressants and gender on the risk of developing malignancy and I found no strong evidence supporting these factors incurring any substantial impact.

One limitation of this analysis is that the follow-up from this study may be insufficient to delineate the effect of the Campath on future development of malignancy, because malignancy tends to develop late following transplantation (106, 118).

Despite this potential shortcoming, it is worth noting that there is a low incidence of PTLD in this large cohort. A previous report from a large registry suggested that PTLD is commonest in the first year, with the incidence reaching approximately 0.3% and 0.7% at 1 and 5 years (163). Our results showed only 1 case of PTLD [0.17%] in this large cohort and it was unarguably substantially lower than published literatures (163, 164). This might be related to the spared use of antiproliferative agents [MMF] and the lymphopenic nature of Campath-destroyed EBV-harboured lymphocytes that could potentially evolve into PTLD (165).

New Onset Diabetes After Transplantation [NODAT] is a common complication following renal transplantation and it is associated with a higher cardiovascular risk and poorer long-term graft and patient survival. It has long been established that long-term use of corticosteroids and calcineurin inhibitors is associated with an increased risk of diabetes, even with a small maintenance dose.

Campath induction is associated with a 48% reduction in risk of NODAT (21), which is most likely related to a spared use of steroids in immunosuppressant maintenance following Campath induction.

Recipients' pre-transplant factors, e.g. obesity, being Afro-American and Hispanic in origin, and older age, are associated with a higher risk of NODAT. The effects of risk factors developed after transplantation, e.g. weight gain, treatment change following rejection, were not well studied.

56 patients developed NODAT in our non-diabetic cohort consisting of 433 patients. I demonstrated 1- and 6-year NODAT survival of 92.2% and 84.2%. My model confirms that Afro-Caribbean and South Asian have a 5.3- and 3.4-fold increased risk of NODAT and that age is also a significant risk factor, which increased the risk of NODAT by 3% for every year of a recipient's age.

I further developed this mathematical model to account for the changes in body habitus and events occurring following transplantation. My model showed that ethnicity has a similar effect on the risk of NODAT as the pre-transplant risk factor model. Afro-Caribbean and South Asian have an 8.1- and 6.9-fold increase in risk of NODAT respectively.

More importantly, although the pre-transplant weight has little impact on NODAT, weight gain after a transplant is a highly significant risk factor of NODAT. My data showed that patients who weigh above 90kg increase the risk of NODAT by 50%, as well as a further 180% if their weight reaches above 100kg.

Treatment of rejection with recommencing steroids and MMF is also associated with an increased risk of NODAT; my model estimated that NODAT risk increases by 3.5 times after an episode of rejections.

12 Discussion

The incidence of end-stage renal failure has gradually been rising over the last decade in the UK and renal transplantation has long been established as the treatment of choice. Renal transplantation not only improves a patient's quality of life, it has been shown to be associated with a significant survival benefit.

The key difficulties of renal transplantation is how to maintain the balance of immunosuppression to reduce an alloimmune response to an allograft and the associated risk of long-term immunosuppression, e.g. risk of infection, malignancy and metabolic side effects associated with immunosuppressants in the long term.

The US UNOS registry confirmed the increased risk of malignancy in transplant recipients compared to the general population. It has long been established that the risk of acquiring infection is higher in transplant recipients and, in particular, the first 6 months following transplantation. And yet, allograft failure with rejection is inevitable without long-term immunosuppression.

Long-term exposure to an immunosuppressant itself is associated with chronic allograft damage and other metabolic side effects. A previous report suggested that 100% of patients exposed to long-term cyclosporine developed features of long-term allograft damage after 10 years. Furthermore, it is well known that long-term exposure of antiproliferative agents and oral steroids is associated with skin malignancies, raised cardiovascular risk and mortality.

Therefore, the Imperial College Kidney and Transplant Centre aimed to develop an immunosuppression regimen to provide adequate alloimmune control, yet sparing patients from excessive risk of complications. This became possible with the development of Campath 1H.

Campath 1H is a humanised monoclonal antibody that targets CD52 antigens. CD52 antigen is one of the most abundant antigens on human lymphocytes. Once CD52 antigen is stimulated, it causes profound lymphopenia by cell lysis and a marked cytokine release. It had been hypothesised that

this period of lymphopenia is crucial for development of immune tolerance and therefore facilitates a lower intensity of long-term immunosuppression.

Although it had been shown by Kirk et al. that Campath 1H on its own is insufficient to generate immune tolerance, maintenance immunosuppression is crucial for successful long-term survival following Campath induction. It had been shown that Campath 1H allowed a safe reduction in intensity of maintenance immunosuppressant. Calne et al. showed that a medium dose of cyclosporine maintenance following Campath induction is as efficacious as conventional triple therapy maintenance consisting of high-dose cyclosporine, steroids and azathioprine.

This leads to the development of this simple minimalistic regimen in our unit consisting of Campath induction and medium-dose tacrolimus maintenance. Albeit tacrolimus and cyclosporine both exert their pharmacological property via a similar pathway and tacrolimus delivers similar therapeutic efficacy, it has been shown that tacrolimus has an improved risk profile in the long term. We hoped that this lower intensity of maintenance immunosuppressant would translate to improvements in long-term allograft function, patient and allograft survivals.

Our unit converted to this Campath regimen in 2004; I described the results from the pilot study in chapter 3. The results showed that 1-year cumulative patient and graft survival were comparable to the historic cohort consisting of patients receiving daclizumab induction and tacrolimus and mycophenolate mofetil maintenance regimes with steroids at 1 year. This pilot study also showed a substantial reduction in rejection, albeit not statistically significant. The incidence of new onset diabetes after transplantation [NODAT] and calcineurin inhibitor toxicity remained low, likely as a result of spared use of steroids. This pilot study was a retrospective short cohort study and number of subjects were small, these small retrospective cohot studies was subjected to bias related to difference in patient characteristics between the two groups, as well as difference in practice between the control and test group eras. Neverthelss, the results from this cohort study was encouraging, this showed Campath was safe and delivers very similar if no better results in the short term.

In order to examine the effect of Campath induction in transplantation in more details, we have therefore proceeded to develop a formal randomised controlled study to compare the Campath and tacrolimus monotherapy regimes with daclizumab induction, tacrolimus and MMF maintenance regimes in 2005.

This open-label RCT was designed as a non-inferior study, aimed at detecting a non-inferiority efficacy with a 10% inferiority margin in patient survival with a functioning graft with the Campath regimen at 1 year. Details of this trial are described in Chapter 4. I demonstrated the difference of survival between the two groups was in favour of the Campath-treated group with 2.6% improvement [95%CI -1.7%, 6.9%]. The results from this RCT supported our previous findings with the retrospective cohort study that graft survival and function in patients receiving the Campath regimen were comparable to the daclizumab-treated cohort. Rejection risk was once again slightly lower in the Campath-treated group; however, it did not reach statistical significance.

Although this randomised controlled study supported our hypothesis that the Campath and tacrolimus monotherapy protocol was at least "as good as" the conventional daclizumab induction protocol. This supported our argument that this cheaper and miminalstic protocol were at least as good as the standard but substantially lower in cost.

The study was designed to be a non-inferiority study, and therefore the interpretations of the results were limitations by trial design and the statistical power. We were not able to detect any potential superiority survival signals if that should exist. In order to detect a superiority signal, a considerable bigger study is required and is out of scope of resources on both participants and financially for a single centre. If resources were no restriction, I would propose a multicentre study to examine the risk benefit of this regime in more details. Benefit of a multicentre study does not only provide the number of subjects needed to produce the evidence to support the hypothesis of superiority with this regime, this also allow to results of the study be more applicable to a wider range of subjects and difference in practice across centres. Furthermore, a large study would also reduce the effects of confounder bias. In this RCT, there were a bigger proportion of sensitised subjects in the

daclizumab group compared to the study group. One would argue the results observed in this RCT could be confounded by the imbalance of sensitised subjects between groups. Small clinical studies are more prone to these confounder bias caused by randomness. A larger study and will balance the effect of randomness, as well as provide the statistical power for post hoc adjusted analysis if necessary.

We were only able to report the results on 1-year outcome because of trial design. An extended follow up study is ongoing, this will take a longer follow up period to test if there is a true difference in the long term. We would have to rely on the results from the cohort study to provide us an idea on the long-term outcomes of this regime in the time being.

I examined the medium-term outcomes in patients in our original cohort and reported the findings in Chapter 5. Results from this medium-term follow-up cohort showed a comparable 5-year patient and graft survival in those receiving the Campath protocol. Unlike some of the reported studies, our cohort did not suffer a significant level of "catch-up" acute rejection episodes beyond 6 months. After correcting for risk of rejection in the first year, adjusted 5-year cumulative rejection-free survival was 86.7% and 92.0% in the Campath and daclizumab group. A time-adjusted risk model did not suggest an increased risk of late rejection. I also examined the trend of allograft function on individuals over time using a repeat-measurements model and confirmed that there were no overall net differences in function or rate of changes in function between the two groups. Another interesting finding was that incidences of CNI toxicity were low in both groups, compared to the incidence reported in the literature based on a cyclosporine regimen.

This study was a long-term longitudinal cohort study and was subjected to issues with loss to follow-up; however, thanks to the nature of our practice, the majority of patients within this cohort were followed up in our centre and were provided with an excellent source of data to examine the risk and benefit of this regimen.

Furthermore, one should interpret the results with caution, as with any long term cohort study. The results were by the study's retrospective nature and the difference in chronologies between the two

groups. The daclizumab control cohort consisted of mainly subjects transplanted between 2002 and 2004, whereas the Campath group were transplanted after 2004. Therefore, the results were subjected to the change of practice over time.

As discussed previously, a large long-term follow up study is needed in order to test the long-term effect of Campath. The choice of long-term outcome measure to examine is also crucial. Most transplant studies were designed with primary outcome measure of graft/patient survival or allograft function. These outcomes had substantially improved with advance in immunosuppressant maintenance in the last decade and therefore the outcome measures used in these study should reflect the concern with long-term allograft pathology, for example CNI toxicity or transplant glomerulopathy. Even if the right clinical measure was selected, the difference in these outcomes is often very small. Mathematically, it is very difficult to detect a difference in these outcomes without a large-scale clinical trial, and will require trial size similar to conventional cardiovascular studies – which is extremely hard in transplantation trial due to disease prevalence. One would argue these clinical trial design difficulties could be resolved using adaptive clinical trial design and Bayesian statistically methods.

I have demonstrated the short- and long-term efficacies of Campath induction in Chapter 3-5. However, it was unclear as to what dosage of Campath would provide a most favourable benefit risk balance in transplant recipients. Studies in the literature described a dosage range from a body weight-adjusted dosage of up to 1.2mg/kg to a standard dosing of 30 to 60mg in total, and there was no clear evidence supporting a higher dose of Campath improving transplant outcomes. There was some anecdotal evidence suggesting that the risk of systemic fungal and mycobacterial infections was higher in patients who have received high or repeated doses of Campath as maintenance therapy. This supported the argument that Campath doses should be adjusted to avoid excess dosage and the associated infective complications. Therefore, I performed a dose-finding analysis on our cohort of Campath-treated patients and reported the findings in Chapter 6. 608 patients were included in the analysis and were divided into quadrants according to their Campath exposure per

weight [<0.35mg/kg, 0.35-0.40mg/kg, 0.40-0.48mg/kg and >0.48mg/kg]. The study showed there was an approximate 49.8% increased risk of rejection in the first 12 months with patients who received less than 0.35mg/kg of Campath and there was no evidence to suggest an additional protective effect against rejection with a higher-dose Campath. Alarmingly, patients who received the highest dose of Campath >0.48mg/kg appeared to have the highest risk of infection. Compared to the group who received an average dose of Campath, this group exposed to the highest dose of Campath have a 2.6-fold increased risk of infection in the first 6 months and this increased infective risk persisted after 2 years. The results did not change after adjusting the effect of gender difference between groups. This analysis showed that patients receiving less than 0.35mg/kg of Campath were subjected to an increased risk of rejection and a higher dose of Campath did not offer additional protection against rejection, yet raised infective risk. As a result of this study, we changed our immunosuppressive protocol to a weight-adjusted Campath induction dose at 0.40mg/kg followed by a targeted tacrolimus monotherapy in January 2012.

Although this dose finding retrospective study was conducted using the objective evidence based on laboratory results, the results of this study were affected its study design – as discussed earlier. Moreover, the severity and seriousness of the events were not assessed.

A large clinical trial is needed to confirm the beneficial risk balance of weight adjusting dosage of Campath. Apart from the standard clinical outcomes, one should examine the adverse events using the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline and reporting requirement. Defining events based on ICH definition of seriousness and causality assessment. This approach would provide an objective, repeatable assessment of seriousness and severity of events related to the treatment regime.

Little data was available to examine the causes of graft loss in Campath-treated patients. In Chapter 7, I examined the cause of graft loss in our Campath cohort. Patient death with a functioning graft remained the commonest cause of graft loss in our unit, accounting for 34% of graft losses, of which sepsis was the commonest cause of patient death. Contrary to previous UNOS reports,

cardiovascular death only accounted for a moderate portion of total patient deaths [8/33, 24.2%]. The reduction of cardiovascular death was likely because of a proactive cardiac screening prior to transplantation in our transplant recipients, and this was reported by my colleague, Dr. Nichola Kumar, in her MD thesis. Although data from my short- and long-term studies on Campath both showed a low risk of rejection, rejection remained an important cause of graft loss. This study once again highlighted the importance of patient compliance – 8 grafts were lost due to non-compliance despite the minimalistic maintenance regimen.

As discussed earlier in this thesis, Campath is a powerful lymphopenic agent and the rates of repopulation of lymphocytes are variable depending on the lymphocyte subset. Therefore, I developed a study to examine the haematological profiles, the relationship between lymphocyte and monocyte repopulation and rejection, as well as examined the association of lymphopenia and infective risk on patients from the earlier study, based on 96,695 results collected from their routine haemological blood work. Details of the results and analysis were reported in chapters 8 and 9. Data from this large cohort confirmed previous results that lymphocyte repopulation differs between subsets. Lymphocyte repopulation starts weeks after Campath – 96.8% of patients remained lymphopenic at 3 months and this fell to 52.3% at 6 months. By 2 years, 75.6% of patients in this cohort had recovered their lymphocyte compartment. Interestingly, monocytes depleted immediately following Campath and their level remained suppressed for the first 4 weeks after transplantation. Monocytes repopulated substantially faster compared to the lymphocyte compartment. Monocyte counts in 45% of cohort patients reached a normal level within the first 6 months and 65% of patients' routine blood tests demonstrated a normalised monocyte level by 1 year. There were a few cases of transient thrombocytopenia and neutropenia in the Campath cohort; however, there was no evidence to suggest an overall effect on either platelets or neutrophils, and this will be discussed in detail by my colleague, Dr. Rawya Charif, in her thesis concentrating on the complications of Campath.

It had been hypothesised that the recovery of a leukocyte compartment following Campath is associated with occurrences of rejection. I developed a mathematical model to examine the rate of lymphocyte and monocyte changes over time between rejecters and non-rejecters. The model showed that both lymphocyte and monocyte recovery follows a cubic trajectory and the trajectories on lymphocyte and monocyte recovery were not different in patients who experienced rejection and those who did not. Furthermore, the residual lymphocyte and monocyte counts were not statistically different between the two groups.

This study was the first of it's kind to examine the profiles of lymphocyte repopulations on individuals and its impact on rejection risk. This approach did take into account of changes in the individual profile over time and the effect of missing data between time points. However, this approach requires a heavy statistical input and mathematical modelling.

The results of this study were indeed very interesting, the model was built using total lymphocyte counts. Therefore, we could not conclude whether lymphocyte subset repopulation has any effects on rejection.

Long-term success in transplant immunosuppression is dependent on the balance between immunosuppressive effects to reduce an alloimmune response with the associated infection and malignancy risk and metabolic side effects. It was thought that the powerful lymphodepleting ability of Campath might result in a high risk of infection. Therefore, in Chapter 10, I examined the incidence and pattern of infection, malignancy and NODAT in Campath-treated patients over time, as well as tried to answer the question on whether lymphopenia induced by Campath is associated with an increased risk of infection. Data from the study showed that the infection rate was highest in the first 6 months after transplantation, and urinary tract infection was the commonest form of infection and remained high for 12 months. The incidence of infection remained low and I was not able to elicit a relationship between lymphopenia and infection risk in our centre. This was likely a result of the spared use of MMF; our data showed treatment of rejection by restarting corticosteroids, and MMF increased risk of infection by 71%, albeit it just reached statistical

significance. Despite the lymphopenia, the incidence of viral infection remained low late after transplantation. There were no evidence suggesting a high risk of CMV disease or fungal infection

Malignancy remained rare in this cohort, and the cumulative risk of developing malignancy in this cohort was 0.5%, 3.1% and 3.5% at 1, 3 and 5 years, respectively. Skin cancer was the commonest malignancy. A major limitation of this analysis was that the follow-up from this study might be insufficient to delineate the effect of the Campath on future development of malignancy, giving malignancy a tendency to develop late following transplantation. Despite the limitation, we were surprised to note the low incidence of post-transplant lymphoproliferative disorder in this cohort. It was estimated that there was a cumulative risk of 1% at 5 years and this is much lower than published literature. This low-risk PTLD is likely as a result of the spare use of antiproliferative agents as well as the lymphopenic nature of Campath-destroyed EBV-harboured lymphocytes that could potentially evolve into PTLD.

The metabolic effect of this regime was best reflected by the low incidence of NODAT; 6-year NODAT survival of 84.2%. The statistical model suggested that ethnicity, recipient age, weight gain and treatment of rejection increase the risk of NODAT.

In this thesis, I described the short- and medium-term clinical outcomes of this minimalistic regimen consisting of Campath induction and tacrolimus maintenance in renal transplantation. I was the junior investigator of the CamTac trial, which provided evidence to support clinical outcomes with this regimen being “as good” as the conventional regimen, consisting of daclizumab induction with tacrolimus and MMF maintenance. I also demonstrated that Campath dosage should be weight-adjusted to maximise the immunosuppressive effect without risking an increased risk of over-immunosuppression and related infection; as a result of my study, the immunosuppression regimen has changed from a standard dose to a weight-adjusted regimen in the Imperial College Kidney and Transplant Centre. I have also showed that patient death with a functioning graft remained the commonest cause of graft loss and that a transplant clinician should endeavour to reduce the risk of infection in the long term.

Regarding the long-term efficacy of this regimen, I demonstrated that the incidence of CNI toxicity, transplant glomerulopathy and recurrent disease remains low despite the reduction in the immunosuppression burden.

I developed a mathematical model to examine the haematological profile in a large cohort of patients using their routine blood work; I confirmed previous finding that recovery varies between lymphocyte subsets. Importantly, I showed evidence that there were no differences in the patterns of lymphocyte recovery between rejecters and non-rejecters.

Although there were limitations in the clinical studies included in this thesis because of study design, data from these study supported the notion that this minimalist regimen is efficacious and safe.

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14 Appendix

14.1 Risk factors for infection following Campath induction

14.1.1 Risk of any infection

Table 14-1 Risk factor analysis for any infection after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
0-6 months	Week	0.93 [0.91,0.95]	<0.001	0.94 [0.92,0.96]	0.000	0.93 [0.91,0.94]	0.000
	Lymphopenia	1.24 [0.77,2.03]	0.370	0.65 [0.38,1.13]	0.127		
	Neutropenia	NA	0.999	N/A	0.997		
	Diabetes	1.75 [1.07,2.88]	0.027	2.10 [1.30,3.39]	0.002	2.12 [1.31,3.44]	0.002
	Reflux nephropathy	3.74 [1.76,7.95]	0.001	3.61 [1.73,7.54]	0.001	3.80 [1.81,7.95]	0.000
	Female gender	1.88 [1.26,2.81]	0.002	1.70 [1.14,2.53]	0.009	1.80 [1.22,2.68]	0.003
	Post-rejection	1.09 [0.63,1.87]	0.765	1.54 [0.90,2.63]	0.115	1.71 [0.99,2.96]	0.054
6-12 months	Week	1.02 [0.99,1.05]	0.197	1.02 [0.99,1.06]	0.114	1.02 [0.99,1.05]	0.216
	Lymphopenia	1.74 [0.97,3.13]	0.065	1.55 [0.85,2.83]	0.150		
	Neutropenia	NA	0.999	NA	0.999		
	Diabetes	1.22 [0.51,2.90]	0.656	1.60 [0.72,3.56]	0.247		
	Reflux nephropathy	5.17 [1.69,15.79]	0.004	4.28 [1.60,11.50]	0.004	5.13 [1.67,15.77]	0.004
	Female gender	1.97 [1.02,3.78]	0.043	1.85 [1.00,3.42]	0.049		
	Post-rejection	3.28 [1.67,6.42]	0.001	1.53 [0.78,2.99]	0.212		
12-24 months	Week	1.00 [0.98,1.01]	0.728	1.00 [0.99,1.02]	0.844	1.00 [0.99,1.02]	0.743
	Lymphopenia	2.19 [1.22,3.94]	0.008	1.93 [1.06,3.51]	0.031	2.22 [1.24,3.96]	0.007
	Neutropenia	2.84 [0.31,26.30]	0.358	2.67 [0.30,24.08]	0.382		
	Diabetes	1.36 [0.54,3.42]	0.518	1.78 [0.77,4.10]	0.176		
	Reflux nephropathy	4.72 [1.66,13.41]	0.004	4.59 [1.76,11.94]	0.002	3.96 [1.56,10.03]	0.004
	Female gender	1.07 [0.53,2.16]	0.849	1.10 [0.58,2.10]	0.772		
	Post-rejection	4.58 [2.19,9.60]	0.000	1.79 [0.90,3.55]	0.098		
24-36 months	Week	0.98 [0.96,1.01]	0.181	0.99 [0.97,1.02]	0.458	0.98 [0.96,1.00]	0.095
	Lymphopenia	1.69 [0.71,4.03]	0.238	1.52 [0.66,3.49]	0.325		
	Neutropenia	NA	0.999	NA	0.999		
	Diabetes	0.29 [0.05,1.65]	0.161	0.35 [0.07,1.74]	0.201		
	Reflux nephropathy	9.80 [2.59,37.13]	0.001	5.82 [2.12,15.95]	0.001	6.16 [1.99,19.10]	0.002
	Female gender	3.92 [1.39,11.05]	0.010	2.65 [1.15,6.09]	0.022	3.01 [1.23,7.35]	0.016
	Post-rejection	4.47 [1.53,13.01]	0.006	1.79 [0.75,4.27]	0.189	4.04 [1.66,9.87]	0.002
36-48 months	Week	1.00 [0.97,1.04]	0.812	1.00 [0.96,1.04]	0.968	1.00 [0.97,1.04]	0.884
	Lymphopenia	1.38 [0.35,5.36]	0.643	1.33 [0.33,5.42]	0.689		
	Neutropenia	0.00 [0.00,0.00]	0.000	#VALUE!	1.000		
	Diabetes	1.66 [0.36,7.53]	0.513	2.22 [0.56,8.72]	0.255		
	Reflux nephropathy	4.73 [1.25,17.86]	0.022	3.09 [0.73,13.07]	0.125	3.25 [0.95,11.03]	0.059
	Female gender	3.88 [1.24,12.14]	0.020	3.74 [1.19,11.77]	0.024	3.19 [1.03,9.93]	0.045
	Post-rejection	1.52 [0.39,5.85]	0.546	1.33 [0.38,4.70]	0.657		

14.1.2 Surgical infection

Table 14-2 Risk factor analysis for surgical infection after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	0.64 [0.57,0.72]	0.000	0.63 [0.56,0.71]	0.000	0.63 [0.56,0.71]	0.000
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	2.41 [1.42,4.10]	0.001	2.66 [1.51,4.68]	0.001	2.49 [1.43,4.34]	0.001
	Reflux nephropathy	1.33 [0.47,3.78]	0.588	1.70 [0.57,5.01]	0.339		
	Female gender	1.18 [0.71,1.97]	0.524	1.24 [0.73,2.10]	0.426		
	Post-rejection	1.22 [0.57,2.61]	0.608	2.91 [1.30,6.55]	0.010	2.89 [1.29,6.48]	0.010

14.1.3 Urinary tract infection

Table 14-3 Risk factor analysis for urinary tract infection after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	0.95 [0.93,0.97]	0.000	0.97 [0.95,1.00]	0.061	0.97 [0.95,1.00]	0.042
	Lymphopenia	0.72 [0.42,1.24]	0.240	0.53 [0.28,1.00]	0.052	0.52 [0.28,0.98]	0.044
	Neutropenia	NA		NA			
	Diabetes	1.49 [0.78,2.83]	0.225	1.90 [1.00,3.60]	0.049	1.90 [1.00,3.60]	0.049
	Reflux nephropathy	4.82 [1.98,11.75]	0.001	3.54 [1.42,8.86]	0.007	3.65 [1.46,9.09]	0.006
	Female gender	2.96 [1.77,4.97]	0.000	2.75 [1.62,4.65]	0.000	2.71 [1.61,4.58]	0.000
	Post-rejection	0.72 [0.33,1.57]	0.404	0.77 [0.35,1.69]	0.520		
6-12 months	Week	1.01 [0.98,1.05]	0.518	1.02 [0.98,1.06]	0.351	1.01 [0.97,1.04]	0.720
	Lymphopenia	1.42 [0.70,2.88]	0.335	1.10 [0.54,2.24]	0.799		
	Neutropenia	NA		NA			
	Diabetes	1.56 [0.54,4.50]	0.411	2.22 [0.85,5.80]	0.102		
	Reflux nephropathy	6.35 [1.62,24.93]	0.008	4.85 [1.45,16.15]	0.010	4.94 [1.42,17.27]	0.012
	Female gender	4.06 [1.76,9.34]	0.001	3.27 [1.50,7.15]	0.003	3.23 [1.46,7.14]	0.004
	Post-rejection	3.57 [1.55,8.22]	0.003	1.97 [0.87,4.46]	0.102	3.26 [1.47,7.23]	0.004
12-24 months	Week	1.00 [0.98,1.02]	0.956	1.00 [0.98,1.02]	0.815	1.00 [0.98,1.02]	0.877
	Lymphopenia	2.13 [0.94,4.81]	0.070	1.79 [0.79,4.07]	0.164		
	Neutropenia	11.38 [0.94,137.72]	0.056	8.85 [0.77,101.89]	0.080	10.57 [0.92,121.63]	0.059
	Diabetes	1.23 [0.35,4.33]	0.752	1.55 [0.46,5.18]	0.476		
	Reflux nephropathy	7.78 [2.14,28.29]	0.002	6.56 [1.89,22.78]	0.003	5.82 [1.76,19.29]	0.004
	Female gender	2.50 [0.99,6.31]	0.052	2.33 [0.96,5.66]	0.061	2.41 [1.01,5.75]	0.047
	Post-rejection	3.26 [1.47,7.23]	0.004	1.43 [0.56,3.66]	0.457		
24-36 months	Week	0.98 [0.95,1.01]	0.181	0.02 [1.02,0.00]	0.960	0.99 [0.96,1.03]	0.692
	Lymphopenia	3.01 [1.00,9.07]	0.051	2.57 [0.89,7.36]	0.080	2.63 [0.99,7.04]	0.053
	Neutropenia	NA		NA			
	Diabetes	0.16 [0.01,1.89]	0.144	0.22 [0.02,2.29]	0.203		
	Reflux nephropathy	14.71 [3.69,58.70]	0.000	10.04 [2.95,34.18]	0.000	13.08 [4.00,42.80]	0.000
	Female gender	7.18 [1.88,27.39]	0.004	4.14 [1.28,13.39]	0.018	3.49 [1.16,10.52]	0.027
	Post-rejection	4.47 [1.20,16.72]	0.026	1.78 [0.57,5.53]	0.319		
36-48 months	Week	1.00 [0.97,1.04]	0.850	1.00 [0.96,1.04]	0.960	1.00 [0.97,1.04]	0.928
	Lymphopenia	1.52 [0.37,6.20]	0.559	1.60 [0.38,6.68]	0.519		
	Neutropenia			NA			
	Diabetes	1.81 [0.37,8.82]	0.461	2.51 [0.62,10.22]	0.198		
	Reflux nephropathy	5.36 [1.30,22.13]	0.020	3.20 [0.74,13.95]	0.121	3.44 [0.97,12.17]	0.055
	Female gender	4.86 [1.41,16.67]	0.012	4.50 [1.31,15.45]	0.017	3.95 [1.16,13.43]	0.028
	Post-rejection	1.08 [0.24,4.91]	0.920	0.95 [0.23,3.90]	0.947		

14.1.4 BK nephropathy

Table 14-4 Risk factor analysis for BK nephropathy after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	1.13 [1.01,1.26]	0.040	1.12 [1.00,1.26]	0.056	1.12 [0.99,1.25]	0.062
	Lymphopenia	NA		NA			
	Neutropenia	NA		NA			
	Diabetes	0.70 [0.09,5.69]	0.739	0.76 [0.09,6.36]	0.803		
	Reflux nephropathy			NA			
	Female gender	0.57 [0.12,2.85]	0.497	0.64 [0.12,3.36]	0.601		
	Post-rejection	5.23 [1.25,21.90]	0.024	4.12 [0.89,19.12]	0.071	4.15 [0.98,17.56]	0.053
6-12 months	Week	1.16 [0.82,1.64]	0.402	NA		1.12 [0.99,1.25]	0.062
	Lymphopenia	NA		NA			
	Neutropenia	NA		NA			
	Diabetes	NA		NA			
	Reflux nephropathy	NA		NA			
	Female gender	NA		NA			
	Post-rejection	NA		NA		4.15 [0.98,17.56]	0.053
12-24 months	Week	1.04 [0.94,1.15]	0.417	1.04 [0.94,1.15]	0.427	1.04 [0.94,1.15]	0.417
	Lymphopenia	NA		NA			
	Neutropenia	NA		NA			
	Diabetes	NA		NA			
	Reflux nephropathy	14.42 [0.90,230.57]	0.059	14.15 [0.88,226.38]	0.061		
	Female gender	NA		NA			
	Post-rejection	NA		NA			
24-36 months	Week	0.97 [0.84,1.12]	0.685	0.97 [0.84,1.12]	0.685	0.97 [0.84,1.12]	0.685
	Lymphopenia	NA		NA			
	Neutropenia	NA		NA			
	Diabetes	NA		NA			
	Reflux nephropathy	NA		NA			
	Female gender	NA		NA			
	Post-rejection	NA		NA			
36-48 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					

14.1.5 CMV infection

Table 14-5 Risk factor analysis for CMV disease after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	1.14 [1.02,1.27]	0.021	1.21 [1.06,1.38]	0.004	1.14 [1.02,1.27]	0.021
	Lymphopenia	0.72 [0.09,5.88]	0.761	2.40 [0.27,21.14]	0.430		
	Neutropenia	NA		NA			
	Diabetes	0.61 [0.08,4.90]	0.644	0.78 [0.09,6.52]	0.820		
	Reflux nephropathy	2.50 [0.31,19.98]	0.389	3.12 [0.35,27.87]	0.308		
	Female gender	1.38 [0.37,5.14]	0.632	1.60 [0.39,6.61]	0.517		
	Post-rejection	2.49 [0.52,11.99]	0.256	1.42 [0.26,7.63]	0.685		
6-12 months	Week	1.00 [0.90,1.11]	0.984	1.02 [0.91,1.13]	0.778	1.00 [0.90,1.11]	0.984
	Lymphopenia	1.57 [0.29,8.60]	0.601	2.01 [0.35,11.68]	0.437		
	Neutropenia	NA		NA			
	Diabetes	NA		NA			
	Reflux nephropathy	3.71 [0.43,31.81]	0.231	2.97 [0.34,26.28]	0.327		
	Female gender	0.34 [0.04,2.90]	0.323	0.28 [0.03,2.44]	0.250		
	Post-rejection	0.87 [0.10,7.45]	0.899	0.43 [0.05,3.96]	0.459		
12-24 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
24-36 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
24-36 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					

14.1.6 Bacteraemia

Table 14-6 Risk factor analysis for bacteraemia after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	0.94 [0.90,0.98]	0.005	0.97 [0.92,1.02]	0.193	0.94 [0.90,0.98]	0.005
	Lymphopenia	2.07 [0.55,7.85]	0.285	1.44 [0.35,6.02]	0.615		
	Neutropenia	NA		NA			
	Diabetes	2.12 [0.78,5.82]	0.143	2.74 [1.12,6.73]	0.027		
	Reflux nephropathy	3.92 [0.91,16.93]	0.067	6.53 [1.77,24.13]	0.005		
	Female gender	0.98 [0.41,2.34]	0.961	0.72 [0.31,1.67]	0.441		
	Post-rejection	1.78 [0.57,5.49]	0.319	2.28 [0.77,6.71]	0.135		
6-12 months	Week	1.02 [0.95,1.09]	0.623	1.03 [0.96,1.10]	0.486	1.02 [0.95,1.09]	0.623
	Lymphopenia	3.94 [0.80,19.41]	0.092	3.27 [0.64,16.69]	0.153		
	Neutropenia	NA					
	Diabetes	1.42 [0.19,10.37]	0.729	2.14 [0.36,12.79]	0.406		
	Reflux nephropathy	8.83 [0.74,104.71]	0.084	6.19 [0.75,50.90]	0.090		
	Female gender	1.90 [0.39,9.29]	0.430	1.41 [0.34,5.79]	0.632		
	Post-rejection	6.02 [1.18,30.74]	0.031	2.25 [0.50,10.12]	0.292		
12-24 months	Week	1.01 [0.97,1.06]	0.488	1.02 [0.98,1.07]	0.267	1.01 [0.97,1.06]	0.488
	Lymphopenia	3.16 [0.66,15.07]	0.149	3.74 [0.72,19.38]	0.116		
	Neutropenia	0.00 [0.00,0.00]	0.000	#VALUE!	0.998		
	Diabetes	1.51 [0.21,10.91]	0.685	2.12 [0.34,13.37]	0.424		
	Reflux nephropathy	1.75 [0.12,26.23]	0.686	1.66 [0.12,22.34]	0.704		
	Female gender	1.69 [0.36,7.85]	0.502	1.43 [0.31,6.68]	0.648		
	Post-rejection	3.32 [0.62,17.92]	0.163	0.90 [0.16,4.98]	0.908		
24-36 months	Week	0.98 [0.96,1.01]	0.181	1.02 [0.95,1.09]	0.600	0.99 [0.94,1.05]	0.841
	Lymphopenia	1.69 [0.71,4.03]	0.238	0.51 [0.05,5.40]	0.577		
	Neutropenia	NA					
	Diabetes	0.29 [0.05,1.65]	0.161				
	Reflux nephropathy	9.80 [2.59,37.13]	0.001				
	Female gender	3.92 [1.39,11.05]	0.010	11.86 [1.15,122.62]	0.038		
	Post-rejection	4.47 [1.53,13.01]	0.006	13.65 [1.26,148.39]	0.032	7.89 [1.44,43.07]	0.017
24-36 months	Week	Unable to estimate - 1 case only					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					

14.1.7 Fungal infection

Table 14-7 Risk factor analysis for fungal infection after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	1.19 [0.81,1.75]	0.376			1.19 [0.81,1.75]	0.376
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
6-12 months	Week	1.20 [0.81,1.77]	0.358			1.20 [0.81,1.77]	0.358
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
12-24 months	Week	0.99 [0.90,1.09]	0.806			0.99 [0.90,1.09]	0.806
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
24-36 months	Week	1.03 [0.94, 1.13]	0.553			1.03 [0.94, 1.13]	0.553
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					
24-36 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					

14.1.8 Other infections

Table 14-8 Risk factor analysis: Other infections after Campath induction

		Univariant model		Adjusted model		Final model	
	Risk Factors	Odds Ratio	p value	Odds Ratio	p value	Odds Ratio	p value
6 months	Week	0.94 [0.89,1.00]	0.069	0.95 [0.88,1.02]	0.151	0.94 [0.89,1.00]	0.069
	Lymphopenia	0.85 [0.19,3.88]	0.835	0.48 [0.08,2.72]	0.405		
	Neutropenia	NA					
	Diabetes	1.21 [0.35,4.20]	0.767	1.28 [0.36,4.52]	0.705		
	Reflux nephropathy	1.02 [0.11,9.90]	0.984	1.19 [0.12,11.57]	0.881		
	Female gender	1.48 [0.56,3.95]	0.429	1.35 [0.49,3.75]	0.561		
	Post-rejection	1.73 [0.43,6.98]	0.438	2.00 [0.48,8.43]	0.344		
6-12 months	Week	1.05 [0.97,1.12]	0.213	1.07 [0.98,1.16]	0.122	1.05 [0.97,1.12]	0.213
	Lymphopenia	3.55 [0.77,16.43]	0.105	3.57 [0.72,17.62]	0.119		
	Neutropenia	NA	1.000				
	Diabetes	1.44 [0.40,5.16]	0.576	1.68 [0.34,8.20]	0.522		
	Reflux nephropathy	3.10 [0.69,13.87]	0.139	4.28 [0.86,21.30]	0.076		
	Female gender	0.68 [0.21,2.16]	0.512	0.78 [0.22,2.70]	0.693		
	Post-rejection	4.36 [1.53,12.45]	0.006	1.68 [0.48,5.86]	0.415		
12-24 months	Week	1.00 [0.98,1.01]	0.728	0.99 [0.96,1.02]	0.445	0.98 [0.95,1.01]	0.163
	Lymphopenia	2.19 [1.22,3.94]	0.008	1.31 [0.52,3.30]	0.570		
	Neutropenia	2.84 [0.31,26.30]	0.358				
	Diabetes	1.36 [0.54,3.42]	0.518	3.35 [1.10,10.24]	0.032	3.09 [1.00,9.56]	0.051
	Reflux nephropathy	4.72 [1.66,13.41]	0.004	1.80 [0.29,11.04]	0.523		
	Female gender	1.07 [0.53,2.16]	0.849	0.30 [0.09,1.02]	0.054		
	Post-rejection	4.58 [2.19,9.60]	0.000	3.32 [1.16,9.51]	0.026	5.18 [1.85,14.50]	0.002
24-36 months	Week	0.98 [0.93,1.03]	0.366	0.97 [0.92,1.03]	0.310	0.98 [0.93,1.03]	0.366
	Lymphopenia	0.65 [0.09,4.63]	0.670	0.61 [0.08,4.92]	0.646		
	Neutropenia	NA					
	Diabetes	0.71 [0.07,7.62]	0.779	0.65 [0.07,6.26]	0.706		
	Reflux nephropathy	NA					
	Female gender	0.55 [0.09,3.28]	0.508	0.65 [0.11,3.71]	0.623		
	Post-rejection	2.12 [0.39,11.38]	0.382	1.45 [0.26,8.16]	0.674		
36-48 months	Week	No cases					
	Lymphopenia	NA					
	Neutropenia	NA					
	Diabetes	NA					
	Reflux nephropathy	NA					
	Female gender	NA					
	Post-rejection	NA					