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ORIGINAL ARTICLE

Corticosteroid avoidance in adult kidney transplant recipients under rabbit anti-T-lymphocyte globulin, mycophenolate mofetil and delayed cyclosporine microemulsion introduction

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Summary

We conducted the first prospective, randomized, open-label multicenter study in low-immunologic risk adult recipients of primary cadaver kidney transplants receiving rabbit anti-T-lymphocyte globulin, mycophenolate mofetil, cyclosporine microemulsion introduced on day 5, with and without corticosteroids. Patients were randomly assigned according to age and cold ischemia time to receive corticosteroids for at least 6 months or no corticosteroids at all. The main efficacy evaluation criterion was acute rejection (including all treated episodes and those biopsy-confirmed) during the first year following transplantation. For this purpose, this report includes the actual results of the whole 12-month follow-up of all randomized patients. For efficacy analysis, 98 patients were evaluated in the Steroid avoidance group and 99 in the Steroid maintenance group. Taken as a whole, 81% of the patients ($n = 159$) never received anti-rejection treatment. From the 38 patients who received anti-rejection treatment, 25 (25.5%) were in the Steroid avoidance group and 13 (13.1%) in the Steroid maintenance group ($P < 0.031$), experiencing respectively 17 (17.3%) and 7 (7.1%) biopsy-proven first episodes of acute rejection ($P < 0.031$). Borderline changes (6 vs. 3) were not considered as biopsy-proven acute rejections. Onset of first rejection was significantly shorter in the Steroid avoidance group ($P < 0.027$). First-line anti-rejection treatment response, need for any rescue therapy, as well as histologic severity of rejection episodes did not statistically differ between the groups. One-year post-transplantation analysis showed no differences in delayed graft function, serum creatinine, creatinine clearance, 24-h proteinuria, as well as serious adverse events between the groups. *De novo* diabetes ($P < 0.07$) or dyslipidemia ($P < 0.01$) as well as newly diagnosed malignancies ($P < 0.059$) were however more frequently observed in the Steroid maintenance group. At the end of the first post-transplant year, 99% of patients in the Steroid avoidance group and 97% of patients in the Steroid maintenance group were respectively alive ($P = 0.34$), with respectively 95% and 93.2% of functioning kidney grafts ($P = 0.62$). Our results showed

that total avoidance of corticosteroids from the day of transplantation was associated with a significantly increased number of clinically diagnosed and treated, and biopsy-proven acute rejections during the first year of transplantation. Nevertheless, overall outcome, 1-year patient and graft survival as well as renal function were similar, and the patients in the Steroid avoidance group exhibited a lower incidence of *de novo* dyslipidemia, diabetes mellitus and malignancies often associated with steroid treatment (Clinical Trials.gov NCT00200551).

Introduction

Immunosuppressive treatments after organ transplantation are principally directed towards reduction of allograft rejection (acute and chronic), and if possible, should have a minimum of adverse and side-effects. These aims have not been fully achieved up to date. Transplant recipients still experience episodes of graft rejection, infections and cancer complications, as well as toxic effects linked to immunosuppressants intake.

Only 20 years ago, most kidney transplant recipients had to cope with rejection episodes. At this stage, the acute rejection incidence varies from 10% to 40% [1–4]. The immunosuppressive protocols applied by most transplant centers consist of a triple therapy based on calcineurin inhibitor (CNI) drugs such as cyclosporine (CyA) or tacrolimus (Tac), anti-proliferative agents such as mycophenolate mofetil (MMF), enteric-coated mycophenolic acid (EC-MPA) or azathioprine (Aza), and corticosteroids (CS; prednisone or prednisolone). Many centers also use a short induction (or prophylactic) therapy, within the first days or weeks of transplantation, based on polyclonal anti-lymphocyte and anti-thymocyte globulins, or monoclonal antibodies against anti-CD25 and anti-CD52, principally.

Elimination at an earlier stage or total avoidance of CS from immunosuppressive regimens remains a major challenge for transplant physicians. Numerous studies have investigated their withdrawal, as early as 7 days after transplantation, or later on after transplantation. Very few studies explored their total avoidance [5–13].

In a retrospective analysis of more than 1500 kidney transplant recipients of a single center treated with anti-thymocyte globulin (Thymoglobuline®; Genzyme) for induction, CNI and Aza or MMF, discontinuation of CS was feasible and safe in more than 80% of patients as early as 2–3 months after transplantation, with an overall incidence of acute rejection of nearly 20% [14]. Whether induction with polyclonal antibodies against T cells should be given in CS avoidance protocols remains debatable. The immunosuppressive mechanism of action and the possibility of inducing a state of 'operational tolerance' with anti-lymphocyte or anti-thymocyte globulins have been studied in various models since 1960 [15–19]. Apoptotic effects on activated lymphocytes have been demonstrated *in vitro* [20]. Cell death induced by an interaction between Fas (Apo-1, CD95) and Fas-L requires positive transcription of genes of several cytokines including interleukin-2. This interaction is blocked by concomitant CNI drugs and/or CS [20]. These experimental findings and the encouraging results of recent CS-avoidance studies showing a low incidence of acute rejection [21–23], contributed to the rationale of our study design: the absence of CS in the context of T-cell depletion and with a delayed introduction of CNI, may create a favorable state of unresponsiveness with respect to the allograft and decrease the number of patients experiencing acute rejection. To test and validate this hypothesis, we conducted for the first time a prospective, randomized, open-label and multicenter study combining ATG-F, MMF and CNI, with and without concomitant CS, following primary cadaver kidney transplantation. ATG-F was selected because of its good clinical tolerance profile and the lesser incidence of PTLD complications described as compared with other polyclonal antibody preparations [24,25].

The 1-year efficacy and safety data of this trial named FRANCIA (for FResenius ATG, No Corticosteroids, In Kidney Allotransplantation), which includes results from the first to the last randomized patient having all full 1-year follow-up, are presented in this report.

Methods

Study design

The trial was a French multicenter (six university hospitals), prospective, randomized open-label study in accordance with a 1/1 plan, on parallel groups and two arms of treatment. The study (study number BRD00/6-G), with direct individual benefit, was approved by the Ethics Committee of Nantes and was sponsored by the Nantes University Hospital. Recruitment of the 204 patients enrolled started in 2001 and was continued until April 2005, when the required patient number was reached. Follow-up of all patients was scheduled for 5 years (final analysis in April 2010). One group of

patients was treated with a standard regimen of CS for at least 6 months (Steroid maintenance group) and the second group of patients did not receive any CS at all, except for a single dose of methyl prednisolone on the day of transplant surgery (Steroid avoidance group).

Randomization, performed before transplantation and after obtaining the patient's signed informed consent, took into account the patient's age (≤50 and >50 years on the day of transplantation) and cold ischemia time (≤24 or >24 h). A series of sealed numbered envelopes containing the name of each branch of the study (i.e., with or without CS) was stored centrally. Patients were recipients of a first cadaver kidney graft, aged from 18 to 65 years, and had actual and historical panel-reactive cytotoxic T-cell antibodies ≤20%. All patients received ATG-F, one injection postoperatively and four injections on alternate days after transplantation; CyA was commenced from the fifth postoperative day onwards, overlapping with the last ATG-F infusion and MMF was started on the day of transplantation (Fig. 1).

Patients

Patients with chronic renal failure, listed in the French national waiting list and who received a first cadaver renal transplant were included in this study (Table 1). All organs were taken from brain-dead subjects (Table 2). Six French university hospitals participated in this study (University Hospitals of Nantes, Toulouse, Montpellier, Strasbourg, Besançon, and Nice). Inclusion and exclusion criteria are shown in Table 3.

Immunosuppression

The administration schedules and dosages of ATG-F, MMF and CyA were identical in both groups (Steroid

Table 1. Recipient baseline characteristics (full analysis set).

Renal allograft recipients	+CS	−CS
Number of patients	99	98
Mean age (years)	48 (17–65)	48 (19–65)
Gender (male/female)	64/35	70/28
Pre-emptive transplantation	8	7
Mean duration ± SD (months) of pretransplantation dialysis	40 ± 40	30 ± 23
End-stage renal disease etiologies		
Polycystic kidney disease	19	23
Chronic pyelonephritis	2	0
Chronic glomerulonephritis	23	12
Hypertension	7	5
Type 1 diabetes	2	1
Type 2 diabetes	9	4
Other nephropathy	42	54
Missing data	0	1

+CS, Steroid maintenance group; −CS, Steroid avoidance group.

Table 2. Donor characteristics (full analysis set).

Kidney donors	+CS	−CS
Number of donors	99	98
Mean age (years)	46 (5–72)	45 (5–72)
Gender (male/female)	66/33	57/40
CMV status, donor and recipient		
D^-/R^-	22 (22.2)	29 (29.6)
D^-/R^+	30 (30.3)	31 (31.6)
D^+/R^-	24 (24.2)	13 (13.3)
D^+/R^+	23 (23.2)	25 (25.5)
EBV status		
D^-	7 (7.1)	10 (10.2)
D^+	67 (67.7)	64 (65.3)
Unknown	25 (25.3)	24 (24.5)

+CS, Steroid maintenance group; −CS, Steroid avoidance group. Results represent number of donors and percentage.

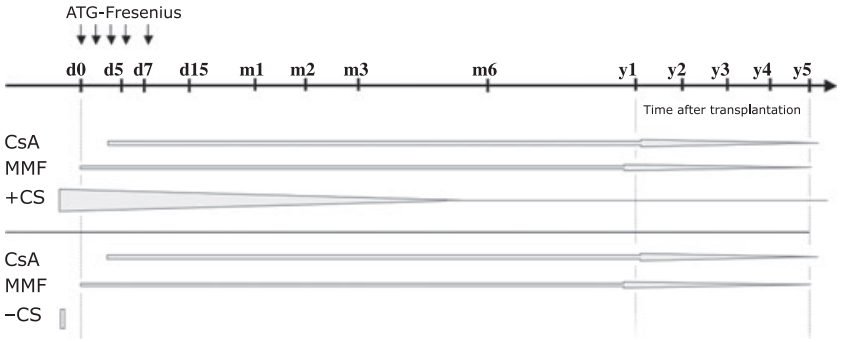

Figure 1 Immunosuppressive regimen. All patients with a first cadaver renal allograft received ATG-F as an induction therapy. Group one: patients with corticosteroids for 6 months (+CS, Steroid maintenance) group; group two: patients only received one corticosteroid bolus (500 mg of methyl-prednisolone) prior to transplantation (−CS, Steroid avoidance group). In both groups cyclosporine (CyA) therapy was started on day 5 post-transplantation; mycophenolate mofetil (MMF) was initiated on the day of transplantation.

Table 3. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
First cadaver renal transplant candidate	Pregnancy or breast-feeding
Included on the national French waiting list	Anti-HLA T-cell antibodies ≥20%
Man or woman between 18 and 65 years of age	Known allergy to any rabbit protein
Use of reliable contraception in women within the first year of the study	Cold ischemia time > 36 h
Donor age between 18 age 65 years	Allergy to macrolide antibiotics, CyA, MMF
Written informed consent	Any immunosuppressive treatment (including CS, before transplantation)
	Malignant tumor or known malignant neoplasia (excepted treated baso- or spino-cellular skin cancers)
	Waiting for an additional transplant
	Recipient of a previous organ or tissue graft
	Leukocyte count <2000/mm^3 and/or platelet count of <50 000/mm^3
	End-stage renal disease secondary to focal and segmental glomerular sclerosis

maintenance group and Steroid avoidance group). The first infusion of ATG-F was of 9 mg/kg dosage and was preceded by an injection of 500 mg methyl prednisolone. Infusion was started immediately after surgery was completed. Duration of the first infusion was at least 6 h. The four subsequent infusions were of 3 mg/kg, fixed-dose, on postoperative days 1, 3, 5 and 7. They were preceded by an infusion of 1 ml of dexchlorpheniramine 1 h earlier. ATG-F was given either via a central line, an arterio-venous shunt, or a large peripheral vein over at least 4 h.

The initial dose of MMF was of dosage 1 g b.i.d., administered orally every 12 h. The first dose was administered either 12 h preceding the removal of the vascular clamps or following surgery. The daily dosage was adjusted in accordance with each center's practice.

The initial dose of CyA was 8 mg/kg/day administered orally in two doses every 12 h. The first dose was administered on day 5 (after the fourth injection of r-ATG). Doses of CyA were adjusted to achieve recommended CyA trough levels of 150–200 ng/ml throughout follow-up.

All patients in both the randomized arms were treated preoperatively with a 500 mg methyl prednisolone bolus injection preceding the first injection of r-ATG. CS (prednisolone or prednisone) were administered to patients from the Steroid maintenance group, usually in the morning, according to the following schedule: 1 mg/kg/day (day 0–5), 0.5 mg/kg/day (day 6–10), 0.25 mg/kg/day (day 11–15), 0.20 mg/kg/day (day 16–30), and 0.10 mg/kg/day (day 31–180). It was permissible to change these doses in cases of medical need. After day 180, CS therapy could be discontinued or continued according to each center's practice.

Each center was authorized to adapt or modify the immunosuppressive regimen according to medical need. In addition, the introduction of Tac, sirolimus, everolimus, EC-MPA, Aza or other immunosuppressive drug was allowed and not considered as a drop-out from the study.

Acute-rejection episodes therapy

Any suspicion of acute rejection had to be, if technically possible, proved by a core needle biopsy. Histopathological results were graded where possible according to the Banff classification [26]. No guidelines were given for first-line treatment for an acute rejection episode. Each center was allowed to use any anti-rejection therapy judged medically necessary. The use of high-dose steroids as first-line therapy, followed by the addition of depleting or nondepleting T-cell monoclonal and polyclonal antibodies within 3 weeks, was considered to be a steroid resistant rejection episode.

Co-medication

Prevention of cytomegalovirus (CMV) infection was given according to local protocols. In patients with high risk of CMV disease (i.e. D^+/R^-), prophylaxis with valacyclovir, ganciclovir or vanganciclovir for 3–6 months was highly recommended. Systematic screening for CMV infection after transplantation was recommended and was performed once a fortnight during the first 3 months. If a blood test (antigenemia pp65 or CMV-DNAemia) was positive and clinical signs were suggestive of a CMV-infection (i.e. fever, leukopenia, asthenia), treatment with IV ganciclovir for 2 weeks was to be administered. This was considered as a symptomatic CMV disease.

In cases of severe leukopenia that required discontinuation of MMF, CS treatment was permissible in the Steroid avoidance group, with dosage dependent on the patient's weight (<60 kg, 10 mg; >60 kg, 15 mg). If a serious viral disease with multi-organ involvement occurred, discontinuation of all immunosuppressive treatment was authorized. Furthermore, patients could be treated with anti-fungal, anti-infectious, diuretic or other preventive or symptomatic treatments according to local center's practices.

Assessment of safety

Adverse drug reactions and all adverse events (AEs) observed, were included into the safety analysis.

Evaluation criteria

The primary end-point was acute rejection during the first year after transplantation: any clinically suspected

and treated episode, biopsy-proved episode, its incidence, its severity and its resistance to anti-rejection treatment. Secondary end-points regarding the 1-year analysis were the baseline glycemia, HbA1c values, blood lipids, homocysteine levels, weight and body-mass index, creatininemia and endogenous creatinine clearance, systolic and diastolic blood pressure, and 24-h proteinuria, as well as graft and patient survivals.

Statistical analyses

The primary end-point was the efficacy of the immunosuppressive prophylaxis assessed by the incidence of acute, clinically defined, rejection episodes during the first year after transplantation. The sample size estimation was based on an incidence of acute rejection of 30% in patients with CS and an incidence of 10% in patients without CS. For a two-sided test for superiority with alpha = 5% two times 62 patients were needed to obtain a power of 80%. To assure the required number of evaluable participants, the study was planned with 200 patients in total, distributed in two arms of 100 patients each. Eligible patients were assigned to CS or non-CS treatment at a 1:1 ratio using block randomization with stratification according to the recipient's age (≤50 vs. >50 years) and cold ischemia time (≤24 vs. >24 h). Treatment codes were provided in sealed envelopes that were to be opened only after a patient had completed all pretreatment examinations, was determined to be eligible, and had signed an informed consent.

For the primary outcome measure, the treatment groups were compared using Kaplan–Meier survival analysis with stratification according to the recipient's age and cold ischemia time (with the same cut-off points used during randomization). The treatment groups were compared using log-rank tests; *P*-values and confidence intervals for time to event within the treatment groups were determined. Patients who completed the first year without a rejection were censored at day 365 for the 1-year analysis. Secondary efficacy parameters were analysed descriptively and included the incidence of CS-sensitive and CS-resistant rejections (as an indicator of rejection severity), histology-confirmed rejections and histology severity of lesions, as well as graft and patient survivals. Descriptive *P*-values were determined using *t*-tests for interval data and Fisher's exact tests for categorical data unless otherwise specified. All reported *P*-values are two-sided.

Results

Patient characteristics

A total of 204 patients were enrolled and randomized. Three patients did not receive any ATG-F and did not provide any data after transplantation; they were thus not included in the analyses. In the safety analysis set 103 patients were treated in the Steroid maintenance group (+CS) and 98 in the Steroid avoidance one (−CS). Four patients analysed for safety were not included in the efficacy analysis because of substantial deviations from the immunosuppressant therapy protocol. So far, 197 patients were analysed in the full analysis set: 99 patients in the +CS group and 98 patients in the −CS one. Five patients (+CS 3; −CS 2) terminated their participation in the trial before the end of the first year after transplantation. The reasons for premature termination were death (+CS 3; −CS 1) and chronic graft failure (−CS 1). The mean age of participants was 48 years, mean weight was 74.9 kg for men and 62.2 kg for women; 16.2% of participants were smokers. The randomization procedure resulted in a well-balanced distribution of patients into the +CS and −CS groups (Table 1). Donor data showed a similar well-balanced distribution between the +CS and −CS groups (Table 2). Mean cold ischemia time was 23.5 ± 8.5 h in the +CS group and 22.0 ± 7.9 h in the −CS one ($P = 0.20$). The need for dialysis (independently of the cause) during the post-transplant period was considered as delayed graft function (DGF). DGF occurred in 34 (34.3%) +CS patients and in 38 (38.8%) −CS ones (P = NS).

Rejections

During the first year after transplantation, the number of patients with clinically suspected acute rejection episodes and who received anti-rejection treatment was higher among −CS than +CS patients (25 vs. 13; $P = 0.031$). The total number of episodes was 28 in the −CS group (three patients experienced two episodes) and 14 in the +CS group (one patient experienced two episodes). In the Kaplan–Meier survival analysis (Fig. 2a) the estimated average time until the first rejection episode was 279 days (95% confidence interval: 249–308 days) for −CS and 328 (308–348) days for +CS (primary analysis stratified by recipient age and cold ischemia time: $P = 0.027$; event-free patients censored at day 365). The stratified analysis also revealed that the treatment group differences regarding rejection incidence were entirely attributable to the patients above the age of 50. Whereas, the first-year rejection rates were 20.4% (10 out of 49 patients) in the −CS group and 21.3% (10 of 47) in the +CS group in patients aged 50 or younger, rates of 30.6% (15 of 49) and 5.8% (3 of 52) were observed in patients over 50 for −CS and +CS, respectively. As shown in Table 4, 17 from the 25 patients in the −CS group and seven from the 13 patients in the +CS group had at least one acute rejection confirmed by biopsy ($P = 0.031$). Borderline changes (six

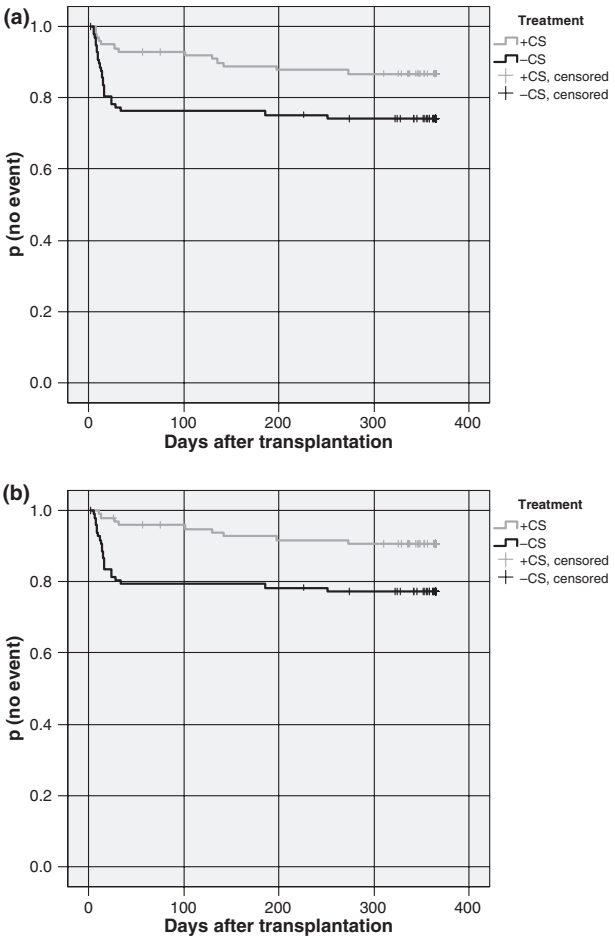

Figure 2 (a) Time until first clinically suspected rejection episode during the first year after kidney transplantation (full analysis set). (b) Time until first biopsy confirmed rejection episode during the first year after kidney transplantation (full analysis set).

Table 4. Acute rejection episodes during the first year following transplantation.

	+CS	−CS	*P*-value
Number of patients	99	98	
Patients with clinically suspected rejection	13 (13.1%)	25 (25.5%)	0.031
Biopsy-proven rejections (Banff grades)	7 (7.1%)	17 (17.3%)	0.031
1a	5	9	
1b	1	2	
2a	0	6	
2b	1	0	
Patients with DGF and rejection	6 (42.6%)	6 (24.0%)	
First-line anti-rejection therapy			
Steroid boluses	9 (69%)	20 (80%)	
Anti-CD3	1 (8.3%)	0	
Anti-CD3 and plasma exchange	2 (16.7%)	5 (21%)	
Other histologic diagnosis			
No rejection	1	2	
Acute pyelonephritis	1	1	
BKV infection	1	0	
No biopsy performed	1	0	
Patients with more than one rejection	1 (1%)	3 (3%)	

+CS, Steroid maintenance group; −CS, Steroid avoidance group; DGF, delay graft function.

Anti-CD3 = Orthoclone OKT3, muromonab-CD3 (Janssen-Cilag). Borderline changes on renal histology (3 in +CS group and 6 in −CS group) were not considered as biopsy-proven acute rejections. Only one patient in the Steroid maintenance group was not able to be biopsied to confirm the rejection. This patient was treated with OKT3 and plasma exchange as first-line therapy on postoperative day 8. In addition, two more patients (both in the Steroid maintenance group) did not receive anti-rejection therapy because of borderline lesions in one case on postoperative day 12, and noncompliance in the second case. This last patient experienced a grade 2b rejection 9 months following transplantation after complete immunosuppression withdrawal several weeks before. The patient returned to hemodialysis without any additional rejection therapy and the graft was removed.

in the −CS and three in the +CS) were not considered as biopsy-proven acute rejections. No statistical difference on severity lesions according to the Banff classification was noted ($P = 0.19$, exact Mann–Whitney U-test based on 17 and 7 patients with biopsy-proven rejection episodes). The majority of biopsy–proven rejections were of grade 1 (12/18 in +CS and 6/7 in −CS). More −CS patients experienced grade 2 episodes. Kaplan–Meier survival analysis for time until biopsy confirmed rejection (Fig. 2b) resulted in an estimated average time to event of 307 days (95% confidence interval: 281–332 days) for −CS and 348 (336–361) days for +CS (log-rank test stratified by recipient age and cold ischemia time: $P = 0.021$; event-free patients censored at day 365).

Responsiveness to first-line anti-rejection therapy did not differ between groups and similar incidence of rescue treatment was observed. The incidence of corticosteroid resistant rejection was comparable; −CS 8 (8.2%) vs. +CS 9 (9.1%), $P = 1.00$. None of the patients had more than one corticosteroid resistant episode.

The need for dialysis (independently of the cause) during the post-transplant period was considered as delayed graft function (DGF). DGF occurred in 34 (34.3%) +CS patients and in 38 (38.8%) transplant recipients treated with −CS ($P = 0.56$). Among the study participants experiencing a rejection episode during the first year after transplantation the percentage of patients with previous DGF was higher in the +CS group (6 patients out of 13, 42.6%) than for −CS (6 of 25, 24.0%). When serum creatinine was compared between patients with and without rejection in either group at 1 year, results showed no statistical differences in rejection-free patients (median of 135 μmol/l in +CS patients vs. 137 μmol/l in −CS

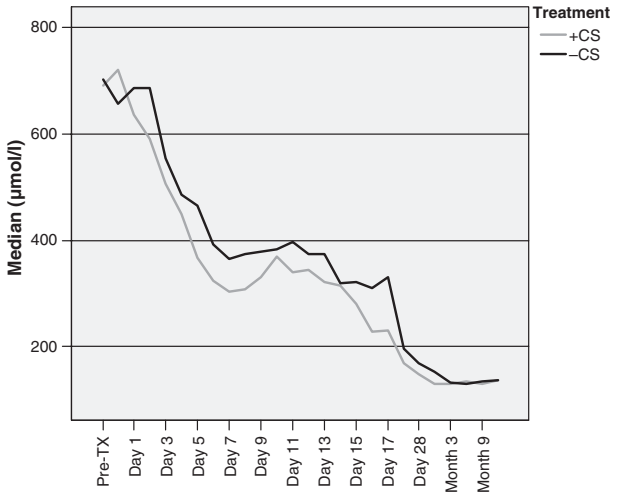

Figure 3 Serum creatinine levels throughout the first year after kidney transplantation (full analysis set).

patients). Interestingly, a statistically higher median serum creatinine level was observed in +CS patients with history of rejection (220 µmol/l) as compared to a median of 131 µmol/l in −CS patients ($P = 0.004$). Graft survival in these patients with history of rejection was also better in patients from the Steroid avoidance group (Fig. 3).

ATG-F administration and concomitant immunosuppressants

In the full analysis set, 90 out of 99 +CS patients and 89 from 98 −CS patients received the entire scheduled ATG-F course of five infusions. The average absolute doses administered did not vary significantly between the two groups (Table 5). The average relative cumulative dose of ATG-F was 20.7 ± 2.7 mg/kg body weight (range: 12.0–31.2 mg/kg) in the +CS group and 20.5 ± 2.2 mg/kg (8.8–25.5 mg/kg) in the −CS group (prespecified cumulative dose according to the protocol: 21 mg/kg). In 79 of 99 patients (79.8%) from the +CS group and in 71 of 98 patients (72.4%) from the −CS group no abnormal vital signs during and/or after the first infusion were observed. In +CS and −CS patients the most frequent adverse events during or after ATG-F administration period were fever (14 vs. 17), tachycardia (8 vs. 10), hypertension (5 vs. 11) and hypotension (4 vs. 5). During follow-up days 1 to 28 increased body temperature was more often observed in −CS compared to +CS patients (43.9% vs. 27.3%).

Corticosteroids were administered in −CS patients as part of first-line anti-rejection treatment ($n = 25$). Additionally, 14 patients in the −CS group (14.3% of 98) received transient steroid treatment for an indication other than rejection during the first year following transplantation. Overall, complete avoidance of corticosteroids was reached in 60 (61.2%) −CS patients. For other immunosuppressants, Tac was given to 14 −CS patients and to 15 +CS ones and sirolimus to three −CS patients and four +CS ones. EC-MPA was given to one patient in each group.

Table 5. Mean absolute doses of r-ATG.

Days after transplantation	+CS	−CS
Number of patients	99	98
Day 0 (day of transplantation)	628 (350–970) mg	634 (320–900) mg
Day 1	220 (100–610) mg	215 (100–400) mg
Day 3	217 (100–300) mg	216 (100–400) mg
Day 5	216 (100–300) mg	214 (100–400) mg
Day 7	215 (100–300) mg	212 (100–300) mg

+CS, Steroid maintenance group; −CS, Steroid avoidance group.

Laboratory measurements

By 1 year after transplantation, no differences in renal function parameters, such as proteinuria, serum creatinine (Fig. 3) and creatinine clearance, were detected between the two groups. Mean homocysteine dosage peaked at day 7 postoperatively in both treatment groups (+CS: 47.35 ± 51.78 µM; −CS: 40.44 ± 24.50 µM); at 12 months, homocysteine values were almost identical (18.8 vs. 18.3 µM). The glycosylated HbA1c findings were also comparable between groups (Table 6). Similarly, thrombocyte, lymphocyte, granulocyte and erythrocyte counts, as well as T-cell subsets (CD2, CD3, CD4, CD8, CD4/CD8 ratio) were not significantly different between the groups (data not shown).

Patient and graft survival

Patient survival during the first year after transplantation was comparably favorable in both groups: 97.1% of patients in the +CS group (100 out of 103) and 99.0% in

Table 6. Laboratory results 12 months after transplantation (mean ± SD; full analysis set).

Parameter (mean values)	+CS	−CS
Number of patients	99	98
HbA1c (%)	5.7 ± 0.9	5.6 ± 0.5
Proteinuria (g/24 h)	0.92 ± 4.96	0.77 ± 2.23
Serum creatinine (µmol/l)	164 ± 129	159 ± 121
Creatinine clearance (ml/min)	57.84 ± 20.40	58.19 ± 16.60
Homocysteine (µm)	18.83 ± 13.77	18.28 ± 6.45

+CS, Steroid maintenance group; −CS, Steroid avoidance group.
All results were not statistically different between both groups.

the −CS group (97 out of 98) were alive at 1-year after surgery (Kaplan–Meir analysis, $P = 0.34$). Three patients from the +CS group died on days 26, 57 and 75 after transplantation because of intra-cerebral hemorrhage, myocardial infarction and unknown cause; one patient from the −CS group died 2 days postoperatively because of multiorgan failure. Similarly, 93.2% of +CS patients (96 of 103) and 94.9% of −CS patients (93 of 98) had fully functioning renal allografts at 1-year after transplantation (safety analysis set). Graft failure during the first year after transplantation was seen in five patients from each group, and furthermore two patients in the +CS group died with a functioning graft. Average graft survival time estimated by Kaplan–Meier analysis was 346 (95% confidence interval, 332–360) days for +CS and 350 (337–363) days for −CS patients (log-rank test stratified, $P = 0.618$; Fig. 4).

In the subset of patients with any acute rejections during the first year the 1-year graft survival rates (counting deceased patients as graft failures) were 76.9% (10 of 13) and 96.0% (24 of 25) for +CS and −CS, respectively. The associated KaplanMeier survival curves are shown in Fig. 5. The estimated average graft survival time was 355 days (95% confidence interval: 337–374 days) for −CS and 311 (249–373) days for +CS (log-rank test stratified: $P = 0.066$; event-free patients censored at day 365).

Adverse events, infections and malignancies

There were no significant differences regarding the safety of both regimens during the postoperative 12 months. The numbers of AEs and serious adverse events (SAEs) per patient, as well as the cumulative numbers of AEs, SAEs and the median number of potentially related AEs per patient, were comparably low in both treatment groups. In the safety analysis set, a total of 1644 AEs were reported in +CS patients (15.9 events/patient) and 1509 (15.4 events/patient) in −CS patients. The majority of them occurred during the first month (943 and 949, respectively). The incidence of SAEs was higher among +CS patients ($n = 232$; 70 during the first month) than in −CS ones ($n = 162$; 61 during the first month). Serum sickness was detected in only one −CS patient. A total of 75 patients (72.8%) experienced 176 infectious episodes in the Steroid maintenance group and 66 patients (67.3%) experienced 143 infectious episodes in the Steroid avoidance group. The most common infectious episodes in respectively +CS and −CS patients were

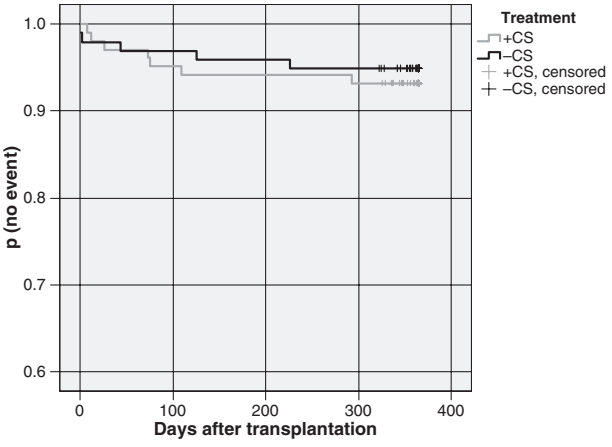

Figure 4 Graft survival, all patients (full analysis set).

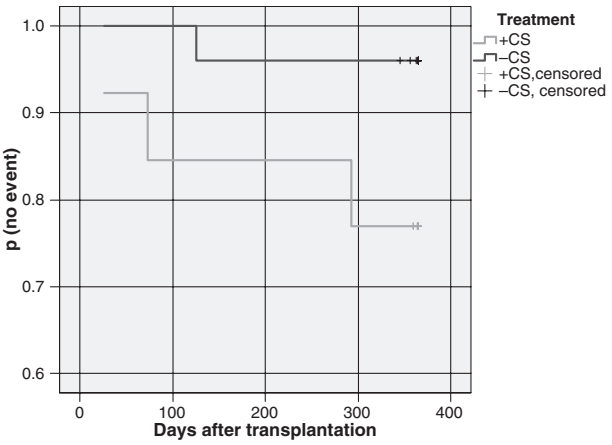

Figure 5 Graft survival in patients with rejection episodes (full analysis set).

Table 7. Medically relevant events at during the 1-year follow-up (safety analysis set unless otherwise noted).

Status parameter	+CS	−CS	*P*-value
Number of patients	103	98	
Deceased patients	3 (2.9)	1 (1.0)	0.62
Graft failures	5 (4.9)	5 (5.1)	1.00
*Delayed graft function	34 (34.3)	38 (38.8)	0.56
Patients with malignancy	5 (4.8)	0 (0.0)	0.059
Newly diagnosed hypertension	12 (11.7)	9 (9.2)	0.65
Persistence of hypertension at 1 year	10 (9.7)	9 (9.2)	1.00
Newly diagnosed dyslipidemia	22 (21.4)	8 (8.2)	0.01
Patients with *de novo* diabetes	15 (14.6)	6 (6.1)	0.07
Insulin requirement	13 (12.6)	5 (5.1)	0.08
Oral anti-diabetic drugs	6 (5.8)	1 (1.0)	0.12
Persistence of diabetes at 1 year	10 (9.7)	3 (3.1)	0.08
Patients with infections	75 (72.8)	66 (67.3)	0.44
Patients with CMV infection	20 (19.4)	16 (16.3)	0.59

+CS, Steroid maintenance group; −CS, Steroid avoidance group.
Results represent number of patients and percentage. *Full analysis set. Hypertension, dyslipidemia and diabetes represent *de novo* (not mentioned at inclusion) complications developed after transplantation. *P*-values: Fisher's exact test.

urinary tract infections (32 vs. 30), CMV infections (20 vs. 16), bronchitis (6 vs. 14) and acute pyelonephritis (9 vs. 5). Concerning CMV, a lower incidence of symptomatic patients requiring anti-viral treatment was seen in −CS patients ($n = 4$, 4.1%) vs. 6 +CS patient ($n = 6$, 5.8%). Malignancies developed in five patients, all in the +CS group: three PTLD and two breast cancers; Fisher's exact test, $P < 0.059$ as compared to −CS group. Medically relevant events are shown in Table 7.

Discussion

Looking at the primary efficacy parameter during the first year following transplantation, our study showed that patients who did not receive maintenance CS from the day of transplantation experienced significantly more clinically suspected (25.5% vs. 13.1%) and biopsy-proven (17.3% vs. 7.1%) acute rejection episodes than patients under maintenance CS. Despite this observation, no difference on severity of histologic lesions was noted and resistance to ongoing first-line rejection therapy was, on the contrary, less evident in the −CS group (probably because of the earlier onset of diagnosis). This increased number of rejection episodes did not adversely affect the 1-year graft and patient survivals; graft failure rates in both groups were comparable: 7% in the +CS group vs. 5.0% in the −CS one, as well as survival of patients: 99% in the +CS group vs. 97% in the −CS group. In addition, graft survival in the subgroup of patients with history of rejection was similar in −CS and +CS patients. Interestingly, although parameters of renal function were similar in all patients without rejection, statistically better results were seen in −CS patients with history of rejection as compared with +CS ones, at 1 year. If confirmed in the long-term, this result may be of clinical relevance, suggesting that many of the acute rejections occuring on steroi-free regimens might be less harmful and/or have good prognosis.

Despite the increased incidence of biopsy-proven acute rejections observed in the Steroid avoidance group, the number of biopsy-proven, rejection-free patients (overall 87.8%) at 1 year was very close to that observed in trials where CS-free regimens with an anti-CD25 monoclonal antibody induction and Tac/MMF as maintenance from the day of transplantation were studied [26]. As our CNI choice was CyA and not Tac, one may expect better results with Tac, considered to be more efficient than CyA in preventing acute rejection [27–29].

The avoidance of CS may modify clinical, biological, and possibly histologic and genetic parameters [30] following renal transplantation. Symptoms such as fever and graft tenderness, biological parameters such as low white blood cell count, and histologic features such as edema and cell infiltrates, may be exacerbated in the absence of CS. This new condition could influence transplant physicians to perform more transplant biopsies in CS-free recipients for graft surveillance. The resultant could be an increased anti-rejection therapy consecutive to higher 'biopsy-proven' acute rejections. In this trial, no protocol biopsies were planned and we are not able to analyse whether CS avoidance was responsible for greater graft cell infiltration and interstitial edema than maintenance CS.The fact that neither clinical nor histologic severity of rejection episodes, their resistance to treatment, graft failure, graft survival and graft function, and proteinuria levels differed when compared with +CS patients, indirectly suggest again an over-estimated number of rejection episodes in the −CS group.

Both immunosuppressive regimens were well tolerated during the first postoperative year. The entire r-ATG course was applied to 98.5% of patients (three premature interruptions, one +CS patient and two −CS patients). As expected, about 70–80% of patients in each group were affected by abnormal vital signs during and/or after the first ATG-F infusion. During the first 28 postoperative days, patients without CS showed more increased body temperatures and only one case of serum sickness (a −CS patient) was observed. Serum sickness has been reported, particularly in the early years of r-ATG use, when low dosages were applied over 20 days [31]. An incidence of 10% was noted with anti-thymocyte globulin under CS therapy [1,14]. The low incidence of acute serum sickness encountered in our study, in the context of CS avoidance and delay CyA introduction, confirms the good clinical tolerance of this polyclonal anti-lymphocyte globulin.

Avoiding CS completely was achieved in 61.2% of patients. Main reason for introducing CS in the Steroid avoidance group was rejection episode. For other immunosuppressive drugs, Tac replaced CyA in 14% of cases as compared to 15% in the Steroid maintenance group. m-TOR inhibitors were minimally given (overall seven patients in both groups).

Adverse events, including severe ones and infectious episodes were not more frequently experienced by either group, although a consistent trend for fewer events were observed in the Steroid avoidance group. The development of a new diabetic disorder (need for exogenous insulin therapy) and lipid abnormalities were however more frequent in the Steroid maintenance group suggesting that low CS maintenance doses are more diabetogenic and toxic than CS boluses given for ongoing rejection. HbA1c levels, in patients without diabetes, were similar at 1 year in both groups.

Recent data from a comparative study between anti-thymocyte globulin and an anti-CD25 monoclonal antibody showed a lower incidence of CMV infection

than in the past, and particularly low in the polyclonal group [32]. The incidence of symptomatic CMV disease in our study was also low 5.0%, and although not statistically different, avoidance of maintenance CS seems to protect patients from this viral complication as only 4% of −CS patients developed symptoms of viral disease requiring therapy despite higher number of rejection episodes. This result must be considered of clinical relevance as it could positively impact long-term graft and patient survivals [33]. Novel and more efficient strategies of CMV infection monitoring, as well as medical anti-viral prophylaxis are likely responsible for these recent observations of CMV disease in patients under polyclonal antibodies.

Five malignancies (including three cases of PTLD) were reported in our study, all in the Steroid maintenance group. PTLD is one of the most redoubtable complications of any immunosuppressive regimen following transplantation, mainly when an anti-lymphocyte preparation is included [24,25]. A lesser prevalence of PTLD with r-ATG use was obtained from large retrospective analysis performed by Opelz *et al.* [25] when compared with other induction agents or no induction at all. Sinha *et al.* [34] showed that the Jurkat cell line used for the production of ATG-F expresses the EBV/C3d receptor. This anti-EBV receptor antibody might contribute for the lower risk for EBV-mediated PTLD encountered with this product. Among the 201 patients of this study, only one EBV infection was recorded (a +CS patient).

The percentage of DGF did not differ in −CS (38.8%) when compared to +CS patients (34.3%), indicating that CS avoidance does not seem detrimental for the immediate renal graft function resumption. ATG-F induction, in the absence of CNI, was however able to reduce DGF after renal transplantation when compared with anti-CD25 monoclonal antibodies [35]. ATG-F may cause a reduction of the expression of adhesion and inflammation molecules both in endothelium and reperfused tissue. The inhibition of the expression of molecules required for firm cellular adhesion (ICAM-1, VCAM, PECAM, CD11b, CD62E), may contribute to decreasing cellular graft infiltration after postischemic reperfusion [36]. The relatively high incidence of DGF observed in our study, contrasting with other studies [35,36], could be in part attributed to the fact the ATG-F was given after surgery and not before or during the transplant procedure, as previously reported.

Analysis of T-cell subsets (CD2, CD3, CD4, CD8, CD4/CD8 ratio) following administration of ATG-F (see Ref. 37 for a detail report on short- and long-term follow-up of white blood cell count phenotype in a limited population of this study cohort) did not differ between +CS and −CS groups, suggesting for the first time that CS avoidance did not modify ATG-F-induced T-cell depletion and T-cell homeostasis. Our findings are in accordance to previous observations of Müller *et al.* [38] and Lange *et al.* [38].

In conclusion, the 1-year analysis of this first randomized evaluation of a regimen based on ATG-F induction (including a first high-dose infusion and four consecutive alternate-day schedule), MMF, delaying CyA introduction for 5 days, and without consecutive CS treatment, can be considered as safe as a same regimen with maintenance CS. However, our primary hypothesis based on a favorable state of unresponsiveness with respect to the allograft in the absence of CS, was not confirmed. This CS-free regimen gives a higher incidence of acute rejection episodes (two times higher) than a regimen containing CS. Our 1-year overall results did not indicate, nevertheless, a negative impact of acute rejection on graft survival and function. CS avoidance could open a long-term perspective for renal transplant patients to being less exposed to their numerous side-effects (principally diabetes and cardiovascular-related complications). The 5-year planned analysis of this trial is necessary to confirm these results.

Authorship

The study was designed by D. Cantarovich. It is an Investigator Origin Protocol, performed by six university Hospitals in France. The reagents analysed were all recorded in Europe with no experimental drugs evaluated. Data were collected and monitored by the University Hospital of Nantes. Statistical analyses were performed by an independent statistician, Dr Andreas Voelp (Frankfurt, Germany). The paper was written by D. Cantarovich, PI of the study. Authors are two members of each participant center, and they were all co-investigators of the study.

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References

1. Soulillou JP, Cantarovich D, Le Mauff B, *et al.* Randomized controlled trial of a monoclonal antibody against the interleukin-2 receptor (33B3.1) as compared with rabbit antithymocyte globulin for prophylaxis against rejection of renal allografts. *N Engl J Med* 1990; **322**: 1175.

2. Giral M, Taddei C, Nguyen JM, *et al.* Single-center analysis of 468 first cadaveric kidney allografts with a uniform ATG-CsA sequential therapy. In: Cecka, Terasaki, eds. *Clin Transplant*. UCLA Tissue Typing Laboratory, LA, California, 1996: 257–264.
3. Halloran P, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C. Mycophenolate mofetil in renal allograft recipients: a pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. The International Mycophenolate Mofetil Renal Transplant Study Groups. *Transplantation* 1997; **63**: 39.
4. Cantarovich D, Karam G, Giral-Classe M, *et al.* Randomized comparison of triple therapy and antithymocyte globulin induction treatment after simultaneous pancreas-kidney transplantation. *Kidney Int* 1998; **54**: 1351.
5. Brown MW, Forwell MA. Rejection reaction after stopping prednisolone in kidney transplant recipients taking cyclosporine. *N Engl J Med* 1986; **314**: 183.
6. Hricik DE, O'Toole MA, Schulak JA, Herson J. Steroid-free immunosuppression in cyclosporine-treated renal transplant recipients: a meta-analysis. *J Am Soc Nephrol* 1993; **4**: 1300.
7. Ratcliffe PJ, Dudley CR, Higgins RM, Firth JD, Smith B, Morris PJ. Randomised controlled trial of steroid withdrawal in renal transplant recipients receiving triple immunosuppression. *Lancet* 1996; **348**: 643.
8. Ahsan N, Hricik D, Matas A, *et al.* Prednisone withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mofetil – a prospective randomized study. Steroid Withdrawal Study Group. *Transplantation* 1999; **68**: 1865.
9. Vanrenterghem Y, Lebranchu Y, Hené R, Oppenheimer F, Ekberg H. Double-blind comparison of two corticosteroid regimens plus mycophenolate mofetil and cyclosporine for prevention of acute renal allograft rejection. *Transplantation* 2000; **70**: 1352.
10. Vanrenterghem Y, van Hoff JP, Squifflet JP, *et al.* Minimization of immunosuppressive therapy after renl transplantation: results of a randomized controlled trial. *Am J Transplant* 2005; **5**: 87.
11. Vincenti F, Schena FP, Paraskevas S, Hauser IA, Walker RG, Grinyo J. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. FREEDOM Study Group. *Am J Transplant* 2008; **8**: 307.
12. Woodle ES, First MR, Pirsh J, Shihab F, Gaber AO, van Veldhuissen P. A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early (7 days) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. Astellas Corticosteroid Withdrawal Study Group. *Ann Surg* 2008; **248**: 564.
13. Matas AJ. Minimization of steroids in kidney transplantation. *Transpl Int* 2009; **22**: 38.
14. Cantarovich D. Prevention of acute rejection with antithymocyte globulin (Thymoglobuline): its potential to reduce corticosteroids. *J Nephrol* 2004; **8**: S40.
15. Woodruff MF, Reid B, James K. Effects of antilymphocytic antibody and antibody fragments on human lymphocytes in vitro. *Nature* 1967; **215**: 591.
16. Monaco AP, Abbot WM, Othersen HB, *et al.* Antiserum to lymphocytes: prolonged survival of canine allografts. *Science* 1966; **153**: 1264.
17. Bonnefoy-Bérard N, Vincent C, Revillard JP. Antibodies against functional leukocyte surface molecules in polyclonal antilymphocyte and antithymocyte globulins. *Transplantation* 1991; **51**: 669.
18. Rebellato LM, Gross U, Verbanac KM, Thomas JM. A comprehensive definition of the major antibody specificities in polyclonal rabbit antithymocyte globulin. *Transplantation* 1994; **57**: 685.
19. Merion RM, Howell T, Bromberg JS. Partial T-cell activation and anergy induction by polyclonal antithymocyte globulin. *Transplantation* 1998; **65**: 1481.
20. Genestier L, Fournel S, Flacher M, Assossou O, Revillard JP, Bonnefoy-Berard N. Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. *Blood* 1988; **7**: 2360.
21. Birkeland SA. Steroid-free immunosuppression after kidney transplantation with antithymocyte globulin induction and cyclosporine and mycophenolate mofetil maintenance therapy. *Transplantation* 1998; **66**: 1207.
22. Cantarovich D, Giral-Classe M, Hourmant M, *et al.* Prevention of acute rejection with antithymocyte globulin, avoiding corticosteroids, and delaying cyclosporin after renal transplantation. *Nephrol Dial Transplant* 2000; **15**: 1673.
23. Cantarovich D, Giral-Classe M, Hourmant M, *et al.* Low incidence of kidney rejection following simultaneous kidney-pancreas transplantation after antithymocyte globulin induction and in the absence of corticosteroids: results of a prospective pilot study in 28 consecutive cases. *Transplantation* 2000; **69**: 1505.
24. Ducloux D, Kazory A, Challier B, *et al.* Long-term toxicity of antithymocyte globulin induction may vary with choice of agent: a single-center retrospective study. *Transplantation* 2004; **77**: 1029.
25. Opelz G, Naujokat C, Daniel V, Terness P, Döhler B. Disassociation between risk of graft loss and risk of non-Hodgkin lymphoma with induction agents in renal transplant recipients. *Transplantation* 2006; **81**: 1227.
26. Solez K, Axelsen RA, Benediktsson H, *et al.* International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int* 1993; **44**: 411.
27. Rostaing L, Cantarovich D, Mourad G, *et al.* Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807.
28. Charpentier B, Rostaing L, Berthoux F, *et al.* A three-arm study comparing tacrolimus therapy with antithymocyte globulin induction therapy followed by tacrolimus or

cyclosporineA in adult renal transplant recipients. *Transplantation* 2003; **75**: 844.
29. Gonwa T, Johnson C, Ahsan N, *et al.* Randomized trial of tacrolimus + mycophenolate mofetil or azathioprine versus cyclosporine + mycophenolate mofetil after cadaveric kidney transplantation: results at three years. *Transplantation* 2003; **75**: 2048.
30. Grinyo J, Vanrenterghem Y, Nashan B, *et al.* Association of four DNA polymorphisms with acute rejection after kidney transplantation. *Transpl Int* 2008; **21**: 879.
31. Grosse-Wilde H, Jakubowski HD, Eigler FW, Kuwert EK. In vitro immunoresponsiveness in recipients of cadaveric renal allograft during ATG therapy. *Proc EDTA* 1981; **18**: 481.
32. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N Engl J Med* 2006; **355**: 1967.
33. Kliem V, Fricke l, Wollbrink T, Burg M, Radermacher J, Rohde F. Improvement in long-term renal graft survival due to CMV prophylaxis with oral ganciclovir: results of a randomized clinical trial. *Am J Transplant* 2008; **8**: 975.
34. Sinha SK, Todd SC, Hedrick JA, Speiser CL, Lambris JD, Tsoukas CD. Characterisation of the EBV/C3d receptor on the human jurkat T-cell line. *J Immunol* 1993; **150**: 5311.
35. Kyllönen LE, Eklund BH, Pesonen EJ, Salmela KT. Single bolus antithymocyte globulin versus basiliximab induction in kidney transplantation with cyclosporine triple immunosuppression: efficacy and safety. *Transplantation* 2007; **84**: 75.
36. Beiras-Fernandez A, Chappell D, Hammer C, Beiras A, Reichart B, Thein E. Impact of polyclonal anti-thymocyte globulins on the expression of adhesion and inflammation molecules after ischemia-reperfusion injury. *Transplant Immunol* 2009; **20**: 224.
37. Louis S, Audrain M, Cantarovich D, *et al.* Long-term cell monitoring of kidney recipients after antilymphocyte globulin induction with and without steroids. *Transplantation* 2007; **83**: 712.
38. Müller TF, Grebe SO, Neumann MC, *et al.* Persistent long-term changes in lymphocyte subsets induced by polyclonal antibodies. *Transplantation* 1997; **64**: 1432.
39. Lange H, Müller TF, Ebel H, *et al.* Immediate and long-term results of ATG induction therapy for delayed graft function compared to conventional therapy for immediate graft function. *Transplant Int* 1999; **12**: 2.

