Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Alemtuzumab induction and triple maintenance immunotherapy in kidney transplantation from donors after cardiac death

Erik Schadde,[1] Anthony M. D'Alessandro,[2] Stuart J. Knechtle,[2] Jon Odorico,[2] Yolanda Becker,[2] John Pirsch,[2] Hans Sollinger[2] and Luis A. Fernandez[2]

1 Department of Surgery, Kansas University, Kansas City, KS, USA
2 Division of Organ Transplantation, Department of Surgery, University of Wisconsin-Madison, Madison, WI, USA

Keywords
alemtuzumab, Campath-1H, graft survival, induction, kidney transplantation, rejection.

Correspondence
Luis Fernandez, MD, Division of Organ Transplantation, University of Wisconsin School of Medicine, 600 Highland Avenue, CSC H4/733, Madison, WI, USA. Tel.: 1 608 263 9903; fax: 1 608 263 9903; e-mail: luisf@surgery.wisc.edu

Received: 30 June 2007
Revision requested: 19 July 2007
Accepted: 8 January 2008

doi:10.1111/j.1432-2277.2008.00642.x

Summary

We have used alemtuzumab in combination with triple maintenance immunosuppression in renal transplantation from donors after cardiac death between 2002 and 2006. We compared outcomes of induction therapy with alemtuzumab with interleukin-2 (IL-2) receptor antagonists (RA) and anti-lymphocyte antibodies. We used a retrospective sequential study design to examine 170 recipients of kidneys from donor after cardiac death (DCD) for survival, graft survival, time to first rejection, glomerular filtration and complications. Patients were stratified into high-risk and low-risk groups based on the following criteria: panel of reactive antibodies >20%, retransplants, Afro-American race. Induction with alemtuzumab was compared with anti-thymocyte globulin (ATG) in the high-risk and with IL-2RA in the low-risk group. Patients received triple immunosuppression with steroids, mycophenolate mofetil and calcineurin inhibitors. Patient survival, graft survival, rejection rate and glomerular filtration rate did not significantly differ between patients treated with alemtuzumab versus IL-2RAs or ATG. There was a trend towards reduced graft- and patient survival in the alemtuzumab group. There was an increased incidence of cytomegalovirus (CMV) infections in the alemtuzumab-induced group and a trend towards increased BK virus and bacterial infections. Induction of DCD kidney transplants with alemtuzumab compared to IL-2RA and ATG has no significant impact on acute rejection. It appears however that CMV infections are increased in patients induced with alemtuzumab. We therefore conclude that induction with alemtuzumab does not confer any advantage over traditional induction agents.

Introduction

In the United States, there were 476 kidney transplants performed from donors after cardiac death in 2004, representing about 5.1% of all renal transplants performed nationwide (http://www.ustransplant.org/annual_reports/current/504_KI.htm). Reports from the United States and from Europe have demonstrated no difference in long-term graft survival despite a significantly higher incidence of delayed graft function (DGF) [1,2]. At University of Wisconsin 170 kidney transplants were performed from donors after cardiac death between 1996 and 2005. Kidneys from donors after cardiac death (DCD) represent 13% of all deceased donor kidney transplants in this 10-year period. The percentage of DCD kidneys in our institution increased from 7% in 1996 to 22% in 2005 contributing significantly to the donor pool. We have demonstrated in the past that there is no statistical difference in 5-, 10- and 15-year allograft survival and graft function when kidney transplant recipients from DCD donors were compared with those from DBD donors. We also reported a rate of

DGF of 27.5% for DCD donors in the past [3]. An analysis of our data upto 2005 shows a DGF rate of 46%, which is consistent with published reports [2].

Some advocate more potent induction therapy to be used for kidney transplants from donors after cardiac death to protect the grafts from immunologic injury during the period of DGF. Depleting induction therapy with anti-thymocyte globulin (ATG) is felt by many to be more potent than therapy with IL-2RA. A randomized study comparing rabbit ATG (thymoglobulin®; Genzyme, Cambridge, MA, USA) with basiliximab induction in high-risk patients including but not limited to recipients of DCD kidneys demonstrated a significantly reduced rate of acute rejection with thymoglobulin than with basiliximab [4]. Many centers including ours convert induction with interleukin-2 (IL-2) receptor antagonists (RA) by adding ATG as secondary induction in cases of DGF. Not much is known about the effectiveness of the newer monoclonal-depleting anti-CD52 antibody alemtuzumab in renal transplantation, which has been used in our institution since 2002 in combination with triple immunosuppression with steroids, calcineurin inhibitors and mycophenolate mofetil (MMF). According to UNOS statistics alemtuzumab was used in only 2.3% of all cases between 2000 and 2004 [5].

The purpose of the present study was to retrospectively evaluate three groups of recipients of DCD kidneys induced with IL-2RA, ATG and alemtuzumab for patient survival, graft survival, time to acute rejection, infections, malignancy and glomerular filtration.

Methods

Study design

University of Wisconsin maintains a prospectively collected database of all solid-organ transplants performed in our institution. We obtained permission from the Institutional Review Board to retrospectively review this database. Between January 1996 and December 2005, we performed 1335 deceased donor kidney transplants, 170 from controlled donation after cardiac death.

Immunosuppression and medical therapy

Retrospectively risk stratification was undertaken to account for the fact that since 1996 low-risk patients were mostly induced with IL-2RAs, either basiliximab (Simulect®, 20 mg intravenously day 0 and day 4; Novartis, Basel, Switzerland) or daclizumab (Zenapax®, 20 mg intravenously day 0 and day 4; Roche, Basel, Switzerland). High-risk patients were induced with preparations of ATG, either horse antithymocyte serum (ATGAM®, 15 mg/kg i.v.; Pfizer, New York, NY, USA) or rabbit anti-thymocyte serum (Thymoglobulin®, 1.5 mg/kg intravenously daily for 5 doses; Genzyme, Cambridge, MA, USA).

Both high- and low-risk patients were induced with alemtuzumab (Campath-1H®, 30 mg intravenously once daily; BERLEX, Richmond, CA, USA) since 2002.

Risk stratification was performed in the following fashion (Fig. 1): Of the 170 recipients of DCD kidneys from controlled donors (Maastricht type III), four patients with

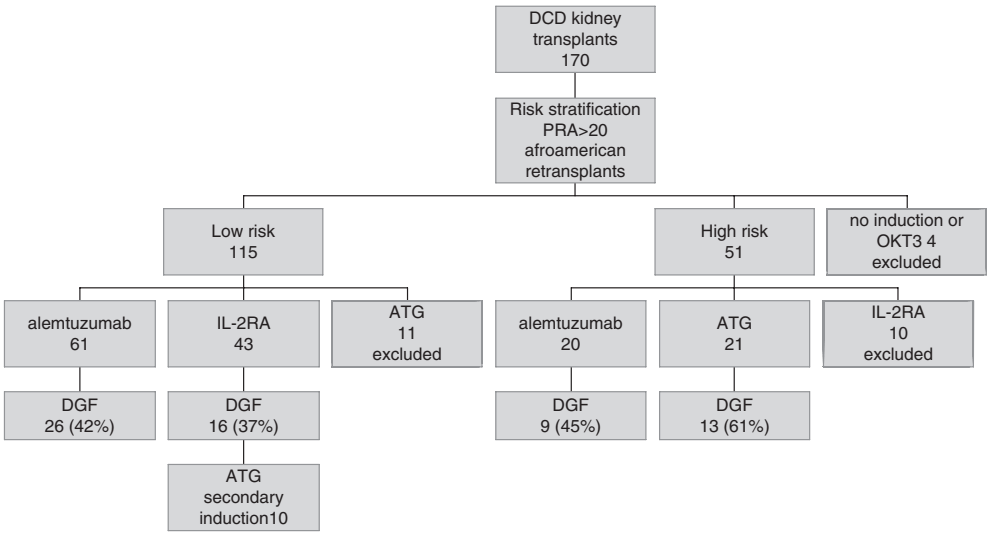

Figure 1 Flow chart demonstrating the study cohorts. Out of 170 DCD kidney transplants 145 were selected based on the risk stratication to examine alemtuzumab versus IL-2RA induction in low-risk patients and alemtuzumab versus ATG in high-risk patients. High-risk patients induced with IL-2RA or low-risk patients induced with ATG were excluded as were patients induce with OKT-3 or no induction.

OKT-3 induction or with no induction as the case may be, were excluded. 104 patients were considered low immunologic risk [panel of reactive antibodies (PRA) < 20%, no retransplants or no Afro-Americans] and were induced with IL-2RA. In cases of DGF, ATG was used in 10 patients. In six patients with a contraindication against depletional therapy no second induction was given. Sixty-one low-risk patients were induced with alemtuzumab.

Forty-one patients were considered to be high-risk patients (PRA > 20%, retransplants or Afro-Americans) and were induced either with ATG (21 patients) or alemtuzumab (20 patients).

Eleven patients were low-risk and induced with ATG and 10 patients were high risk and induced with IL-2RAs. They were excluded from this analysis as this choice of induction was felt to be atypical for our practice.

Patients induced with IL-2RA and ATG received 500 mg of prednisone on the day of transplantation and were then slowly tapered to a daily dose of 30 mg daily at the end of the first month and then further as per the clinician's discretion to reach 10 mg daily at 6 months after the transplant. Patients induced with alemtuzumab received 100 mg of dexamethasone during the transplant and were then rapidly tapered to a dose of 10 mg prednisone daily on day 2. Mycophenolate mofetil was given to all patients at 1000 mg twice daily and was replaced for most patients by mycophenolate EC (Myfortic®; Roche) 720 mg twice daily since 2004. Tacrolimus at 0.05–0.1 mg/kg per mouth (Prograf®; Astellas Pharmaceuticals, Tokyo, Japan) and cyclosporine at 7–9 mg/kg per mouth (Neoral®; Novartis Pharmaceuticals, Basel, Switzerland) were started in twice per day doses once the serum creatinine fell below 3 mg/ml. There were no strict prospective guidelines on dosing and differences between groups in maintenance immunosuppression were not planned.

Antiviral therapy for cytomegalovirus (CMV)-negative recipients of CMV-positive organs and patients being treated for rejection (additional 12 weeks ganciclovir or valganciclovir according to the era) consisted of ganciclovir (Hoffmann-La Roche Inc., Nutley, NJ, USA) at a dose of 500–1000 mg three times daily for 12 weeks or adjusted to renal function. Since 2001, valganciclovir (Hoffmann-La Roche Inc.), at a dose of 450 mg twice per day for 12 weeks, has replaced ganciclovir for the positive to negative patient population. CMV-positive recipients of CMV-positive or -negative organs receive acyclovir at 800 mg four times daily. CMV-negative recipients of CMV-negative organs receive acyclovir at a dose of 400 mg twice daily for Herpes prophylaxis. Sulfamethoxazole/trimethoprim at 160 mg/800 mg was used for PCP prophylaxis except in patients with sulfa allergies who received inhaled pentamidine at 300 mg monthly or dapsone for 1 year. Oral clotrimazole or nystatin was given for fungal prophylaxis for 3 months.

None of the patients had a CDC-positive cross match. Flow cytometry cross match was not performed.

Demographics

We performed a pair-wise comparison of the demographic variables for the high- and low-risk groups: Recipient variables like age, body mass index, percentage of patients with more than one transplant, CMV prevalence, rate of DGF and the immunologic variates PRA, HLA-A, B and DR matching and maintenance immunosuppression and donor variables such as age, donor CMV status, donor-related cause of death, percentage of expanded criteria donor (ECD) kidneys, warm- and cold ischemia time and pump status were compared.

Statistical analysis

Categorical variables were compared with Fisher's exact test and continuous variables were compared between groups using a Wilcoxon rank sums test. For survival curves, the Kaplan–Meier method and the log-rank sum test were used. *P*-values <0.05 were considered significant. Graft loss was not death corrected to also reflect impact on patient survival. All episodes of rejection were biopsy-proven and classified according to the Banff classification.

All statistical analyses were performed using SAS statistical software version 6.12, SAS Institute Inc. (Cary, NC, USA). Continuous variables are summarized by reporting the means and standard deviations (mean ± SD). Percentages are used to summarize categorical variables.

Glomerular filtration rate (GFR) was calculated by the following formula: GFR (ml/min) = 6.7/creatinine (mmol/l) + BW (kg)/4-urea (mmol/l)/2 − 100/height $(m)^2$ + [35 (male) or 25 (female)] [6] for 1 and 6 months, 1, 3 and 5 years post-transplant to assess long-term function.

Results

Comparison of donation after cardiac death to donation after brain death

The analysis of our overall outcome of DCD kidney transplants compared with 1165 transplants from donors after brain death (DBD) over the same time period showed that primary nonfunction rate and graft loss within the first 30 days is not significantly different between the groups. The incidence of DGF in recipients of DCD transplants is about double as high (46%) compared to DBD recipients (24%). GFR is initially significantly depressed in DCD kidneys but returns to the same level as in DBD kidneys within 1 year, which confirms

Table 1. Kidney allograft function comparing donation after cardiac death with donation after brain death.

	DBD donors (n = 1165)	DCD donors (n = 170)	P-value
Primary nonfunction (%)	0.5%	2.4%	0.2
Graft loss during first 30 days (%)	9%	13%	0.32
Delayed graft function (%)	24%	46%	**<0.001**
GFR 1 month (ml/min)	63.3 (n = 1129)	57.7 (n = 166)	**0.005**
GFR at 6 months (ml/min)	64.9 (n = 1023)	61.2 (n = 141)	**0.03**
GFR at 1 year (ml/min)	66.4 (n = 929)	63.3 (n = 116)	0.08
GFR at 5 years (ml/min)	67.3 (n = 339)	68.7 (n = 31)	0.67
Graft-survival after 5 years (%)	69%	67%	0.41
Freedom from acute rejection at 2 years (%)	62%	63%	0.46

GFR, glomerular filtration rate; DBD, donation after brain death; DCD, donation after cardiac death. $P < 0.05$.

previously published data [7]. Graft survival at 5 years and overall rates of biopsy-proven acute rejection at 2 years do not differ (Table 1).

Demographics low-risk group

Within the low-risk group, the patients induced with alemtuzumab had older donors (mean 39 vs. 49 years) and had a shorter cold-ischemia time reflecting the change of organ allocation over the 10-year time period and our sequential study design (Table 2a). We also noticed a lower number of CMV-positive to -negative patients in the alemtuzumab-induced group, which is the group at higher risk for CMV disease. HLA-B matching was better for the IL-2RA group and HLA-DR matching was better in the alemtuzumab-induced group. We found significant difference in drug-dosing for MMF and drug levels for both tacrolimus and cyclosporine between the alemtuzumab- and the IL-2RA-induced groups. It is difficult to interpret this finding in the context of a retrospective study. There were no clear prospective guidelines for dosing and the reasons for drug dosing are unknown.

Demographics high-risk group

The only difference we found in the high-risk group was a shortened cold ischemia time and less HLA-B matching for the alemtuzumab group (Table 3).

Patient survival/graft survival and time to acute rejection

We did not find a significant difference for time to acute rejection in both high and low-risk groups comparing alemtuzumab to either IL-2RA or ATG (Figs 2 and 3) There was no statistically significant difference in graft survival (Figs 2 and 3) over 3 years. Patient survival (Fig. 4) seemed to be worse for patients induced with alemtuzumab in the high-risk group with a P-value approaching significance ($P = 0.055$). The antibody induction strategies did not lead to significantly different outcomes in patients receiving kidney transplants from donation after cardiac death.

However at 1 year we noticed a reduced graft survival of 84% (confidence interval 71–91%) for the

Table 2. Demographics of (a) donors and (b) recipients for low-risk DCD kidney transplants.

Variable Time period	IL-2RA (n = 43) 1996–2005	Alemtuzumab (n = 61) 2002–2005	P-value
(a)			
Donor age	39 ± 16	49 ± 12	**<0.01**
Donor gender (% fem)	33%	33%	1.0
Donor race (% caucasian)	97%	97%	1.0
Crea at death (mean)	0.96	0.97	0.96
Cause of death (% stroke)	35%	25%	0.37
ECD (%)	16%	26%	0.62
WIT (min)	23 ± 26	27 ± 21	0.10
CIT (h)	23 ± 25	18 ± 4	**<0.05**
Pumped (%)	93%	100%	0.06
(b)			
Age (years)	53 ± 10	52 ± 12	0.49
Gender (% fem)	49%	49%	1.0
Race (% afroamerican)	0	0	
Weight (kg)	78 ± 13	81 ± 19	0.33
Peak PRA (%)	2.3 ± 3	1.7 ± 2.6	0.60
PRA at transplant (%)	0.3 ± 0.9	0.6 ± 2.1	0.57
Hemodialysis pretransplant	67%	80%	0.07
Diabetes (in %)	21	34	0.18
Retransplants (%)	0	0	
On hemodialysis at Tx	63	75	0.06
Full A match (%)	2.3	3.2	0.8
Full B match (%)	11.6	4.9	**<0.01**
Full DR match (%)	4.9	23.3	**0.02**
CMV pos → CMV neg	37%	19%	**0.008**
CMV neg → CMV pos	25%	20%	
CMV pos → CMV pos	14%	44%	
CMV neg → CMV neg	23%	16%	

PRA, panel of reactive antibodies; CMV, cytomegalovirus; ECD, expanded criteria donor; WIT, warm ischemia time; CIT, cold ischemia time. $P < 0.05$.

Table 3. Demographics of (a) donors and (b) recipients for high-risk DCD kidney transplants.

Variable Time period	ATG (*n* = 21) 1996–2005	Alemtuzumab (*n* = 20) 2002–2005	*P*-value
(a)			
Donor age	50 ± 10	46 ± 11	0.29
Donor gender (% fem)	52%	25%	0.11
Donor race (% caucasian)	95%	100%	0.80
Cause of death (% stroke)	39%	43%	0.80
ECD (%)	11%	15%	0.33
WIT (min)	22 ± 16	26 ± 12	0.13
CIT (h)	22 ± 7	18 ± 5	**0.02**
Pumped (%)	95	100	0.51
(b)			
Age (years)	46 ± 11	50 ± 11	0.29
Gender (% fem)	50%	23%	0.11
Race (% afroamerican)	28	35	0.24
Weight (kg)	76 ± 24	78 ± 19	0.57
Peak PRA (%)	32 ± 36	16 ± 25	0.19
PRA at transplant (%)	11 ± 22	6 ± 13	0.28
Hemodialysis pretransplant	85%	60%	0.08
Diabetes (in %)	23	30	0.25
Retransplants (%)	76	55	0.19
Recip CMV pos (%)	76	60	0.32
On hemodialysis at Tx	86	60	0.08
Full A match (%)	5	15	0.54
Full B match (%)	10	5	**0.04**
Full DR match (%)	19	10	0.53
CMV pos → CMV neg	19%	20%	0.51
CMV pos → CMV pos	14%	44%	
CMV neg → CMV pos	28%	20%	
CMV neg → CMV neg	4%	20%	

PRA, panel of reactive antibodies; CMV, cytomegalovirus; ECD, expanded criteria donor; WIT, warm ischemia time; CIT, cold ischemia time. $P < 0.05$.

alemtuzumab patients in the low-risk group and of 72% (confidence interval 46–88%) for the alemtuzumab patients in the high-risk group. Patient survival in the high-risk group was only 70% (confidence interval 43–87%) for the high-risk patients induced with alemtuzumab compared to ATG. At the same time the chance of rejection appeared decreased at 1 year for the alemtuzumab-induced patients in both the low- (78%) and the high-risk (62%) groups (Fig. 9).

Infections

In the low-risk group, there was a significantly higher incidence of CMV infections found in the alemtuzumab group as well as trend towards a higher incidence of BK virus and bacterial infections (Table 4b). There was no difference seen in either invasive or non-invasive fungal infections (Table 4b) There was no significant difference observed in the high-risk group, presumably due to small sample size (Table 5b).

Malignancy and post-transplant lymphoproliferative disorder

There was no difference observed in post-transplant lymphoproliferative disorder (PTLD) or malignancies between induction groups (Tables 4b and 5b).

Long-term graft function/glomerular filtration

There was no difference or trend in 3-year renal function as assessed by estimated glomerular filtration in either low (Table 4a) or high-risk groups (Table 5a).

Maintenance immunosuppressive therapy

The majority of low-risk patients received maintenance immunosuppression with cyclosporine. Cyclosporine levels were significantly lower in the group induced with alemtuzumab. The doses of MMF were reduced in the alemtuzumab cohort throughout reflecting the higher incidence of leukopenia in the alemtuzumab-treated patients (Table 6).

The majority of patients in the high-risk group received maintenance immunosuppression with tacrolimus. There was no significant difference in tacrolimus level observed between the induction groups, but the sample size was small. There was a trend towards reduced dosing of MMF in the alemtuzumab group (Table 7). These results are difficult to interpret as there were no prospective guidelines on dosing.

Discussion

Patients who receive kidneys from DCD donors are considered to be at high-risk for DGF and initial dysfunction presumably due to prolonged warm ischemia during the recovery process [8]. We and others have shown in the past that this does not impact on graft survival or acute rejection [1–3].

Donation after cardiac death is therefore an important addition to the deceased donor pool, but it poses several management problems. Due to the increased incidence of DGF and the elevated creatinine levels acute rejection is difficult to detect unless protocol biopsies are performed. Undetected acute rejection puts the graft at risk. Antibody induction therapy has successfully reduced the incidence of early acute rejection while long-term effects are controversial. There is good evidence that use of induction therapy with IL-2R antagonists and ATG reduces the risk of acute rejection by about 30% and mixed evidence that it

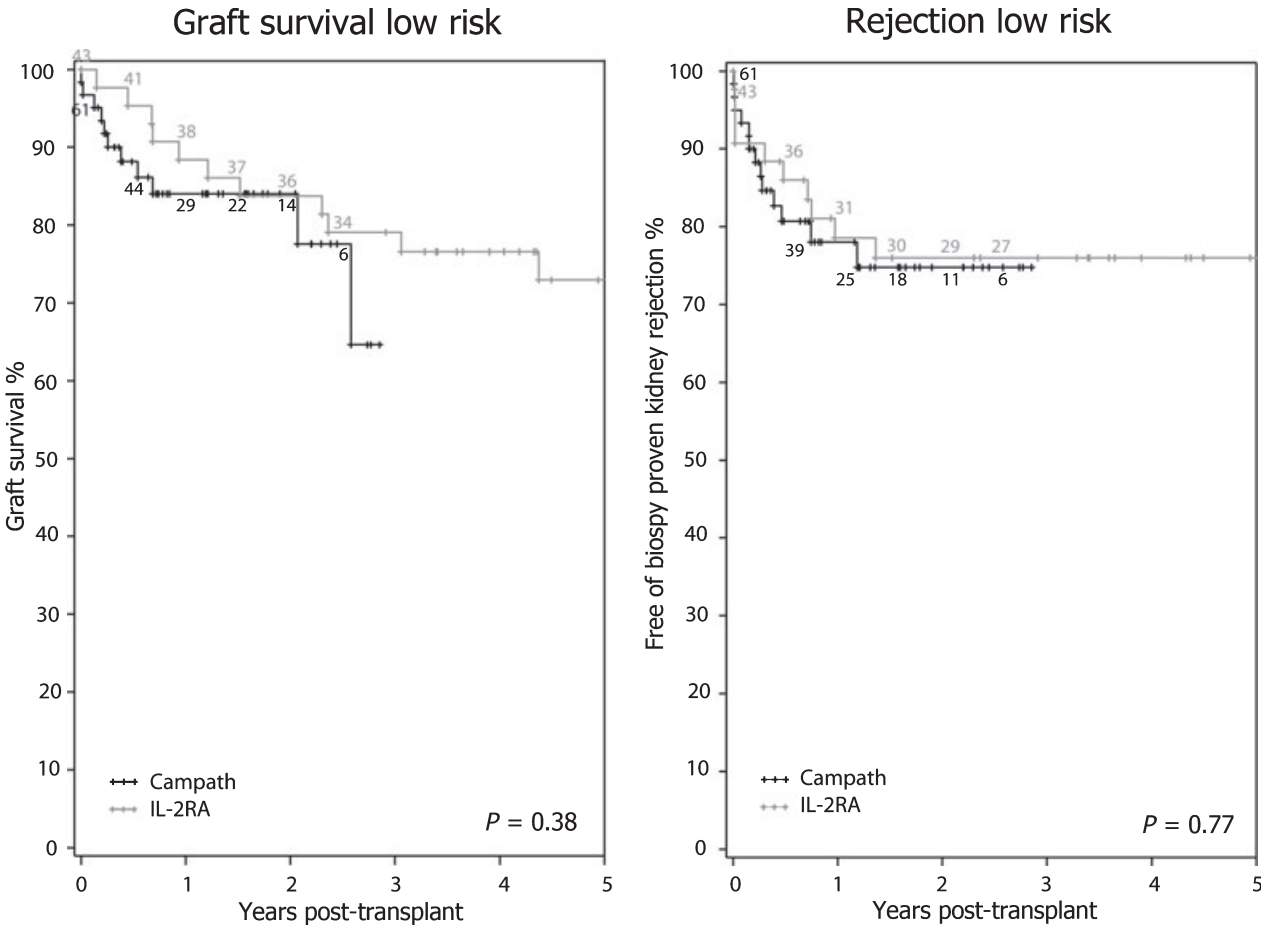

Figure 2 Graft survival and freedom from acute rejection for low-risk DCD kidney transplants stratified for IL-2RA (basiliximab and daclizumab) induction versus alemtuzumab.

improves graft survival in renal transplant patients [9]. Between 1997 and 2002, a threefold increase in induction therapy was attributed to the surging use of IL-2RA and ATG [10]. Potent induction therapy also has the potential to attenuate initial ischemia reperfusion injury.

Anti-thymocyte globulin in the form of TThymoglobulin® has enjoyed soaring popularity in recent years. The Thymoglobulin Induction Study Group performed a multi-center randomized study of thymoglobulin against basiliximab induction in high-risk patients and demonstrated a significant decrease in acute rejection with the use of rabbit ATG versus basiliximab (15.6% vs. 25.5%) but similar incidences of graft loss, DGF and patient survival. They also found a greater incidence of overall infection and a lower incidence of cytomegalovirus disease in patients induced with rabbit ATG. DCD status of the donor was one high-risk criterion together with CIT >24 h, donor age >50, donor ATN, six antigen mismatch PRA > 20%, six antigen mismatch or African descent. Many centers therefore routinely induce recipients of DCD kidneys and other high-risk patients with ATG.

We use induction therapy in every deceased donor kidney transplant including DCD kidney transplants. Generally high-risk recipients receive ATG and low-risk recipients receive IL-2 RA. We have not considered DCD status alone to be a criterion for high-risk. Our definition of high-risk generally includes PRA > 20, Afro-Americans and retransplants. In low-risk patients induced with IL-2RA usually secondary induction therapy with ATG is added in cases of DGF if there are no contraindications. For high-risk patients (PRA > 20, Afro-Americans, retransplants) ATG was used upfront. Since 2002, we have used the induction agent alemtuzumab for all patients, both low- and high-risk patients with the presumption that alemtuzumab potently prevents acute rejection. Besides being a potent T-cell depleting agent, alemtuzumab presents several advantages. It is easy to administrate as it does not require a central line and is less expensive than rabbit ATG (Table 8).

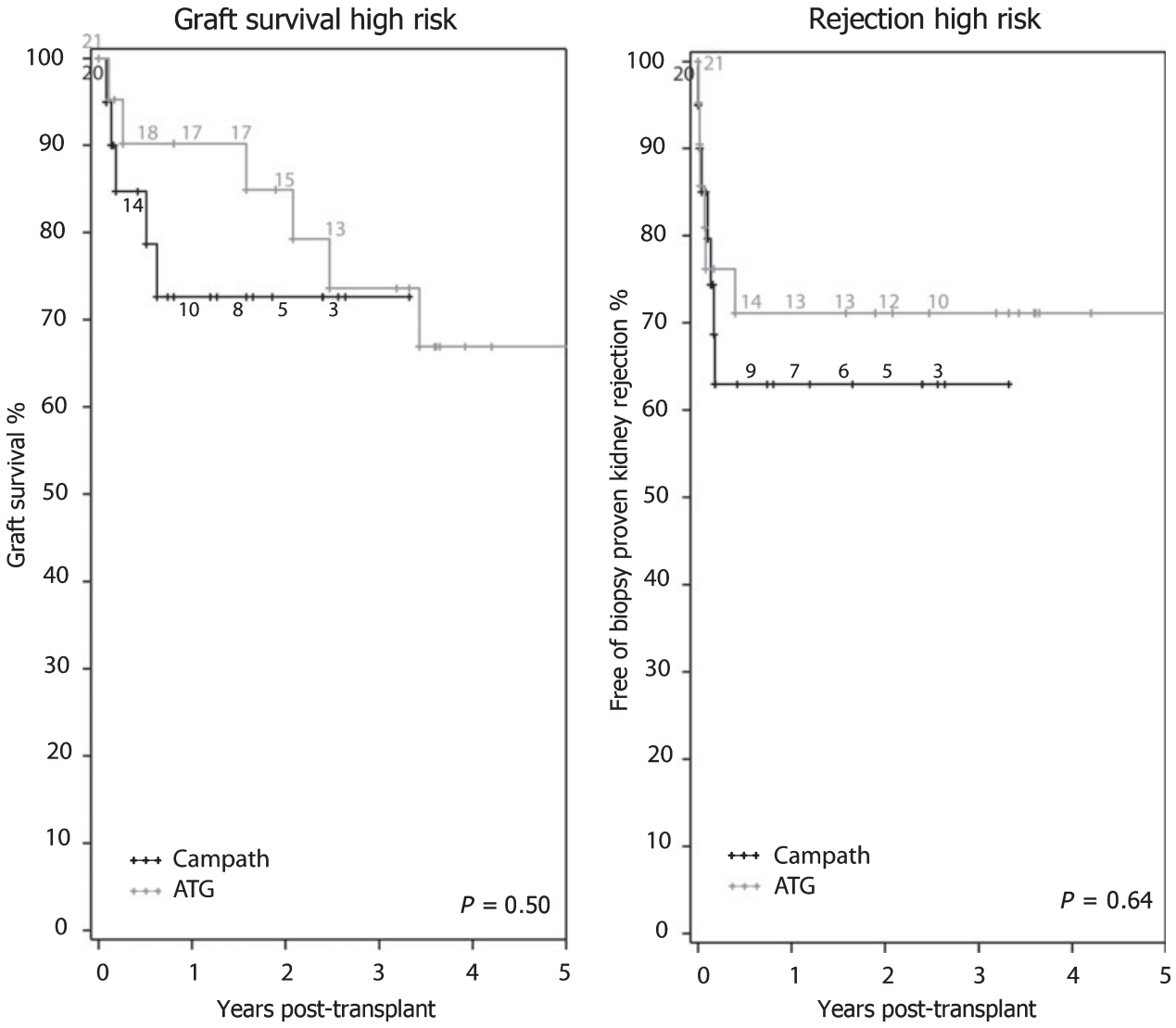

Figure 3 Graft survival and freedom from acute rejection for high-risk DCD kidney transplants stratified for ATG (ATGAM and thymoglobulin) versus alemtuzumab.

The effect of alemtuzumab on outcome for the subgroup of recipients of DCD kidneys has to our knowledge never been examined, neither prospectively or retrospectively. Due to lack of prospective data we decided to retrospectively analyze our experience with alemtuzumab in DCD kidney transplantation.

In our retrospective analysis, we realized that the majority of low-risk patients were either induced with IL-2RA or alemtuzumab and that the bulk of high-risk patients were induced with either ATG or alemtuzumab. To simplify the analysis and because of low numbers for the other groups, we compared IL-2RA and alemtuzumab in the high-risk and ATG and alemtuzumab in the low-risk group. In the demographic comparison there were differences in the low-risk group between alemtuzumab and IL-2RA as donor age and cold ischemia time, recipient CMV disease and B and DR match. For the high-risk group slightly shorter cold ischemia time potentially advantages the alemtuzumab group and lower donor age potentially advantages the ATG group for all outcomes. Better DR matching in the alemtuzumab could predispose this group to less rejection. In the high-risk group less differences were noted, but the groups were significantly smaller. Shorter cold ischemia time in donors represents an advantage for the alemtuzumab group, whereas the impact of less B matching in recipients is unlikely to play a role. The combined impact of these differences on outcome is difficult to judge.

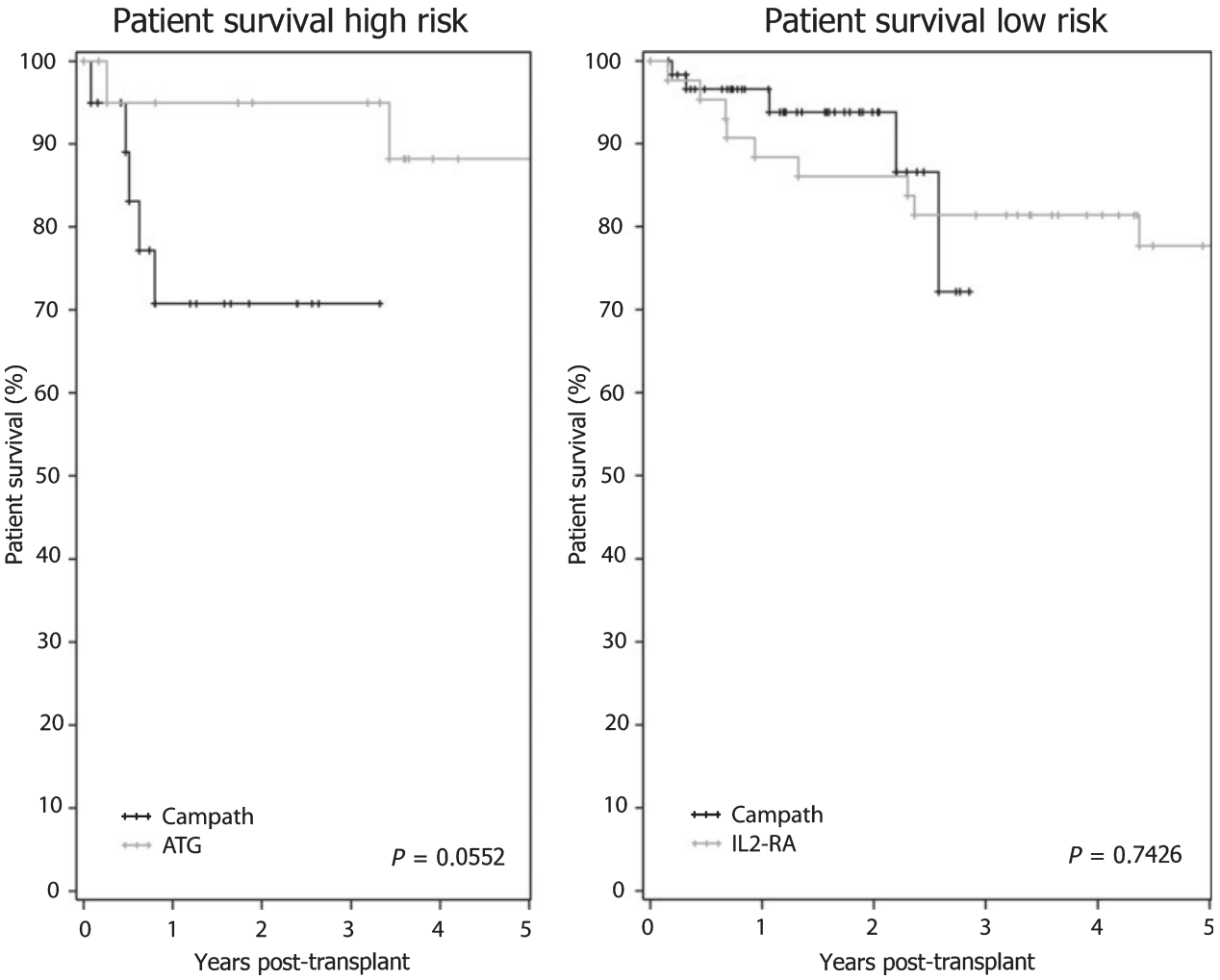

Figure 4 Patient survival for high-risk DCD kidney transplants and low-risk DCD kidney transplants stratified according to conventional induction versus alemtuzumab.

There are several studies about induction in DCD kidney transplantation.

Wilson *et al.* randomized 51 recipients of DCD kidney transplants to induction with daclizumab and daily MMF and compared them with no induction but a standard tacrolimus-based triple therapy without induction. Although they found a decreased incidence of DGF in daclizumab-induced recipients with delayed introduction of calcineurin inhibitors, outcome beyond 3 months was not presented [11]. Rivera *et al.* [12] showed that induction with basiliximab compared with no induction reduced the incidence of DGF in patients who receive allograft at high-risk for DGF which also included but not limited to recipients of DCD kidneys. We have not found the incidence of DGF significantly different between induction regimen in our series (see Fig. 1).

Several studies have examined alemtuzumab in kidney transplantation. Alemtuzumab has held the promise to allow reduction of maintenance immunosuppressant regimen. Watson *et al.* reported the successful use of alemtuzumab followed by low-dose cyclosporine to facilitate steroid-free immunosuppression. He observed an early delay in acute rejection and no increase in infectious complications [13]. We observed a high acute rejection rate of 28% when we used alemtuzumab with sirolimus monotherapy for maintenance immunosuppression [14]. The 3-year results of this study demonstrated good graft- and patient outcomes [15]. The addition of mycophenolate mofetil to sirolimus maintenance therapy after alemtuzumab induction resulted in 36% incidence of acute rejection [16]. Kirk *et al.* [17] attempted alemtuzumab monotherapy without maintenance immunosuppression,

Table 4. (a) Estimated glomerular filtration of recipients of low-risk DCD kidney transplants; (b) Incidence of infections and malignancy in recipients of low-risk DCD kidney transplants at 3 years.

GFR at time after transplant	IL-2RA (*n*)	Alemtuzumab (*n*)	*P*-value
(a)			
1 month	60 ± 19 (15)	57 ± 18 (25)	0.58
6 months	60 ± 17 (15)	60 ± 21 (21)	0.83
1 year	63 ± 18 (13)	62 ± 18 (17)	0.75
2 year	64 ± 20 (11)	54 ± 14 (10)	**0.02**
	IL-2RA (*n* = 43)	Alemtuzumab (*n* = 61)	*P*-value
(b)			
Viral			
CMV	4	15	**0.01**
EBV	0	0	–
BK	0	4	0.07
Fungal and parasitic			
Noninvasive	7	2	0.22
Invasive	8	9	0.80
Bacterial	22	31	0.06
PTLD	1	0	0.32
Other malignancies	8	1	0.25

GFR, glomerular filtration; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PTLD, post-transplant lymphoproliferative disorder.
$P < 0.05$.

Table 5. (a) Estimated glomerular filtration of recipients of high-risk DCD kidney transplants; (b) Incidence of infections in recipients of high-risk DCD kidney transplants at 3 years.

GFR at time after transplant	ATG (*n*)	Alemtuzumab (*n*)	*P*-value
(a)			
1 month	57 ± 20 (13)	51 ± 18 (9)	0.39
6 months	64 ± 10 (12)	61 ± 15 (7)	0.80
1 year	69 ± 17 (11)	60 ± 14 (4)	0.32
2 years	56 ± 25 (10)	62 ± 50 (4)	1.0
Infections	ATGAM/rATG (*n* = 21)	ALM (*n* = 20)	*P*-value
(b)			
Viral			
CMV	1	4	0.10
EBV	1	0	0.78
BK	0	0	
Fungal and parasitic			
Noninvasive	2	1	0.63
Invasive	1	3	0.24
Bacterial	8	8	0.84
PTLD	0	0	–
Other Malignancy	4	0	0.62

GFR, glomerular filtration; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PTLD, post-transplant lymphoproliferative disorder.

Table 6. Maintenance immunosuppression of recipients of low-risk DCD transplants.

	BAS/DAC (*n*)	ALM (*n*)	*P*-value
No. patients started on TAC	5 (43)	12 (61)	
TAC level at 1 month	6 ± 3.6 (5)	8.1 ± 8.2 (12)	0.55
TAC level at 6 months	4.6 ± 3.3 (9)	5.2 ± 3.7 (12)	0.85
TAC level at 12 months	5.1 ± 3.3 (7)	4.3 ± 3.8 (9)	0.44
TAC level at 24 months	5.8 ± 1.9 (5)	4.5 ± 1.8 (6)	0.26
No. patients started on CsA	34 (43)	34 (61)	
CsA level at 1 month	214 ± 107 (34)	179 ± 287 (34)	**<0.01**
CsA level at 6 months	132 ± 46 (31)	118 ± 86 (25)	**0.05**
CsA level at 12 months	123 ± 51 (29)	98 ± 77 (19)	**0.03**
CsA level at 24 months	151 ± 113 (24)	96 ± 79 (8)	**0.02**
No. patients started on MMF	43 (43)	61 (61)	
MMF dose at 1 month	1651 ± 744	1571 ± 650	**0.03**
MMF dose at 6 months	1548 ± 600	1270 ± 599	**0.02**
MMF dose at 12 months	1546 ± 646	1325 ± 538	**0.04**
MMF dose at 24 months	1569 ± 645	1230 ± 560	**0.03**

TAC, tacrolimus; CsA, cyclosporine; MMF, mycophenolate mofetil.
$P < 0.05$.

which resulted in early rejection in all patients and discouraged the hopes in the tolerogenic potential of alemtuzumab without maintenance immunosuppression. Shapiro *et al.* reported the spaced weaning of maintenance immunosuppression (tacrolimus) of alemtuzumab-induced kidney transplant recipients with cumulative rejection rates of 20% at 1 year, which equaled those of historical controls. Only 12% of alemtuzumab-induced patients ultimately required multidrug therapy. There was no increased viral infection rate observed in the alemtuzumab-induced group [18]. Helderman *et al.* [19] in a SRTS databank analysis showed that nationwide graft-survival is reduced for both depleting therapies with alemtuzumab and rabbit ATG but there remains a concern about a negative selection bias. Second, decreased graft survival could be caused by withdrawal or minimization strategies of maintenance immunosuppression. Huang *et al.* analyzed the OPTN/UNOS database for the period January 2003 to December 2004 to evaluate alemtuzumab induction and found an increased incidence of acute rejection in deceased donor transplants induced with alemtuzumab compared to no induction, Il-2RAs and rabbit ATG. There was no difference observed in acute rejection rates of living donor transplants. There was no difference in graft survival observed between induction groups [20].

Since 2003, we have felt that continuing triple maintenance immunosuppression after induction with alemtuzumab is important [21]. We reported our results of alemtuzumab induction combined with a calcineurin inhibitor, mycophenolate mofetil and low-dose steroid

Table 7. Maintenance immunosuppression of recipients of high-risk DCD transplants.

	ATGAM/rATG (n = 21)	ALM (n = 20)	P-value
No. patients started on TAC	13	7	
TAC level at 1 month	7.7 ± 5.5 (13)	7.7 ± 4.3 (7)	0.69
TAC level at 6 months	6.6 ± 4.1 (11)	6.8 ± 4.3 (7)	0.92
TAC level at 12 months	6.6 ± 3.3 (12)	7.6 ± 4.9 (5)	0.52
TAC level at 24 months	7.4 ± 4.1 (10)	8.5 ± 4.0 (4)	0.61
% of patients started on CsA	5	6	
CsA level at 1 month	108 ± 104 (5)	204 ± 128 (6)	0.14
CsA level at 6 months	352 ± 435 (5)	114 ± 26 (6)	0.58
CsA level at 12 months	64 ± 54 (4)	142 ± 91 (6)	0.19
CsA level at 24 months	59 ± 37 (3)	105 ± 2.8 (2)	0.08
% of patients started on MMF	21	20	
MMF dose at 1 month	1814 ± 918	1633 ± 900	0.47
MMF dose at 6 months	1580 ± 791	1032 ± 769	0.08
MMF dose at 12 months	1705 ± 751	1050 ± 724	**0.03**
MMF dose at 24 months	1566 ± 728	1100 ± 741	0.22

TAC, tacrolimus; CsA, cyclosporine; MMF, mycophenolate mofetil.
$P < 0.05$.

Table 8. Cost of induction therapy per treatment.

	Average whole sale price in US$
Simulect alone 20 mg × 2	3719
Simulect 20 mg × 1 converted to thymoglobulin 1.5 mg/kg × 4 doses	20 609
Thymoglobulin 1.5 mg/kg × 4 doses	18 750
Campath 1-H 30 mg × 1	1956

therapy in 2004. One year after induction the incidence of acute rejection was reduced and graft survival was improved in the alemtuzumab-induced patient groups. Especially patient groups with DGF seemed to profit from this approach and infection rates were not different at 1 year [22].

Our study reports our experience with this strategy over the last 3 years in recipients of DCD kidneys, a patient group with a high DGF rates. Patients were treated with triple-drug immunosuppression after their induction. We have found that reduction of MMF doses is necessary on account of alemtuzumab-induced leukopenia. We have also found a decrease in cyclosporine levels in the ALM group. Significance was obtained in the low-risk group but not in the high-risk group due the small sample size. Cyclosporine levels were found to be decreased, most likely, because clinicians felt that lower cyclosporine dosing was adequate in the setting of alemtuzumab induction. We also used lower doses of steroids in our standard protocol for alemtuzumab induction. We therefore present this experience as outcomes of alemtuzumab used in conjunction with reduced triple maintenance immunosuppression.

Our results demonstrate that alemtuzumab with triple immunosuppression protocol yields equivalent rejection rates and achieves statistically not significantly different graft and patient survival compared with IL-2RA or ATG. To avoid misinterpretation of the right head end of the Kaplan–Meier curves the numbers of patients at risk for graft loss are given in Figs 2 and 3. The analysis of the estimated survival rate at 1 year revealed a decreased chance of graft and patient survival with alemtuzumab (Table 9) Long-term renal function as judged by glomerular filtration of kidneys in both groups did not differ at 3 years, but were not available in all patients for follow-up at all time points. In our cohort of DCD transplants we could not observe a decrease of rejection episodes in the first 3 months like Kaufman *et al.* [23] reported for his alemtuzumab-induced cohort with steroid avoidance nor within the first year as we [22] and others [18] had

Table 9. Total numbers of events of graft loss, death, rejection and graft survival estimates and confidence intervals at 1 year.

Groups (total number of patients)	Graft loss (n)	Estimated graft survival on ALM (CI)	Death (n)	Estimated patient survival on ALM (CI)	Rejection (n)	Estimated risk of rejection on ALM (CI)
Low-risk ALM (61)	11	84 (71–91)%	5	96 (87–99)%	12	78 (64–87)%
Low-risk IL-2RA (43)	13		5		9	
High-risk ALM (20)	5	72 (46–88)%	5	70 (43–87)%	7	62 (37–80)%
High-risk ATG (21)	2		1		6	

initially observed in our kidney transplant recipients. We have attributed alemtuzumab's lack of superiority in preventing acute rejection to the slightly reduced calcineurin-hibitor levels and mycophenolate mofetil doses that accompanied its use (Tables 4 and 7).

We found a higher incidence CMV infections in patients induced with alemtuzumab although number of high-risk group of CMV positive to negative transplants was significantly lower (19%) in the alemtuzumab-induced group compared with the IL-2RA induced group (37%). We also found a trend towards higher infection rates for BK-virus and bacterial infections. Similar trends were observed when alemtuzumab was compared with ATG in the high-risk groups, but numbers here were too small for significance. It is interesting that neither Kaufmann *et al.* in their study of alemtuzumab induction with tacrolimus maintenance and steroid avoidance nor Shapiro *et al.* in their study of alemtuzumab induction with tacrolimus monotherapy and spaced weaning nor Flechner in their study of alemtuzumab induction with sirolimus/MMF maintenance found an increase in viral or bacterial infections. Unfortunately the large database studies [19,20] have not reported on infectious complications. It appears likely that the respective withdrawal or minimization strategies of maintenance immunosuppression in these respective studies balanced the infectious risk associated with alemtuzumab induction with triple immunosuppression.

In the face of higher infection rates with a trend towards decreased graft survival and higher mortality compared to IL-2RA in low-risks patients, there seems to be no benefit to alemtuzumab induction in recipients of DCD kidneys. The economic advantage of utilizing less expensive induction therapy does not offset the higher risk of infections. We recently initiated prophylaxis with valganciclovir in every patient with alemtuzumab induction regardless of their CMV status, which adds more costs to the regimen.

It appears unlikely to us that better outcomes could have been achieved with higher doses of maintenance immunosuppression because of the leukopenia and even higher risks of infectious complications.

On the other hand it is possible that novel therapeutic combinations of alemtuzumab with withdrawal strategies of calcineurininhibitors and/or drugs that induce regulation could yield better outcomes than alemtuzumab used with conventional immunosuppression.

The major study limitation is the lack of prospective and controlled study design. Given that the bulk IL-2RA and ATG patients underwent transplantation before the general introduction of alemtuzumab an era effect could be responsible for higher graft loss and mortality and the higher infection rate in the alemtuzumab group. Only a prospective study could rule out these concerns.

Conclusion

Induction of DCD kidney transplants with alemtuzumab with triple immunosuppression compared to IL-2RA and ATG does not delay acute rejection in a retrospective analysis. There are no statistically significant differences in patient and graft survival but trend towards worse outcomes for alemtuzumab. It also appears that CMV infections are increased in patients induced with alemtuzumab. We therefore conclude that induction with alemtuzumab in kidney transplants from DCD donors followed by conventional immunosuppression does not confer any advantage over traditional induction agents.

Authorship

ES: analyzed data and wrote paper. LAF: designed study, analyzed data and corrected and revised paper. AMD: corrected and revised paper. SJK, JO, YB, JP and HS: corrected and revised paper.

References

1. Cho YW, Terasaki PI, Cecka JM, Gjertson DW. Transplantation of kidneys from donors whose hearts have stopped beating. *N Engl J Med* 1998; **338**: 221.
2. Weber M, Dindo D, Demartines N, Ambuhl PM, Clavien PA. Kidney transplantation from donors without a heartbeat. *N Engl J Med* 2002; **347**: 248.
3. Cooper JT, Chin LT, Krieger NR, *et al.* Donation after cardiac death: the university of wisconsin experience with renal transplantation. *Am J Transplant* 2004; **4**: 1490.
4. Brennan D. Thymoglobulin Induction Study Group. Thymoglobulin versus simulect for induction immunosuppression in cadaveric renal transplant recipients: final results from a prospective, randomized, multicenter trial. *Am J Transplant* 2003; **3**: 1268.
5. Flechner SM, Feng J, Mastroianni B, *et al.* The effect of 2-gram versus 1-gram concentration controlled mycophenolate mofetil on renal transplant outcomes using sirolimus-based calcineurin inhibitor drug-free immunosuppression. *Transplantation* 2005; **79**: 926.
6. Nankivell BJ, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1995; **59**: 1683.
7. Chapman J, Bock A, Dussol B, *et al.* Follow-up after renal transplantation with organs from donors after cardiac death. *Transpl Int* 2006; **19**: 715.
8. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* 2004; **364**: 1814.

9. Nashan B. Antibody induction therapy in renal transplant patients receiving calcineurin-inhibitor immunosuppressive regimens: a comparative review. *BioDrugs* 2005; **19**: 39.
10. Bunnapradist S, Takemoto SK. Multivariate analyses of antibody induction therapies. *Clin Transpl* 2003; **1**: 405.
11. Wilson CH, Brook NR, Gok MA, Asher JF, Nicholson ML, Talbot D. Randomized clinical trial of daclizumab induction and delayed introduction of tacrolimus for recipients of non-heart-beating kidney transplants. *Br J Surg* 2005; **92**: 681.
12. Rivera CF, Hernandez AA, Verdejo PV, Garcia JO, Rego SC, Canedo FV. Basiliximab (Simulect) in acute tubular necrosis high-risk kidney transplantation. *Transplant Proc* 2005; **37**: 3733.
13. Watson CJ, Bradley JA, Friend PJ, *et al.* Alemtuzumab (CAMPATH 1H) induction therapy in cadaveric kidney transplantation–efficacy and safety at five years. *Am J Transplant* 2005; **5**: 1347.
14. Knechtle SJ, Pirsch JDH, Fechner JJ, *et al.* Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. *Am J Transplant* 2003; **3**: 722.
15. Barth RN, Janus CA, Lillesand CA, *et al.* Outcomes at 3 years of a prospective pilot study of Campath-1H and sirolimus immunosuppression for renal transplantation. *Transpl Int* 2006; **19**: 885.
16. Flechner SM, Friend PJ, Brockmann J, *et al.* Alemtuzumab induction and sirolimus plus mycophenolate mofetil maintenance for CNI and steroid-free kidney transplant immunosuppression. *Am J Transplant* 2005; **5**: 3009.
17. Kirk AD, Hale DA, Mannon RB, *et al.* Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). *Transplantation* 2003; **76**: 120.
18. Shapiro R, Basu A, Tan H, *et al.* Kidney transplantation under minimal immunosuppression after pretransplant lymphoid depletion with Thymoglobulin or Campath. *J Am Coll Surg* 2005; **200**: 505; quiz A59-61.
19. Helderman J, Gilmore A, Marehbian J, Legorreta A. Patient outcomes assessment of campath induction therapy in renal transplant recipients. *Transplantation* 2006; **82**: 294.
20. Huang E, Bunnapradist S. Alemtuzumab induction in kidney transplantation. *Clin Transpl* 2005; **1**: 343.
21. Magliocca JF, Knechtle SJ. The evolving role of alemtuzumab (Campath-1H) for immunosuppressive therapy in organ transplantation. *Transpl Int* 2006; **19**: 705.
22. Knechtle SJ, Fernandez LA, Pirsch JD, *et al.* Campath-1H in renal transplantation: The University of Wisconsin experience. *Surgery* 2004; **136**: 754.
23. Kaufman DB, Leventhal JR, Axelrod D, Gallon LG, Parker MA, Stuart FP. Alemtuzumab induction and prednisone-free maintenance immunotherapy in kidney transplantation: comparison with basiliximab induction–long-term results. *Am J Transplant* 2005; **5**: 2539.