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Impact of daclizumab, low-dose cyclosporine, mycophenolate mofetil and steroids on renal function after kidney transplantation

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Abstract

Background. Early and long-term use of cyclosporine A (CsA) leads to increased risks of renal toxicity. We hypothesized that administration of daclizumab in combination with mycophenolate mofetil (MMF) allows a relevant reduction in the dose of CsA.

Methods. We carried out a 3-year, prospective, randomized, controlled clinical multi-centre trial in 156 patients. The patients were randomized to standard treatment (CsA, MMF, steroids) or to high-dose daclizumab (first dose: 2 mg/kg), in combination with low-dose CsA, MMF and steroids. We maintained the mean CsA levels of daclizumab patients at 57% of standard patients (132 versus 216 ng/ml) on Day 7 post-transplant, and 84% by 6 months.

Results. Primary outcome, creatinine clearance (with imputation of informative dropouts) at 12 months, was significantly better in daclizumab-treated (34 ± 17) than standard patients (29 ± 17; $P = 0.028$, two sided). Only 5 cases of BPAR were recorded in the daclizumab compared to 22 in the standard group ($P = 0.0016$). Daclizumab patients had 91% event-free survival after 1 year compared to 66% in standard patients ($P = 0.00017$).

Conclusion. We demonstrate here that high-dose daclizumab in combination with lower CsA levels in adult renal transplant recipients is as or more effective than standard regimen (CsA, MMF, steroids) and may result in better outcomes at 12 months post-transplant with no increase in adverse reactions.

Keywords: cyclosporine; daclizumab; immunosuppression; kidney; transplantation

Introduction

Cyclosporine A (CsA) and tacrolimus, which prevent T-lymphocyte activation by calcineurin inhibition (CNI), are currently the anchor medication of most immunosuppressive regimens in kidney transplant recipients [1]. However, there are many efforts to reduce or even withdraw CNIs as their early and long-term administration is associated with a high risk of developing renal complications as well as other adverse side effects such as hypertension, hyperlipidaemia or diabetes mellitus. Virtually all patients treated with CNIs develop nephrotoxicity [2], and a reduction in the use of CNIs could therefore result in a therapeutic benefit.

Standard immunosuppression usually consists of a triple drug therapy of three drug classes: CNIs, anti-proliferative agents including mycophenolate mofetil (MMF) and corticosteroids. As CNIs act against T-cell activation [3], MMF inhibits T- and B-cell proliferation [4], which is of additional benefit because recent data increase the importance of B cells in graft dysfunction and rejection processes [5,6]. The humanized monoclonal antibody daclizumab competitively binds to the CD25 subunit of the IL-2 receptor further inhibiting the activation of T cells. The introduction of

Table 1. Medication regimen in the early post-operative period for standard therapy patients and daclizumab patients

Medication	Standard group	Daclizumab group
Early post-operative period (<6 months)		
Daclizumab 1st dose, day 0	–	2 mg/kg
2nd–5th dose; day 14, 28, 42, 56	–	1 mg/kg
Mycophenolate mofetil	2 × 1 g/day	2 × 1 g/day
Steroids (tapering)	Min. dose 7.5 mg/day	Min. dose 7.5 mg/day
Cyclosporine (trough level)	150–250 ng/ml	75–125 ng/ml
Reaching at 6 months	125–175 ng/ml	50–75 ng/ml
Month 7–12		
Mycophenolate mofetil	2 × 1 g/day	2 × 1 g/day
Steroids (tapering)	Min. dose 5 mg/day	Min. dose 5 mg/day
Cyclosporine (trough level)	125–175 ng/ml	50–100 ng/ml
Reaching at 12 months	100–150 ng/ml	50–75 ng/ml

induction therapies with non-depleting antibodies, such as daclizumab, as a supplement to standard immunosuppression has resulted in further achievements in the reduction of acute rejection [7,8] without increasing the risk for infection.

As it is clear that induction therapies with anti-CD25 antibodies, such as daclizumab, decrease rejection episodes in combination with standard therapy [7,9], it has been hypothesized that the administration of daclizumab can allow a reduction in the dose of CNIs. However, complete avoidance of cyclosporine in *de novo* kidney transplant patients has resulted in increased rejection rates [10,11]. These considerations have led to the current trial design, in which a decreased dose of cyclosporine is administered to patients receiving daclizumab in combination with MMF and steroids with the reasoning that this could be sufficient to provide effective rejection prophylaxis. Decreased exposure to CNIs may result in a reduction in nephrotoxicity but may carry an increased risk for rejection. Over the last years, two large clinical trials ('CAESAR' with 536 patients and 'SYMPHONY' with 1645 patients) [12,13] were published that tested this hypothesis and found no improvement in renal function between daclizumab with low- and standard-dose CsA without daclizumab. Interestingly, both treatment arms had similar rejection rates despite the use of daclizumab, which raises the question of optimal CsA exposure in combination with anti-CD25 antibodies [14]. In these two studies, CsA trough levels in the daclizumab plus low CsA group were set to between 50 and 100 ng/ml, while higher CsA levels between 120 and 130 ng/ml may provide a better balance between rejection prophylaxis and nephrotoxicity. In order to provide more information on the optimal dosing of CsA, we present here the results of a 3-year, prospective, randomized trial investigating renal function, efficacy and safety of daclizumab, MMF, steroids and low-dose CsA in comparison to standard therapy in adult renal transplant recipients.

Patients and methods

Patients and study design

This investigator-initiated trial was a prospective, randomized, controlled, multi-centre study conducted in 14 centres in Germany, Switzerland and Austria. Patients were recruited between December 2000 and February 2003 and data collected until February 2006. This trial was performed in accordance with ICH/GCP guidelines and the amended Declaration of Helsinki following approval from the Institutional Review Committee at each centre. Informed consent was obtained from each patient before enrolment.

Adult (>18 years) primary renal allograft recipients, whose graft was obtained from a deceased donor, were eligible for enrolment. The most important exclusion criteria were cold-ischaemia time >30 h, combined or prior transplants, grafts from living donors and the use of another induction agent. Other factors that precluded participation were white-blood count $<2.5 \times 10^9$/l, platelet count $<100 \times 10^9$/l or haemoglobin <60 g/l. The patients were required to have panel reactive antibodies (current or peak) <20%. Eligible patients were enrolled prior to transplantation and randomized centrally by telephone after stratification by centre and age (< or ≥60 years). Randomization was carried out in a 1:1 manner to either standard therapy or to the experimental group consisting of daclizumab induction and low-dose CsA (Table 1) using computer-generated randomization lists. After verification through the central office, centres were notified by fax. The patients enrolled in the study were treated according to the protocol for 1 year. After this active treatment period, the patients were followed for another 2 years for selected parameters as defined in the study protocol allowing evaluation of long-term efficacy, safety and graft survival. Study monitoring was carried out centrally. Data were recorded in a computer database system and cross-checked by computer with central data verification and monitoring.

Immunosuppression

Both immunosuppressive regimens are summarized in Table 1. Patients with standard therapy received CsA, MMF and steroids without daclizumab. The initial CsA starting dose in this group was 10 mg/kg administered within 6 h after transplantation; thereafter, CsA doses were targeted to achieve trough levels between 150 and 250 ng/ml initially (as per centre practice) with a gradual decrease to reach 125–175 ng/ml at 6 months and 100–150 ng/ml at 12 months. The experimental group received daclizumab (Zenapax®, Roche Pharma AG) induction with 2 mg/kg IV within 24 h pre-transplant, followed by four additional IV doses of 1 mg/kg daclizumab every 2 weeks. CsA reduction was intended to reach 50% of trough levels of the standard group with a gradual taper (Table 1). Both groups received standard therapy with MMF (2 × 1 g/day orally) and corticosteroids as per centre practice with a minimum of 7.5 mg/day in the first 6 months and a minimum dose of 5 mg/day in months 7–12 (Table 1) with the attempt to obtain similar doses in both groups.

Primary and secondary outcomes

Data from patients who had 12 months of follow-up, or left the trial within these 12 months were analysed for the primary endpoint calculation. Renal function 12 months after kidney transplantation determined by creatinine clearance (Cockcroft–Gault formula [15,16]) was the primary endpoint. Importantly, for patients without adequate measurements at 12 months (e.g. graft loss, death), a creatinine clearance of 0 ml/min was imputed.

Secondary endpoints included patient and graft survival at 6 and 12 months, incidence of biopsy-proven graft rejection within the 12-month follow-up, incidence of treated, OKT3/ATG (muromonab/anti-thymocyte globulin) treated and steroid-resistant acute rejection, renal function [with

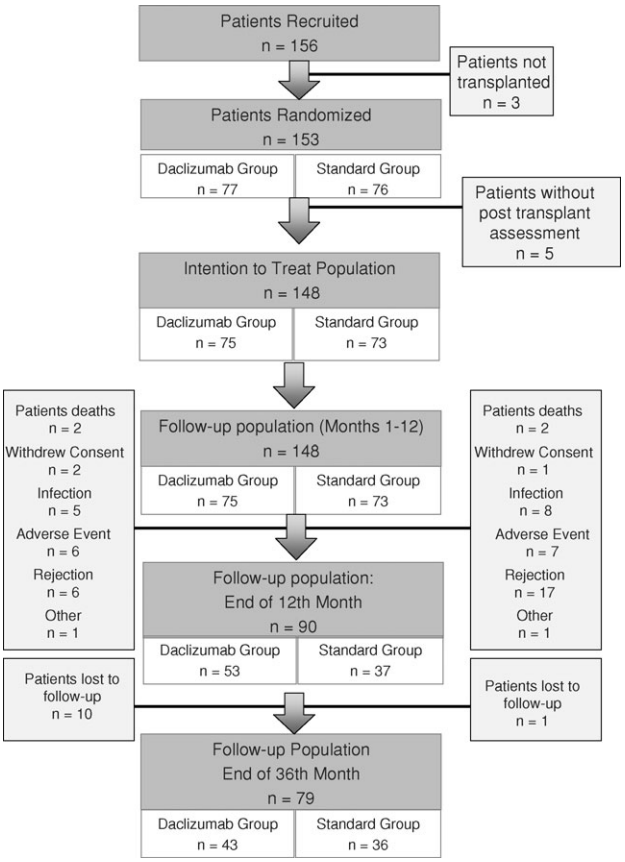

Fig. 1. Flow chart of patients in the trial. Premature withdrawals are further detailed in Table 4 along with exact breakdown of graft dysfunction per group.

and without imputation of missing values according to the last-observed-value-carried-forward (LOCF) method], blood pressure and lipid values. All acute rejections were asked to be biopsy proven and defined according to Banff 97 criteria [16]. High-dose steroids were first-line therapy of acute rejection, depleting antibodies (OKT3/ATG), or tacrolimus could be given in the case of severe rejection; these patients, however, had to be removed from the trial. Adverse events were graded according to the NCI Common Toxicity Criteria. All infections were recorded, and a subset of infections including CMV, EBV, Herpes zoster and Herpes simplex were specifically tracked. Delayed graft function was defined as the need for dialysis in the first week.

Statistical analysis

In order to detect a clinically relevant difference in the creatinine clearance of 10 ml/min (with a standard deviation of 20 ml/min) after 12 months, as the primary endpoint of the study, with a type I error of 0.05 and a power of 80% using a two-sided *t*-test, 64 patients were required per randomization arm. Patients without adequate measurement at 12 months as a consequence of death or graft loss were anticipated. In order to account for these informative dropouts in an intention-to-treat (ITT) analysis, the primary endpoint definition was prospectively extended to a composite criterion, assigning an artificial 'worst possible creatinine clearance' to cases with graft loss or permanent graft failure within the first year. To allow for this from a statistical point of view, a distribution-free test procedure, i.e. the Wilcoxon rank-sum test, was planned, as creatinine clearance under this assumption was not anticipated to be normally distributed. Accounting for up to 10% loss of power by the application of the non-parametric test and, in addition, taking into consideration a 10% uninformative dropout rate, a total of 155 patients were recruited.

In addition to standard methods of descriptive analysis, the Wilcoxon rank-sum test was used to compare groups with respect to creatinine, or other laboratory values. As a consequence of the composite endpoint approach described above, non-ITT-based means or medians could be derived for these parameters. Event-related data such as time to rejection or graft dysfunction were analysed according to the Kaplan–Meier technique [17] and compared with the log rank test [18]. For differences in adverse effects, Fisher's exact test or an exact version of the chi-square test for trend was applied.

All analyses were carried out according to ITT that was defined as the population that included all transplanted patients with a valid randomization, and at least one post-transplant assessment available.

Results

Patient demographics

A total of 156 patients were included in the trial; however, three of these patients were withdrawn from the analysis because they were not transplanted: two who were not transplanted due to the bad quality of the donor kidney and one patient who died before the transplant procedure was completed due to a cardiovascular event (Figure 1). From 5 out of 153 patients, no post-transplant assessments were available, and therefore, according to the pre-specified protocol, those 5 patients had to be excluded from any further ITT analysis; in consequence, the predefined ITT population consisted only of 148 patients, with 75 patients in the daclizumab and 73 patients in the standard therapy group. This trial included an additional 2 years' follow-up period as specified by the protocol. Eleven patients were lost to follow-up after the first 12 months of the core study period.

There were no significant differences in demographic data between the two groups at the time of enrolment (Table 2). The mean age (range) was 52 years (19–73 years) for daclizumab patients and 54 (19–70 years) for standard

Table 2. Baseline patient demographics and transplant characteristics

Demographics ($n = 148$)	Daclizumab ($n = 75$)	Standard ($n = 73$)
Gender male (%)	47 (64%)	42 (60%)
Mean age ± SD	52.3 ± 13.8	54.2 ± 12.3
Median age (range)	55 (19–73)	58 (19–70)
BMI median (range)	23.3 (17.9–36.7)	25.3 (17.1–33.1)
Neg. CMV IgG (%)	24 (34%)	25 (35%)
Patients with delayed graft function in first week	20	20
Donor status		
Donor age <60	66%	66%
Donor age ≥60	34%	34%
Median cold ischaemia in min.[a]	939 (210–2070)	900 (154–1849)
Primary diagnosis leading to transplantation		
Glomerulonephritis	24	17
Nephritis	13	18
Polycystic kidney	8	14
Diabetes mellitus (type I + II)	6	4
Hypertension	7	4
Other (unknown)	17	16

Patients enrolled in the trial were equally distributed to standard therapy and daclizumab groups in regards to gender, age and other transplant relevant factors.

CMV, cytomegalovirus; BMI, body mass index; IgG, immunoglobulin G.

[a]Two patients with a cold ischaemic time >30 h, (one patient in each group) represent protocol violations, which, however, were included in the ITT analysis.

therapy patients with a high proportion (>34%) of elderly donors ≥60 years.

During the first year post-transplant, a total of 58 patients were withdrawn from the study: 22 of the daclizumab patients (22/75, 29%) and 36 of the standard therapy patients (36/73, 49%). Reasons for withdrawal are listed in Figure 1, and included adverse events, rejection (see Table 3 for breakdown), withdrawal of consent and infection. There were seven graft losses in the first 12 months in the standard group and two in the daclizumab group (Table 3).

Immunosuppression

The goal was a 50% reduction of CsA trough levels in the daclizumab group. After 1 week post-transplant, the mean CsA levels of daclizumab patients were maintained at 57% of standard patients [132 versus 216 ng/ml on Day 7 ($P < 0.0001$; Figure 2)]. The levels of CsA in the daclizumab group only slowly and gradually decreased after transplantation, while the levels in the standard group were tapered more rapidly. By the end of Month 6, the mean CsA levels in daclizumab patients were maintained at 84% of standard therapy patients until the end of the first year ($P = 0.018$ Fisher's exact test, two-sided) and continued to remain 10–20% lower than in the control arm.

At Month 12, the dose of MMF in both groups ranged from 500 to 2000 mg/day. Both in the daclizumab group ($n = 46$) and in the standard therapy group ($n = 39$), the average daily MMF dose was 1.7 g/day. The daily dose of corticosteroids (prednisolone equivalent) at 12 months in the daclizumab group ($n = 47$) ranged from 2 to 12.5 mg, with an average value of 5.5 mg. In the standard therapy group ($n = 38$), corticosteroids ranged from 2 to 15 mg, with an average value of 6.5 mg/day.

Primary endpoint: renal function

The primary endpoint renal function at 12 months (with imputation of '0' for informative dropouts), determined by mean calculated creatinine clearance according to the protocol, was significantly better in daclizumab-treated patients compared to standard therapy patients [34.1 ± 17.4 ml/min in daclizumab patients ($n = 74$) versus 29.4 ± 16.5 ml/min in standard therapy patients ($n = 71$; $P = 0.028$, two sided; Table 3)]. It is important to note that this combined analysis included all patients where graft failures and deaths were assigned the lowest rank, thereby providing a more comprehensive view on post-transplant outcome.

In order to provide better insight in the actual renal function of both groups, we calculated the mean GFR (according to the Cockcroft–Gault formula) with and without imputation of missing values. The observed calculated creatinine clearance at 12 months (without any imputation) was ~7 ml/min better in the daclizumab group without reaching statistical significance (Table 3). Similar results were obtained when missing values were imputed (LOCF method) demonstrating better renal function over the 3-year period for the daclizumab group, reaching statistical significance only between Day 14 and Month 3 as well as on Month 9 (Figure 3A).

Similarly, the Wilcoxon rank-sum test results show significance at 12 months in favour of daclizumab patients with respect to creatinine ($P = 0.014$, two sided, Table 3), when graft failures and deaths were assigned the lowest rank. In order to provide a complete picture of renal function parameters, the time course of observed serum creatinine (without any imputations) is shown in Figure 3B. The mean-observed serum creatinine concentrations over the 3-year period were always better in the daclizumab group already beginning at 2 weeks post-transplant reaching statistical significance at various time points after transplantation. At 36 months, the mean creatinine value in the daclizumab patients ($n = 43$) was 1.7 mg/dl, while in the standard therapy patients ($n = 36$), the mean concentration was 2.1 mg/dl ($P = 0.048$).

Acute rejection

The incidence of and time to first biopsy-proven acute rejection (BPAR) (Figure 4A) within the first 12 months as shown on the Kaplan–Meier plot demonstrates a highly significant difference ($P < 0.002$, two sided for both parameters) between the two groups. Within the first 12 months, only 5/75 daclizumab patients (6.6%) experienced BPAR compared to 19/73 patients (26%) in the standard group ($P = 0.0016$). Three of the 19 standard therapy patients had two BPAR for a total of 22 events in the standard therapy group (Table 4). Furthermore, daclizumab patients also had less severe rejections with less antibody use ($n = 4$ versus $n = 10$) for severe or steroid refractory rejections than standard therapy patients. In addition, eight patients in the standard therapy group were converted to tacrolimus due to rejection compared to only four patients in the daclizumab group. Considering a composite endpoint of graft loss or BPAR likewise, daclizumab patients had 91% event-free survival after 1 year, while standard therapy patients had 66% event-free survival ($P = 0.00017$). This represents an almost 4-fold lower risk of rejection or graft loss in the daclizumab group.

Patients with rejection in both groups had significantly worse renal function (Figure 3C) beginning on Week 6. Even patients with borderline rejections had compromised renal function (39.8 ± 16.3 ml/min; $P < 0.05$, Wilcoxon test, $n = 6$) at 1 year compared to rejection-free patients.

In order to further differentiate the effect of better rejection prophylaxis from lower CsA levels on renal function, we performed a *post hoc* analysis comparing renal function from all patients without any rejection episodes (Figure 3D). In these rejection-free patients, differences between both groups were smaller and did not reach statistical significance.

Within the first year in the daclizumab group, no biopsy-proven chronic rejection and/or CsA toxicity were observed, while two patients in the standard therapy groups experienced biopsy-proven chronic rejection, and nine patients had biopsy-proven cyclosporine toxicity (Table 4). In addition, in nine cases, CsA toxicity was suspected by the investigator in the standard therapy arm. Biopsies were only administered if deemed clinically necessary, with more ($n = 43$) biopsies due to renal dysfunction in the standard group compared to the daclizumab group ($n = 25$). Premature withdrawal events due to rejection in the first 12 months

Table 3. Primary endpoint, graft survival, adverse events, infection and premature withdrawal

	Daclizumab			Standard		
Mean creatinine clearance 12 months	34.1 ± 17.4			29.4 ± 16.5		
Median with imputation of informative dropouts* ($P = 0.028$, two sided)	31.9 ($n = 74$)			27.8 ($n = 71$)		
Mean creatinine clearance 12 months	57 ± 22 ($n = 74$)			50 ± 25 ($n = 71$)		
Median (range) with imputation LOCF** ($P = 0.098$, two sided)	53 (11–113)			50 (8–137)		
Mean creatinine clearance 12 months	59 ± 21 ($n = 61$)			52 ± 22 ($n = 53$)		
Median (range) only observed cases ($P = 0.208$, two sided)	54 (22–112)			52 (9–98)		
Mean creatinine 12 months with imputation of informative dropouts* ($P = 0.014$, two sided)	1.80 ± 1.10 ($n = 75$)			2.60 ± 2.20 ($n = 73$)		
Mean creatinine 12 months	1.81 ± 1.07 ($n = 75$)			2.35 ± 2.06 ($n = 73$)		
Median (range) with imputation LOCF** ($P = 0.058$, two sided)	1.50 (0.74–5.64)			1.63 (0.64–11.3)		
Mean creatinine 12 months	1.56 ± 0.66 ($n = 53$)			1.94 ± 1.51 ($n = 42$)		
Median (range) only observed cases ($P = 0.139$, two sided)	1.44 (0.74–4.20)			1.80 (0.74–10.2)		
	($n = 75$)			**($n = 73$)**		
Patient survival at 12 months	97.3%			93.2%		
Graft survival (censured for death) at 12 months	97.3%			89.7%		
Patient survival at 24 months	94.6%			91.8%		
Graft survival (censured for death) at 24 months	95.8%			84.6%		
Patient survival at 36 months	93.3%			89.0%		
Graft survival (censured for death) at 36 months	90.2%			81.6%		
Premature withdrawal (12 months)						
Patients with events	22 (29%)			36 (49%)		
Number of events	30			65		
Death	2			2		
Rejection	6			17		
Refractory rejection	3			7		
Antibody-treated rejection	4			10		
Conversion to tacrolimus due to rejection	4			8		
Graft thrombosis	0			1		
Adverse events	7			15		
Infection	5			8		
Non-functioning graft	1			3		
Other	7			18		
Withdrawal of informed consent	2			1		
Adverse events (12 months)***						
Total adverse events: Patients with events	70 (93%)			70 (96%)		
Total adverse events: Number of events	203			178		
	NCI grade			**NCI grade**		
	2	**3**	**4**	**2**	**3**	**4**
Hematological (bleeding/blood)	7	4	3	8	9	2
Cardiovascular	4	4	1	0	2	3
Gastrointestinal	10	2	1	6	3	1
Metabolic	17	5	0	7	7	0
Neurological	12	4	1	9	3	1
Renal/genitourinary	10	2	2	1	7	7
Infection (12 months)						
Patients with events	51 (68%)			49 (67%)		
Number of events	89			74		
Bacterial	40			39		
Fungal	9			3		
CMV	19			15		
Herpes	6			11		
Other viral	9			4		
Unknown	6			2		

*For informative dropouts (patients who died, lost their graft or were withdrawn due to severe rejection or toxicity), an arbitrary value and worst possible rank was assigned.

**In case of missing values, the last observed value was carried forward (LOCF-method).

***The adverse event profiles did not differ significantly from each other. None of the calculated *P*-values for various events indicated a statistically significant difference between the two groups.

demonstrated a favourable trend in the daclizumab group; there were six severe rejections in the daclizumab group compared to 17 in the standard therapy group because of treatment with ATG or tacrolimus (see Table 3 for more complete data).

Safety profile: infections and lipids

The adverse event profiles of the two treatment groups in the first 12 months did not differ significantly (Table 3). No differences were noted in number of patients with infections between the two groups; 68% (51/75) of daclizumab

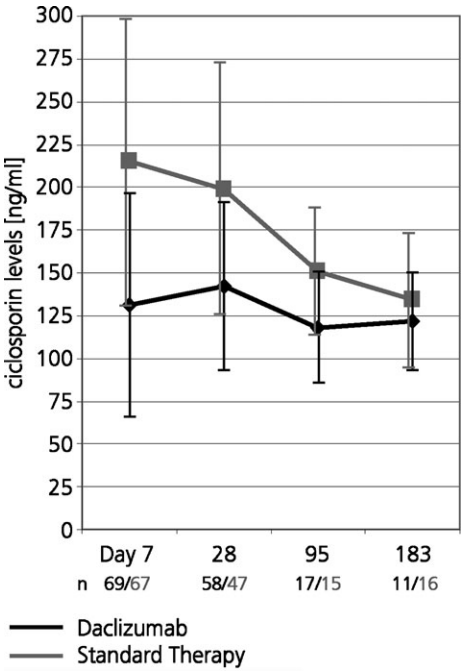

Fig. 2. Cyclosporine trough levels from Day 7 to Day 183 in daclizumab patients (circles) and standard therapy patients (squares).

patients experienced one or more infection episodes versus 67% (49/73) of standard therapy patients. The most frequent adverse occurrence was bacterial infection with 40 reports in the daclizumab group and 39 in the standard therapy group. Finally, there were no differences in blood pressure and lipids between the two groups (data not shown) and no reported malignancies in either group.

Patient and long-term graft survival

Patient survival was similar in both groups. At 12 months, seven deaths were observed within the 148 ITT populations, with two deaths in the daclizumab group and five deaths in the standard therapy group (Table 3). Within the 3 years of follow-up, five deaths occurred in the daclizumab group, while eight were observed in the standard therapy group. Additionally, five patients experienced graft loss or permanent dialysis in the daclizumab group compared to 15 in the standard group ($P = 0.035$, log rank test, two-sided; Figure 4B) over the 3-year observation period. Two out of 5 patients with BPAR in the daclizumab group lost their graft compared to four deaths and seven graft losses in 19 patients with rejection in the standard group.

Discussion

This prospective study demonstrates that high-dose daclizumab induction (2 mg/kg initial dose) in combination with reduced CsA, steroids and MMF is a safe and efficacious regimen following kidney transplantation that allows a relevant reduction in early CsA exposure within the first 12 months in an immunologically low-risk population. This high-dose daclizumab regimen appears to be superior to standard-dose CsA regimen in combination with MMF and steroids with respect to graft survival, renal function and acute rejection episodes. However, with the current study design, we could not prove the hypothesis that a decreased dose of CsA led to improved graft function. Patients without rejection had only minor, not significant differences in renal function despite marked differences in CsA exposure, while patients with rejection clearly had inferior renal function in a *post hoc* analysis suggesting that the superior rejection prophylaxis with high-dose daclizumab induction and sufficient CsA levels could provide a potential explanation for the better outcomes in the daclizumab group.

A major concern regarding CNI immunosuppression is the development of nephrotoxicity [19]. In order to minimize CNI-related side effects, several attempts have been made to reduce, withdraw or even avoid CNIs. Complete CNI-free immunosuppression in the immediate post-transplant period, however, has resulted in an unacceptably high rejection rate of about 50% using a regimen of anti-CD25 antibody, MMF and steroids [10]. Similarly complete CNI withdrawal with MMF and steroids was not successful during the first 2 years post-transplant as demonstrated in two earlier studies [20,21] and a recent multi-centre study [12]. In this latter trial, CNI withdrawal 6 months post-transplant was associated with an increased risk of BPAR compared to a low-dose CsA group (38% versus 25% at 12 months) [12]. Success in complete CNI withdrawal has been obtained only in few studies in which CNI was withdrawn after introduction of MMF in long-term maintenance patients (>3 years post-transplant), without an increase in acute rejection but significantly better renal function [22,23].

Taken together, these results demonstrate that a protocol incorporating complete early CNI withdrawal or even complete CNI avoidance solely with anti-CD25, MMF and steroids is not an optimal strategy. Instead, a CNI minimization strategy could prove to be a successful alternative in order to lower the well-known nephrotoxic effects of CNIs [14]. High-dose anti-CD25 induction with daclizumab (2 mg/kg initially) successfully allowed a significantly reduced CNI exposure while providing excellent rejection prophylaxis. However, we have to emphasize that the participants in this trial were at low risk and that these results may not be applicable to higher risk groups.

Our results confirm in part the results of Vincenti, Nashan and Ingle [7,8,24,25], and indicate significantly decreased acute rejection with induction therapies. Although only a secondary outcome, the biopsy-proven rejection rate in the daclizumab group over the 1-year observation period was only 7% while a rate of 26% was observed in the standard therapy group. The average rate of biopsy-proven rejection in kidney transplant trials with standard therapy consisting of CsA, steroids and MMF is about 25% [12,13]. Administration of high initial daclizumab doses (initial dose was 2 mg/kg) in concert with MMF and CsA trough levels between 120 and 130 ng/ml resulted in improved kidney function early during the study with excellent rejection prophylaxis, similar to those seen with anti-CD25 induction in combination with MPA, steroids and C2-monitored full dose CsA [25]. It is, however, not possible to conclude

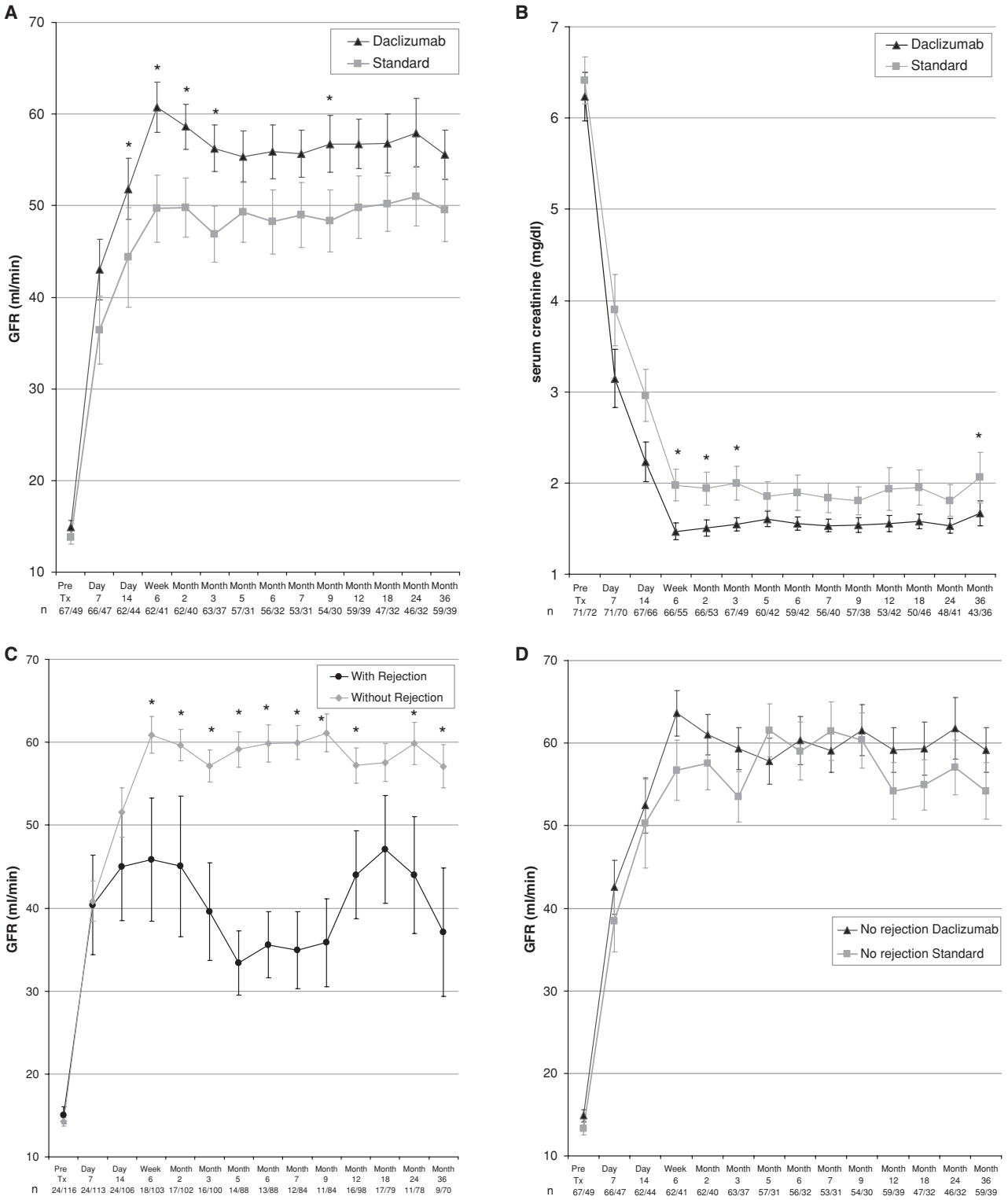

Fig. 3. Evolution of renal function over the course of the study. (**A**) Calculated glomerular filtration rate (Cockcroft method) with the imputation of missing values according to the last-observed-value-carried-forward (LOCF) method and (**B**) observed serum creatinine values. (**C**) Comparison of observed calculated glomerular filtration rate (Cockcroft method) between patients with rejection and without rejection. (**D**) Comparison of observed calculated glomerular filtration rate (Cockcroft method) between the daclizumab group and the control group in patients without rejection.

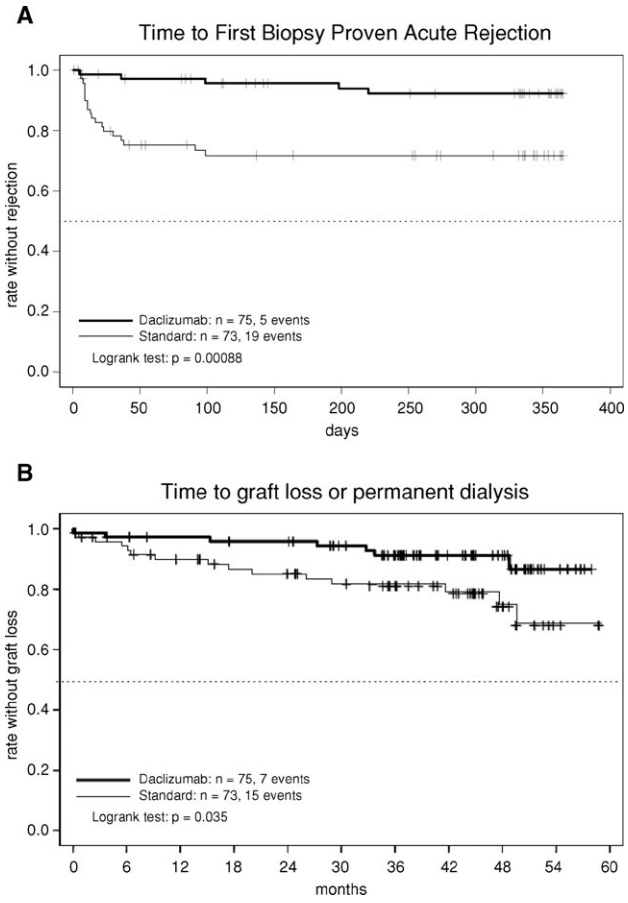

Fig. 4. Time to first biopsy-proven acute rejection and time to graft loss. (**A**) Differences in the rates of acute rejection between daclizumab-treated and standard therapy groups emerged during the trial; a Kaplan–Meier plot shows the time to the first biopsy-proven acute rejection. Daclizumab patients were therefore significantly less likely to have BPAR within the first 12 months ($P = 0.00088$) as compared to standard therapy patients. (**B**) Daclizumab patients demonstrated significantly less graft losses or permanent dialysis over the long-term follow-up ($P = 0.035$, log rank test, two sided).

Table 4. Incidence and severity of acute and chronic rejection

	Daclizumab	Standard
Number of patients with	($n = 75$)	($n = 73$)
Treated acute rejection within the 1st year	6 (8%)	20 (27%)
Biopsy-proven acute rejection (BPAR)	5	19
Steroid-sensitive acute rejection	2	15
Steroid-resistant acute rejection	0	3
Steroid-resistant and antibody-sensitive acute rejection	1	1
Antibody-sensitive acute rejection	2	3
Unknown	1	1
BPAR within the first year	5 (6.6%)	19[a] (26%)
Borderline	0	7[a]
BPAR (excluding Borderline) within the first year	5 (6.6%)	12 (16.4%)
Banff grade I	3	3
Banff grade II	0	7[a]
Banff grade III	2	2
Other biopsy findings		
Number of biopsies	25	43
Biopsy-proven chronic rejection	0	2
Biopsy-proven CsA toxicity	0	9

[a]Two patients with borderline rejection and one patient with Banff II rejection experienced another rejection episodes, resulting in 22 rejection episodes in 19 patients.

that the decreased dose of CsA directly led to better graft function, because our study design cannot discern between the effect of lower CsA doses on renal function and the effect of initial high-dose daclizumab, which might have provided additional potent rejection prophylaxis, leading to subsequent better long-term renal function. The latter hypothesis was partly confirmed by our *post hoc* analysis, demonstrating deleterious effects of rejection episodes on renal function, while different CsA doses exerted only a minor, not significant, effect on renal function in patients without rejection. In theory, this favourable result could also be achieved with daclizumab added to standard CsA doses as evidenced by the excellent outcomes in the DIRECT trial [25]. Thus, we only can conclude that this particular regimen of initial high-dose daclizumab in combination with lower CsA doses was associated with better outcome.

It is, however, important to point out that our primary endpoint, did not only reflect renal function, but in fact was more a composite endpoint, because we imputed a GFR of '0' for informative dropouts such as graft failure or death. As a consequence, it is difficult to compare our GFR result with other studies, where a GFR of '10' was imputed [13] or where no imputation was performed [25]. Our comprehensive analysis on renal function (using observed cases or imputed values according to LOCF) failed to demonstrate significant differences between both groups similar to other studies [12,13]. Given the strong trend at some timepoints and the absolute differences of 5–7 ml/min, it is conceivable that better rejection prophylaxis in combination with lower CsA exposure contributed to the observed outcomes with significant differences in the primary endpoint but non-significant differences in renal function (either observed cases or LOCF method). But as evidenced by the large standard deviation, the current study was not adequately powered for the analysis of such secondary endpoints.

Because we used a similar hypothesis with a similar study design, it is important to discuss the results of the Symphony study and CAESAR study in context of our results [12,13]. Very low CsA exposure with levels between 80 and 100 ng/ml in the first 6 months resulted in higher rejection rates of ∼25% in both trials [20,33]. In our trial, higher CsA levels and higher initial daclizumab doses (2 mg/kg initially) were used; by 183 days post-transplant, CsA trough levels in daclizumab patients achieved levels between 120 and 130 ng/ml resulting in low incidence of rejection in the first month and virtually no rejections beyond the second post-transplant month, which suggests that these levels combined with full dose MMF were effective in obtaining excellent rejection prophylaxis even after anti-CD25 antibody was terminated. Our rejection rates were in a similar range compared to other trials, in which anti-CD25 antibodies were used in combination with MMF, steroids and standard CsA doses [25]. In summary, this demonstrates that initial high-dose daclizumab induction therapy in combination with MMF has the potential to substantially reduce

CsA exposure during the first 6 months post-transplant with no increase in rejection incidence and patient risk.

Other groups corroborate that the use of daclizumab not only reduces acute rejection [26], but permits the reduction of steroids [6,27,28] or the reduction or delayed introduction of the CNIs [24,29–33]. Similarly to our study, lower CsA concentrations in combination with initially high-dose daclizumab were associated with reduced acute rejection, reduced need for biopsy and lower chronic rejection rates. These results (admittedly with a lower power) are in contrast to the findings of two recent large clinical trials ('CAESAR' with 536 patients and 'SYMPHONY' with 1645 patients) [12,13], which demonstrated no improvement in rejection rates and renal function between low- and standard-dose CsA. One obvious difference between these two studies and our trial was the higher CsA levels and initial higher daclizumab dose used in our study. Notably, CsA trough levels in this study came to between 120 and 130 ng/ml, while the two other studies achieved lower CsA levels between 80 and 100 ng/ml. As pointed out, this was associated with better rejection prophylaxis, but actual renal function as determined by serum creatinine and calculated GFR (either observed cases or LOCF method) showed only some small but not significant differences between groups. Further randomized studies are needed to investigate this difference and to determine the optimal CsA level for the early post-transplant period.

Limitations of the study include the rather small number of patients, the use of CsA trough level monitoring instead of C2 monitoring and the high drop out rate. Despite the rather small sample size, we were able to show significant differences in the primary 'composite' endpoint, justifying the assumptions of the power calculation. But as a consequence, the power for secondary endpoints, e.g. graft survival, graft function and rejection rates is limited, especially given the high number of withdrawals. Although the rate of treatment failures is similar to other studies [12,13], the study protocol required that patients who discontinued treatment (e.g. experienced adverse events, or had severe rejection necessitating antibody treatment and/or conversion to tacrolimus) were to be removed from the trial, as they had experienced an obvious treatment failure of the initial immunosuppressive regimen. In 2000, when the study was planned, the safety and efficacy of the experimental daclizumab arm was largely unknown. Due to fear of under-immunosuppression, initial daclizumab dose was even doubled. In retrospect, with current knowledge on the efficacy and safety of both regimens, such patients could (and should) have been kept in the trial and additional safety and efficacy parameters should have been captured.

Lastly, C2 monitoring may have prevented over- or under-exposure in some patients that could have resulted in less rejection and/or less toxicity, and excellent results with C2-monitored full dose CsA in combination with anti CD25 have been reported [25,34]. But the current study was planned before CsA C2 monitoring became popular, and CsA C2 monitoring may also have some limitations [34,35]. Unfortunately, there is little evidence from well-designed large prospective randomized trial to support the theoretical benefits of CsA C2 monitoring studies in *de novo* transplant patients versus trough level monitoring [34], but undoubtedly CsA C2 monitoring with a better assessment of CsA exposure might have resulted in better outcomes, especially in the control group.

Additional trials indicate that daclizumab and MMF also allows reduction in the use of tacrolimus without increased risk for the patient [13], and this data therefore implies that daclizumab combined with MMF may function as a more principal therapeutic to lower CNI use in general. Taken together, the recently published literature together with this trial suggests the more widespread use of anti-CD25 antibodies in combination with corticosteroids and MMF, which allows a profound reduction of CNI dose in low-risk patients. Such a low-dose CNI regimen could lead to improved kidney function, less rejections and better graft outcome.

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Conflict of interest statement. None declared.

References

1. Fredericks S, Holt DW, MacPhee IA. The pharmacogenetics of immunosuppression for organ transplantation: a route to individualization of drug administration. *Am J Pharmacogenomics* 2003; 3: 291–301
2. Nankivell BJ, Borrows RJ, Fung CL *et al*. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004; 78: 557–565
3. Webster A, Woodroffe RC, Taylor RS *et al*. Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients. *Cochrane Database Syst Rev* 2005; 4: CD003961
4. Allison AC, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation* 2005; 80(2 Suppl): S181–S190
5. Noorchashm H, Reed AJ, Rostami SY *et al*. B cell-mediated antigen presentation is required for the pathogenesis of acute cardiac allograft rejection. *J Immunol* 2006; 177: 7715–7722
6. Sarwal MM, Vidhun JR, Alexander SR *et al*. Continued superior outcomes with modification and lengthened follow-up of a steroid-avoidance pilot with extended daclizumab induction in pediatric renal transplantation. *Transplantation* 2003; 76: 1331–1339
7. Vincenti F, Kirkman R, Light S *et al*. Daclizumab Triple Therapy Study Group. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. *N Engl J Med* 1998; 338: 161–165
8. Nashan B, Light S, Hardie IR *et al*. Daclizumab Double Therapy Study Group. Reduction of acute renal allograft rejection by daclizumab. *Transplantation* 1999; 67: 110–115
9. Nashan B. Antibody induction therapy in renal transplant patients receiving calcineurin-inhibitor immunosuppressive regimens: a comparative review. *BioDrugs* 2005; 19: 39–46
10. Vincenti F, Ramos E, Brattstrom C *et al*. Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. *Transplantation* 2001; 71: 1282–1287
11. Vincenti F, Immunosuppression minimization: current and future trends in transplant immunosuppression. *J Am Soc Nephrol* 2003; 14: 1940–1948
12. Ekberg H, Grinyó J, Nashan B *et al*. M. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. *Am J Transplant* 2007; 7: 560–570
13. Ekberg H, Tedesco-Silva H, Demirbas A *et al*. ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; 357: 2562–2575

14. Kaplan B, Budde K. Lessons from the CAESAR Study: calcineurin inhibitors—can't live with them and can't live without them. *Am J Transplant* 2007; 7: 495–496
15. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41
16. Racusen LC, Solez K, Colvin RB *et al*. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55: 713–723
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Ass* 1958; 53:457–481
18. Peto R, Peto J. Asymptotically efficient rank invariation test procedures (with discussion). *J R Stat Soc* 1972; A135: 185–207
19. Fellström Cyclosporine nephrotoxicity. *Transplant Proc* 2004; 36(Suppl 2S): 220S–223S
20. Abramowicz D, Vanrenterghem Y, Squifflet JP *et al*. Efficacy and cardiovascular safety of daclizumab, mycophenolate mofetil, tacrolimus, and early steroid withdrawal in renal transplant recipients: a multicenter, prospective, pilot trial. *Clin Transplant* 2005; 19: 475–482
21. Smak Gregoor P *et al*. Withdrawal of cyclosporine or prednisone six months after kidney transplantation in patients on triple drug therapy: a randomized, prospective, multicenter study. *J Am Soc Nephrol* 2002; 13: 1365–1373
22. Dudley C, Pohanka E, Riad H *et al*. Mycophenolate Mofetil Creeping Creatinine Study Group. Mycophenolate mofetil substitution for cyclosporine a in renal transplant recipients with chronic progressive allograft dysfunction: the 'Creeping creatinine' study. *Transplantation* 2005; 79: 466–475
23. Suwelack B, Gerhardt U, Hohage H. Withdrawal of cyclosporine or tacrolimus after addition of mycophenolate mofetil in patients with chronic allograft nephropathy. *Am J Transplant* 2004; 4: 655–662
24. Ingle GR, Moudgil A, Vo A *et al*. Cyclosporine-sparing effects of daclizumab in renal allograft recipients. *Am J Health Syst Pharm* 2005; 62: 391–396
25. Vincenti F, Friman S, Scheuermann E *et al*. DIRECT (Diabetes Incidence after Renal Transplantation: Neoral C Monitoring Versus Tacrolimus) Investigators. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007; 7: 1506–1514
26. Poorrezagholi F, Einollahi B, Firoozan A *et al*. Effect of daclizumab (zenapax) on prevention of acute rejection of renal transplantation. *Transplant Proc* 2003; 35: 2735–2736
27. Rostaing L, Cantarovich D, Mourad G *et al*. CARMEN Study Group. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; 79: 807–814
28. ter Meulen CG, van Riemsdijk I, Hene RJ *et al*. Steroid-withdrawal at 3 days after renal transplantation with anti-IL-2 receptor alpha therapy: a prospective, randomized, multicenter study. *Am J Transplant* 2004; 4: 803–810
29. Light JA, Sasaki TM, Ghasemian R *et al*. Daclizumab induction/tacrolimus sparing: a randomized prospective trial in renal transplantation. *Clin Transplant* 2002; 16(Suppl 7): 30–33
30. Kuypers DR, Evenepoel P, Maes B *et al*. The use of an anti-CD25 monoclonal antibody and mycophenolate mofetil enables the use of a low-dose tacrolimus and early withdrawal of steroids in renal transplant recipients. *Clin Transplant* 2003; 17: 234–241
31. Wilson CH, Brook NR, Gok MA *et al*. Randomized clinical trial of daclizumab induction and delayed introduction of tacrolimus for recipients of non-heart-beating kidney transplants. *Br J Surg* 2005; 92: 681–687
32. Sanchez-Fructuoso AI, Marques M, Conesa J *et al*. Use of different immunosuppressive strategies in recipients of kidneys from nonheart-beating donors. *Transpl Int* 2005; 18: 596–603
33. Sundberg AK, Rohr MS, Hartmann EL *et al*. Conversion to sirolimus-based maintenance immunosuppression using daclizumab bridge therapy in renal transplant recipients. *Clin Transplant* 2004; 18 (Suppl 12): 61–66
34. Einecke G, Schütz M, Mai I *et al*. Limitations of C2 monitoring in renal transplant recipients. *Nephrol Dial Transplant* 2005; 20: 1463–1470
35. Nashan B, Bock A, Bosmans JL *et al*. Use of Neoral C monitoring: a European consensus. *Transpl Int* 2005; 18: 768–778

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Survival of transplanted and dialysed patients in a French region with focus on outcomes in the elderly

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Abstract

Background. Impact of kidney transplantation on survival of French end-stage renal disease (ESRD) patients is unknown.

Methods. A total of 1495 adults living in the Lorraine region and starting renal replacement therapy from 1997 to 2003 were included. A propensity score (PS) of registration on the renal transplant waiting list was estimated. Patient survival was studied using a time-dependent Cox multivariate regression and a Cox model stratified by PS tertiles. Survival of older patients (≥60 years) was detailed.