TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

The efficacy and safety of cyclosporine reduction in *de novo* renal allograft patients receiving sirolimus and corticosteroids: results from an open-label comparative study

Ferdinand Mühlbacher,[1] Hans-Helmut Neumayer,[2] Domingo del Castillo,[3] Sergio Stefoni,[4] Anthony J. Zygmunt,[5,*] and Klemens Budde[2] for the European Rapamune Cyclosporine Minimization Study Group†

1 University Clinic of Surgery, Vienna, Austria
2 Department of Nephrology, Charité Universitätsmedizin, Berlin, Germany
3 Hospital Reina Sofia, Cordoba, Spain
4 Azienda Ospedaliera S. Orsola Malphigi, Bologna, Italy
5 Wyeth Research, Collegeville, PA, USA

Keywords
cyclosporine, renal transplantation, sirolimus.

Correspondence
Prof. Ferdinand Mühlbacher, University Clinic of Surgery, 1 Waehringer Guertel 18-20, A-1090 Vienna, Austria.
Tel.: +43 1 40 400 6897;
fax: +43 1 40 400 6898;
e-mail: ferdinand.muehlbacher@meduniwien.ac.at

Conflict of interest
The authors have declared no conflicts of interest.

*At the time of this study, Anthony J. Zygmunt was an employee of Wyeth Research, which was acquired by Pfizer in October 2009.

†See Appendix for authors in European Rapamune Cyclosporine Minimization Study Group.

(ClinicalTrials.gov identification number: NCT00507793)

Received: 1 November 2012
Revision requested: 7 December 2012
Accepted: 28 October 2013
Published online: 25 November 2013

doi:10.1111/tri.12228

Summary

This study evaluated the safety and efficacy of a sirolimus, corticosteroid, and cyclosporine reduction regimen in an open-label, 12-month trial of 420 *de novo* renal allograft recipients at 49 European transplant centers. One month post-transplantation, 357 patients were randomized to receive standard-dose cyclosporine (sCsA, $n = 179$) or reduced-dose cyclosporine (rCsA, $n = 178$). All patients also received sirolimus and corticosteroids. The primary end points were the rate of biopsy-confirmed acute rejection (BCAR) and renal function, as measured by serum creatinine. Baseline demographic and donor characteristics were similar between groups. BCAR rates at 12 months were not significantly different: 11.2% for rCsA patients and 16.2% for sCsA patients. Mean serum creatinine (±SEM) was significantly lower (1.75 ± 0.10 vs. 1.97 ± 0.07 mg/dl, $P < 0.001$), and creatinine clearance (±SEM; Nankivell method) was significantly higher (57.8 ± 1.78 vs. 49.5 ± 2.46 ml/min, $P < 0.001$) in patients receiving rCsA versus sCsA at 1 year, respectively. Patient and graft survival exceeded 98% in both groups. No significant differences in infection or malignancy were noted between groups. The rCsA with sirolimus and corticosteroid regimen resulted in excellent 12-month patient and graft survival, a low incidence of BCAR, and improved renal function in renal allograft recipients. Sirolimus administered with rCsA and corticosteroids provided adequate immunosuppression while reducing the potential for the nephrotoxic effects of cyclosporine. These findings may help to improve long-term renal allograft outcomes.

Introduction

The introduction of cyclosporine into clinical practice led to dramatic improvements in renal allograft survival. Calcineurin inhibitors, such as cyclosporine and tacrolimus, remain the cornerstone of immunosuppressive therapy despite their broadly recognized acute and chronic nephrotoxic properties [1]. Both cyclosporine and tacrolimus attenuate T-cell response via inhibition of calcineurin phosphatase [2], thereby preventing transcription of interleukin-2, a cytokine critical to T-cell activation and proliferation. The immunosuppressive benefits of calcineurin inhibitors are limited, however, by adverse effects, including nephrotoxicity, neurotoxicity, and diabetes mellitus [1,3].

Sirolimus (Rapamune; Wyeth Pharmaceuticals [Pfizer], Philadelphia, PA) is a potent immunosuppressive drug that, unlike calcineurin inhibitors, blocks the mammalian target of rapamycin (mTOR), a key regulator of cell cycle progression [4]. Specifically, sirolimus blocks cytokine-driven T-cell proliferation through inhibition of DNA and protein synthesis, resulting in cell cycle arrest from the G1 to S phase [5].

Pivotal phase 3 studies comparing fixed-dose sirolimus (2 or 5 mg) with azathioprine (US) or placebo (global) controls administered in combination with standard-dose cyclosporine and corticosteroids in *de novo* renal allograft recipients showed significantly lower rates of acute rejection in patients receiving either dose of sirolimus [6,7]. Despite this, renal function was worse in patients treated with sirolimus and standard-dose cyclosporine (sCsA), suggesting that sirolimus exacerbates cyclosporine-induced nephrotoxicity. Results from a long-term study of sirolimus maintenance therapy after early withdrawal of cyclosporine confirmed that renal function improved significantly up to 5 years after renal transplantation in patients who underwent elimination of cyclosporine [8–10]. Although acute rejection rates were significantly higher at 1 year in the sirolimus arm than in those who remained on cyclosporine and sirolimus [8], by 2 years and thereafter, these rates were not significantly different between groups [9,10].

As renal allograft function emerges as an important indicator of long-term outcomes [11,12], immunosuppressive strategies that minimize exposure to calcineurin inhibitors are now increasingly common. Acute rejection, however, remains an important consideration, particularly in the first year after transplant. Several clinical trials have examined the use of mTOR inhibitors in combination with reduced doses of calcineurin inhibitors to determine whether this regimen results in comparable efficacy and improved renal allograft function. Recent studies examined cyclosporine minimization or elimination in patients receiving sirolimus and prednisone [13], as well as *de novo* treatment with sirolimus, induction antibody, and cyclosporine doses that were reduced by 50–80% [14]. Both trials found no differences in the incidence of rejection, graft loss, or death, while *de novo* treatment with reduced cyclosporine, antibody induction, and sirolimus improved renal function at 4 years [14].

Given the potent synergism between sirolimus and cyclosporine, this study tested the hypothesis that reduced-dose cyclosporine (rCsA) combined with sirolimus and corticosteroids could minimize acute rejection, as well as the nephrotoxicity associated with standard cyclosporine dosing.

Materials and methods

Study design

This comparative, open-label, randomized, 12-month trial was conducted at 49 European transplant centers between 2000 and 2002. Approval from institutional review boards of all participating centers was obtained, and all studies were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all enrolled patients.

Baseline data were collected before transplantation. Within 48 h of transplantation, all study subjects received sirolimus, sCsA, and corticosteroids. Patients were given a 6-mg loading dose of sirolimus followed by sirolimus 2 mg/day for up to 12 months. Sirolimus was administered to achieve target whole-blood trough levels of 4–12 ng/ml using high-performance liquid chromatography (HPLC) methodology. Cyclosporine levels were targeted within 125–300 ng/ml. Corticosteroids were administered in accordance with local standard of care.

At 1 month post-transplantation, patients were randomly assigned (1:1) to receive rCsA or to continue sCsA. Randomization was performed using sealed envelopes. Target cyclosporine trough levels ranged from 150 to 200 ng/ml for patients receiving sCsA and from 75 to 100 ng/ml for those receiving rCsA (Fig. 1). It was recommended that the cyclosporine dose be titrated to achieve target levels in the rCsA arm over a period of 2 months. Cyclosporine doses were adjusted using cyclosporine whole-blood trough levels measured at the centers using a monoclonal antibody technique. Sirolimus whole-blood trough levels were determined using Abbott IMx (Abbott Diagnostics, Abbott Park, IL, USA) or HPLC UV (Covance, Princeton, NJ, USA). Therapeutic drug monitoring was performed at seven study visits, and before any change in dosing, then repeated within 5–9 days after dosing change, per standardized protocols.

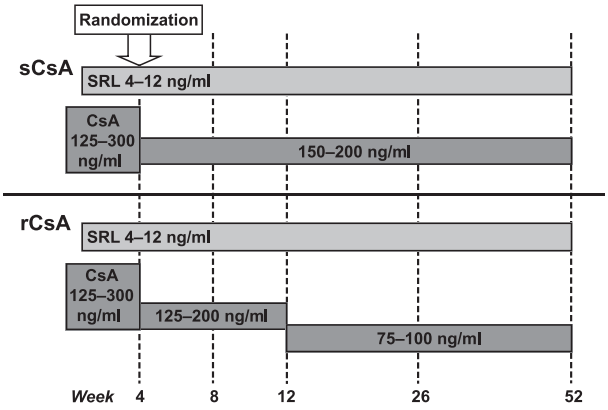

Figure 1 Target trough concentrations of treatment regimens. All targets received corticosteroids per local practice. CsA, cyclosporine; rCsA, reduced-dose CsA; and sCsA, standard-dose CsA.

Eligibility criteria

Patients older than 18 years of age with end-stage renal disease and a recipient of a primary or secondary renal allograft from a deceased, living-unrelated, or living-related mismatched donor were eligible for enrollment. Patients being retransplanted must have maintained their primary allograft for at least 6 months (with the exception of patients who lost their primary allograft within 6 months secondary to a technical/nonimmunologic complication). Women of childbearing potential were required to have a negative pregnancy test.

Patients were excluded if they had evidence of systemic or localized infection. Patients planning to use medications known to interact with sirolimus were excluded, as were those receiving multiple organ transplants. Patients at high risk of rejection were excluded (i.e., those with a panel of reactive antibodies >50%, black patients, and patients who lost their primary renal allograft within the first 6 months due to immunologically mediated rejection). The use of planned antibody induction therapy within 1 week before or at the time of the current transplant was prohibited; however, antibody therapy for treatment of acute rejection, acute tubular necrosis, or delayed graft function was permitted according to local standard of care. Concurrent use of other immunosuppressive therapies, such as tacrolimus, mycophenolate mofetil, or azathioprine, was prohibited. Patients were also excluded if their baseline/screening fasting cholesterol level was >7.8 mmol/l (>300 mg/dl) or if their triglycerides were >4.6 mmol/l (>400 mg/dl). Exclusion criteria for randomization at month 1 included Banff Grade 3 [15] acute rejection between transplantation and randomization, steroid-resistant rejection in the first month after transplantation, patients who were dialysis dependent, and those with inadequate renal function to support cyclosporine reduction, in the opinion of the investigator. Additionally, patients in whom sirolimus trough concentrations were <4 ng/ml were not eligible for randomization.

Efficacy evaluation

The two primary end points were the incidence of biopsy-confirmed acute rejection (BCAR) and renal function, as assessed by serum creatinine, at 12 months.

Secondary end points included the incidence of BCAR at 6 and 9 months after transplantation, patient and graft survival at 6 and 12 months, and renal allograft function, as assessed by serum creatinine and Nankivell-calculated creatinine clearance [16]. Additional secondary end points included incidence of presumed acute allograft rejection and repeated episodes of acute rejection, the time to first BCAR, and the severity of rejection, including the histologic grade (Banff 97 criteria [15]). Documented infection (culture, biopsy, or serologic), presumptive and opportunistic infections, histologically confirmed lymphoproliferative disease or malignancy, and premature withdrawal from study medication were also analyzed as secondary end points.

Safety assessment

Safety was monitored at scheduled office visits through physical examinations and routine laboratory studies including electrocardiography, comprehensive blood chemistries, complete blood counts, and fasting lipid profiles. All adverse events were documented, externally monitored, and grouped according to COSTART. Patients who prematurely discontinued from study medication underwent follow-up evaluations within 1 week of the date of discontinuation and at 1 month post-discontinuation.

Delayed graft function was predefined as the need for dialysis within the first 7 days post-transplantation, excluding a single dialysis during the first 24 h. All suspected episodes of acute rejection had to be biopsied employing Banff-97 criteria for analysis [15]. Clinical and laboratory criteria for performing biopsies included decreasing urinary output, fever, graft swelling, tenderness, increase in creatinine, or decreased perfusion on renal scan. Biopsies were required before therapy. Initial therapy with corticosteroids was recommended. Increased cyclosporine dose and antibody therapy were permitted for treatment of rejection; patients requiring other immunosuppressive therapy had to be withdrawn.

Pneumocystis carinii pneumonia prophylaxis was required for all patients at least during the first 6 months. Cytomegalovirus prophylaxis in patients at high risk (donor positive, recipient negative) was mandated for a minimum of 3 months after transplantation. It was also

suggested that cytomegalovirus prophylaxis be used for 3 months in all other patients as well.

Statistical analysis

The statistical analysis was based on the intent-to-treat (ITT) population (all randomized patients). For the two primary end points—BCAR rates and serum creatinine at month 12, the initial analysis plan included: (i) a comparison of standard versus reduced CsA by a test comparing results in the two randomized groups and (ii) an evaluation of dose response by a test using the CsA trough levels. However, the dose–response analyses of BCAR and serum creatinine were not performed because of resource limitations.

The comparison of standard versus reduced CsA was performed using the Wilcoxon rank-sum test for serum creatinine and Fisher's exact test for BCAR at 12 months. Severity of BCAR was compared using the Cochran–Mantel–Haenszel row mean score test. Time to first BCAR was estimated using the Kaplan–Meier method, with group differences assessed using the log-rank test. Blood levels of sirolimus and cyclosporine were analyzed via two-sample *t*-tests (adjusting for unequal variances) at each time point. Patient and graft survival at 6 and 12 months were compared using Fisher's exact test. For evaluation of serum creatinine and creatinine clearance at the final visit, last observation prior to patient withdrawal was carried forward to month 12.

Sample size was determined based on the primary null hypothesis of slope zero for the CsA dose–response relationship with graft rejection rate or change in creatinine levels against the two-sided alternative. Based on at least 200 subjects, the study yielded 80% power to detect a correlation coefficient with absolute magnitude >0.85 (analogous to a nonzero regression coefficient) at 5% significance level.

Results

Demographics

Of 420 enrolled patients, 357 were randomly assigned to receive either rCsA (n = 178) or sCsA (n = 179) and thus comprised the ITT population. Among the 63 patients who were not randomized, primary reasons for discontinuation included adverse events, graft loss, and protocol violations (Fig. 2).

Patient and donor demographics were not significantly different between treatment groups (Table 1). The majority of the study population was male (sCsA group, 69.8%; rCsA group, 65.2%) and white (sCsA group, 95.0%; rCsA group, 93.3%), with a mean age of 46.1 years in the sCsA group and 47.4 years in the rCsA group. The majority of kidneys were obtained from deceased donors (88.3% in the sCsA group; 87.6% in the rCsA treatment group), and the mean number of HLA mismatches was 3.1 and 2.8 in the sCsA and rCsA groups, respectively. The mean ischemia time was 16.7 h in the rCsA group and 16.9 h in the sCsA group.

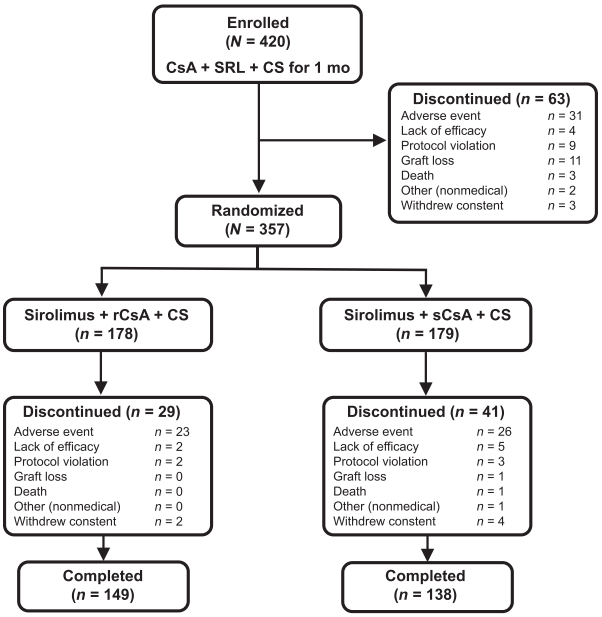

Figure 2 Study flow diagram. CS, corticosteroids; CsA, cyclosporine; rCsA, reduced-dose CsA; sCsA, standard-dose CsA; and SRL, sirolimus.

Table 1. Demographic and baseline characteristics of intent-to-treat patients.

	rCsA (n = 178)	sCsA (n = 179)
Sex male, *n* (%)	116 (65.2)	125 (69.8)
Ethnicity, *n* (%)		
White	166 (93.3)	170 (95.0)
Hispanic	6 (3.4)	4 (2.2)
Other	6 (3.4)	5 (2.8)
Mean recipient age, y ± SD	47.4 ± 13.1	46.1 ± 12.8
Mean donor age, y ± SD	46.2 ± 15.6	45.0 ± 16.7
Donor source, *n* (%)		
Deceased	156 (87.6)	158 (88.3)
Living, related	21 (11.8)	18 (10.1)
Living, unrelated	0 (0)	2 (1.1)
Mean ischemic time, h ± SD	16.7 ± 7.3	16.9 ± 7.4
Mean HLA mismatches, ±SD	2.8 ± 1.2	3.1 ± 1.3
Delayed graft function, *n* (%)	11 (6.2)	16 (8.9)
Deceased	11 (6.2)	15 (8.4)
Living, related	0 (0)	0 (0)
Living, unrelated	0 (0)	1 (0.6)
Cause of end-stage renal disease, *n* (%)		
Diabetes mellitus	6 (3.4)	6 (3.4)
Glomerulonephritis	63 (35.4)	69 (38.5)
Polycystic kidney disease	20 (11.2)	23 (12.8)
Hypertension	18 (10.1)	14 (7.8)
Interstitial disease	15 (8.4)	16 (8.9)
Other/unknown	56 (31.5)	51 (28.5)

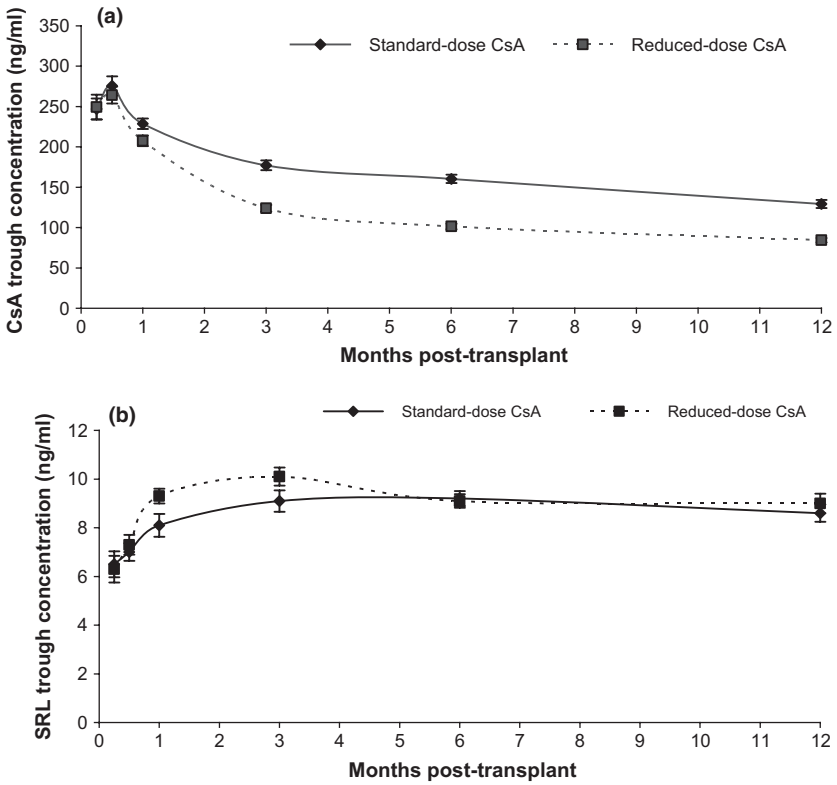

Figure 3 (a) Cyclosporine whole-blood trough levels. (b) Sirolimus whole-blood trough levels. CsA, cyclosporine; SRL, sirolimus.

During the study, 70 patients (rCsA, $n = 29$; sCsA, $n = 41$; Fig. 2) discontinued sirolimus therapy because of an adverse event ($n = 49$), lack of efficacy ($n = 7$), voluntarily withdrawal ($n = 6$), protocol violation ($n = 5$), death ($n = 1$), graft loss ($n = 1$), and other nonmedical events ($n = 1$).

In total, 27 (7.6%) patients in the ITT population—11 (6.2%) in the rCsA group and 16 (8.9%) in the sCsA group—reported delayed graft function. All cases, with the exception of one case in the sCsA treatment group, occurred in kidneys from deceased donors.

Cyclosporine and sirolimus whole-blood trough levels

Among ITT patients, the rCsA group had lower mean cyclosporine trough concentrations compared with sCsA groups (Fig. 3), which is consistent with the lower total exposure to cyclosporine. Sirolimus whole-blood trough concentrations were, on average, within 6–10 ng/ml in both groups and were not significantly different between groups, except at month 1, wherein levels in rCsA (9.3 ng/ml) patients were higher than those in sCsA (8.1 ng/ml, $P = 0.032$) patients. Consistent with these findings, overall exposure to sirolimus was not significantly different between treatment groups.

Efficacy

Primary end points

A lower rate of BCAR at 12 months in the rCsA group versus the sCsA group was observed (11.2% vs. 16.2%; P = NS; Table 2). Among patients reporting for the 12-month follow-up visit, rCsA patients had significantly lower serum creatinine than did sCsA patients ($P < 0.001$; Table 3), independent of donor type.

Secondary end points

Severity of acute rejection was significantly different between groups at 12 months, with a higher proportion of subjects in the sCsA group experiencing Grade 1A and 1B rejection (Table 2; $P = 0.018$). Antibody therapy was used to treat acute rejection in 5/20 (25%) rCsA and 6/29 (20.7%) sCsA patients ($P = 0.740$). Serum creatinine levels were significantly higher in sCsA patients at months 6, 9, and 12 as well as at the final visit (Table 3). Subanalysis of patients based on donor origin revealed significantly lower serum creatinine levels at 12 months in patients in the rCsA group independent of donor type. These observations were confirmed when analyzing renal function according to the Nankivell method.

Table 2. Patient and graft survival (at 6 and 12 months) and biopsy-confirmed acute rejection rates (at 12 months) in intent-to-treat patients.

	rCsA (n = 178)	sCsA (n = 179)	P value*
Patient survival			
6 months			
Patient alive, *n* (%)	178 (100)	178 (99.4)	NS
Patient deceased, *n* (%)	0 (0)	1 (0.6)	
12 months			
Patient alive, *n* (%)	178 (100)	177 (98.9)	NS
Patient deceased, *n* (%)	0 (0)	3† (1.7)	
Graft survival			
6 months			
Graft survival (death censored), *n* (%)	178 (100)	177 (98.9)	NS
Graft loss excluding death, *n* (%)	0 (0)	2 (1.1)	NS
Graft loss including death, *n* (%)	0 (0)	3 (1.7)	NS
12 months			
Graft survival (death censored), *n* (%)	177 (99.4)	177 (98.9)	NS
Graft loss excluding death, *n* (%)	1 (0.6)	2 (1.1)	NS
Graft loss including death, *n* (%)	1 (0.6)	4 (2.2)	NS
BCAR, *n* (%)	20 (11.2)	29 (16.2)	NS
Grade 1A‡	9 (45.0)	14 (48.3)	0.018
Grade 1B‡	3 (15.0)	9 (31.0)	
Grade 2A‡	4 (20.0)	3 (10.3)	
Grade 2B‡	3 (15.0)	2 (6.9)	
Grade 3‡	1 (5.0)	1 (3.4)	
Rejection treated with antibody therapy	5 (25.0)	6 (20.7)	NS

BCAR, biopsy-confirmed acute rejection.
*Between-group comparisons.
†One death was reported in the sCsA group after study completion.
‡Percentages were calculated based on the number of patients with a BCAR.

Table 3. Graft Function as assessed by mean serum creatinine and calculated creatinine clearance (mean ± SEM) in the intent-to-treat population at 6 and 12 months.

	rCsA (n = 178)	sCsA (n = 179)	P value*
Serum creatinine, mg/dl			
6 months			
All patients	1.79 ± 0.07	2.00 ± 0.09	0.03
N	165	163	
Living donor	1.57 ± 0.16	1.97 ± 0.23	NS
N	21	20	
Deceased donor	1.83 ± 0.08	2.01 ± 0.10	NS
N	143	143	
12 months			
All patients	1.75 ± 0.10	1.97 ± 0.07	0.0007
N	147	138	
Living donor	1.32 ± 0.08	1.96 ± 0.25	0.04
N	20	18	
Deceased donor	1.83 ± 0.12	1.98 ± 0.07	0.008
N	126	120	
Final visit (12 months or LOCF)			
All patients	1.94 ± 0.10	2.08 ± 0.08	0.0143
N	178	179	
Living donor	1.35 ± 0.08	2.10 ± 0.26	0.0169
N	21	20	
Deceased donor	2.02 ± 0.11	2.08 ± 0.08	NS
N	156	158	
Creatinine clearance, ml/min (Nankivell method)			
6 months			
All patients	55.9 ± 1.67	51.0 ± 1.67	0.04
N	158	158	
Living donor	59.2 ± 3.50	52.6 ± 5.79	NS
N	21	19	
Deceased donor	55.4 ± 1.86	50.8 ± 1.73	NS
N	136	139	
12 months			
All patients	57.8 ± 1.78	49.5 ± 2.46	0.0005
N	132	127	
Living donor	66.2 ± 3.49	51.4 ± 5.57	0.04
N	18	18	
Deceased donor	56.4 ± 1.97	49.2 ± 2.72	0.004
N	113	109	
Final visit (12 months or LOCF)			
All patients	67.5 ± 13.31	48.2 ± 2.02	0.0060
N	172	178	
Living donor	65.6 ± 3.08	49.1 ± 5.70	0.0257
N	21	20	
Deceased donor	67.9 ± 15.26	47.9 ± 2.17	0.0398
N	150	157	

LOCF, last observation carried forward.
*Between-group comparisons.

Patient and graft Survival

Overall, patient and graft survival for the total study population are provided in Table 2. In addition, it is important to note that among the 63 nonrandomized patients, 19 patients experienced graft loss and six patients died. Among ITT patients, patient survival rates in the rCsA and sCsA groups were similar at 6 (100% and 99.4%, respectively) and 12 (100% and 98.9%) months. Graft survival rates (death censored) were also similar between the rCsA and sCsA groups at 6 (100% and 98.9%, respectively) and 12 (99.4% and 98.9%) months. Three patients died, all in the sCsA group; two patients died from pneumonia and one from gastrointestinal hemorrhage.

Safety

Relevant adverse events reported during the study period are shown in Table 4. No significant differences were observed in overall rates of clinically significant adverse events and infections between treatment groups. Only

Table 4. Relevant adverse events, intent-to-treat population.

	rCsA (n = 178)	sCsA (n = 179)	All randomized (N = 357)
Treatment-emergent adverse events not related to infection, *n* (%)			
At least 1 occurring*	151 (84.8)	166 (92.7)	317 (88.8)
Occurring in >10% of all randomized patients			
Hyperlipemia	52 (29.2)	53 (29.6)	105 (29.4)
Anemia	31 (17.4)	27 (15.1)	58 (16.2)
Lymphocele	24 (13.5)	31 (17.3)	55 (15.4)
Hypercholesterolemia	29 (16.3)	26 (14.5)	55 (15.4)
Hypertension	28 (15.7)	26 (14.5)	54 (15.1)
Peripheral edema	17 (9.6)	28 (15.6)	45 (12.6)
Creatinine increased	18 (10.1)	25 (14.0)	43 (12.0)
Adverse events related to infection, *n* (%)			
At least 1 occurring	89 (50.0)	102 (57.0)	191 (53.5)
Occurring in >10% of all randomized patients			
General infection	23 (12.9)	29 (16.2)	52 (14.6)
Urinary tract infection	40 (22.5)	40 (22.3)	80 (22.4)
Clinically significant infections/adverse events			
≥1 occurring	101 (56.5)	100 (56.0)	201 (56.3)
Occurring in ≥5% of all randomized patients			
Urinary tract infection	47 (26.4)	45 (25.1)	92 (25.8)
Lymphocele	30 (16.9)	33 (18.4)	63 (17.6)
Pneumonia	10 (5.6)	16 (8.9)	26 (7.3)
Candida	11 (6.2)	17 (9.5)	28 (7.8)
Cytomegalovirus	13 (7.3)	14 (7.8)	27 (7.6)
Herpes simplex	10 (5.6)	9 (5.0)	19 (5.3)
CsA toxicity	7 (3.9)	12 (6.7)	19 (5.3)
Wound infection†	13 (7.3)	4 (2.2)	17 (4.8)

*P = 0.02, between-group comparison.
†P = 0.03, between-group comparison.

wound infections occurred significantly more often in the rCsA group compared with the sCsA group (7.3% vs. 2.2%, respectively; P = 0.03). The number of patients experiencing adverse events unrelated to infection was significantly higher in the sCsA group (92.7%) compared with the rCsA group (84.8%; P = 0.02). Overall, the most common adverse events included urinary tract infection/pyelonephritis (25.8%), lymphocele (17.6%), pneumonia (7.3%), *Candida* (7.8%), cytomegalovirus (7.6%), cyclosporine toxicity (5.3%), and wound infection (4.8%). Three malignancies were reported during the study: one lymphoma-like reaction in both groups and one renal carcinoma in the sCsA group.

Lipid Profiles

Total serum cholesterol values (±SEM) increased in both treatment groups from a baseline of 185.3 ± 4.2 mg/dl to 239.4 ± 5.8 mg/dl in rCsA patients and from 181.5 ± 4.6 mg/dl to 235.5 ± 4.6 mg/dl in sCsA patients; these levels were not significantly different between groups. Both treatment groups exhibited similar increases in very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels, and decreases in high-density lipoprotein (HDL) cholesterol levels, with no significant differences between groups.

Triglyceride levels were significantly (P = 0.02) higher at month 12 in the sCsA group (238.9 ± 13.3 mg/dl) compared with the rCsA group (203.5 ± 8.0 mg/dl).

Blood pressure

No significant differences in systolic and diastolic blood pressure were observed between treatment groups. A slight reduction in mean blood pressure was noted in both groups over the 12-month treatment period (rCsA, from 144 ± 1.8 to 137 ± 1.3 mmHg; sCsA, from 144 ± 1.6 to 137 ± 1.4 mmHg for systolic blood pressure; and rCsA, from 83 ± 1.1 to 81 ± 0.8 mmHg; sCsA, from 82 ± 1.0 to 81 ± 0.9 mmHg for diastolic blood pressure).

Laboratory tests

No significant differences were observed between treatment groups with respect to aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), lactate dehydrogenase, fasting serum glucose, hemoglobin, and serum potassium levels.

Four patients in the rCsA group and three patients in the sCsA group reported AST/SGOT levels exceeding five times normal (≥175 IU/l), and one patient in each group exhibited AST/SGOT levels exceeding 10 times normal. In both groups, 11 patients had ALT/SGPT levels in excess of five times normal; two patients in the sCsA group and four patients in the rCsA group had ALT/SGPT levels exceeding 10 times normal. A decrease in white blood cells was noted in the rCsA group at 3 months (7.2×10^9 vs. 7.8×10^9 cells/l; P = 0.01), but no significant difference was evident at 12 months. Leukopenia occurred in <5% of the total population, with no differences between groups. Platelet counts did not show meaningful differences between treatment groups.

Discussion

The results from this study demonstrated that, in this patient population, sirolimus at target trough levels from 4 to 12 ng/ml combined with reduced CsA was as effective in preventing acute rejection as it was when combined with standard CsA doses. Lower CsA exposure resulted in improved renal function and better tolerability, as evidenced by fewer discontinuations over the 1-year period.

Despite an approximate 50% reduction in targeted maintenance cyclosporine trough concentrations, overall rates of acute rejection remained low during the 12-month study and were not significantly different between groups. The BCAR rate at 12 months was even lower in rCSA patients (11.2%) compared with sCSA patients (16.2%), providing further evidence that low-dose cyclosporine

combined with low-dose sirolimus and corticosteroids may provide excellent rejection prophylaxis in patients after kidney transplantation. Additionally, patient and graft survival rates exceeded 98% at 12 months post-transplantation in this selected group of patients. Importantly, minimizing the dose of calcineurin inhibitor cyclosporine allowed for improvements in renal function without compromising efficacy. These data indicate that reducing cyclosporine levels can be achieved without increasing acute rejection rates or compromising renal allograft function, supporting data obtained from other trials [13,14].

Calcineurin inhibitor nephrotoxicity has led to newer protocols that either limit or eliminate these immunosuppressants at varying time points after transplantation. Reduced levels of these agents are advantageous when patient and graft survival as well as graft function are not compromised. This goal is achievable if levels are reduced in the presence of adequate sirolimus blood levels. This study demonstrated that, when combined with sirolimus, cyclosporine can be minimized and used for maintenance immunosuppression in renal transplant recipients. A 6-month phase 3 study by Baboolal examined cyclosporine minimization versus elimination in 133 renal allograft patients receiving sCsA, sirolimus, and steroids [17]. At 3 months after transplantation, patients were subsequently randomized to undergo cyclosporine elimination ($n = 42$) or minimization ($n = 45$). The target cyclosporine concentration in the minimization treatment group was 50–100 ng/ml and similar to our study. At 6 months, patient and graft survival were excellent, with an overall acceptable incidence of BCAR (19.5% at 6 months). In the ITT population, BCAR rates were 7.1% in the cyclosporine elimination group and 2.2% in the cyclosporine minimization group after randomization at month 1. Renal function, as reflected by calculated GFR, was significantly improved after CSA elimination (65 vs. 57 ml/min; $P = 0.027$).

In the present study, we observed slightly lower BCAR rates and significantly improved renal function in patients receiving rCsA compared with those remaining on sCsA. Long-term results from the Rapamune Maintenance Regimen trial also demonstrated significant improvements in renal function and blood pressure in patients receiving sirolimus maintenance therapy after early elimination of cyclosporine [9,18]. From this large trial, we cannot conclude which strategy is favorable but, given the low discontinuation rate in the rCsA group (29/178, 16.3%), we provide evidence that low-dose CsA in combination with sirolimus levels of around 8 ng/ml is a valid alternative treatment option.

Maintenance levels of cyclosporine used to prevent acute graft rejection vary widely across countries in Europe as well as from center to center within a given country [19]. In this study, cyclosporine maintenance levels varied from 150 to 200 ng/ml among transplantation centers. It is apparent from this study that these levels can be safely reduced at 1 month after transplantation to a range of 75–100 ng/ml in the presence of sirolimus. Further studies are needed to determine whether or not these levels can be reduced further.

The benefits of reduced cyclosporine exposure, however, need to be balanced with potential safety concerns due to the exposure to sirolimus. This study showed that reducing the dose of cyclosporine and increasing the levels of sirolimus were not associated with any meaningful differences in cholesterol levels. Both groups exhibited an increase in blood lipids (total cholesterol, VLDL, LDL, and HDL) with the only significant difference occurring with higher levels of triglycerides in the sCsA group. Sirolimus has been associated with increased levels of serum triglycerides [20,21]. This effect may have been offset by the reduction in cyclosporine, which is also known to increase blood lipid levels [22]. Reducing the maintenance level of cyclosporine in the presence of sirolimus appears to be lipid neutral, with the exception of a small positive influence on serum triglycerides levels.

Systolic and diastolic blood pressures were not significantly reduced in the rCsA group compared with the sCsA group. Any effort to affect significant changes in blood pressure would have to include eliminating the calcineurin inhibitor, as was carried out in a trial by Kreis *et al.* [18] and Oberbauer *et al.* [9]. More long-term follow-up relating to the prevention of progressive renal failure and the development of cardiovascular disease with calcineurin-inhibitor minimization or elimination strategies is of interest, and future prospective studies are needed to specifically address these important clinical issues.

The incidence of infection and other safety concerns was within the expected margins, and the incidence of renal tubular necrosis and malignancy was equally distributed between the two treatment groups, indicating that the rCsA regimen utilized in this study provides an overall acceptable safety profile.

Results from this study indicate that reducing the dose of cyclosporine in an immunosuppressive regimen including sirolimus does not increase acute rejection rates or impair renal allograft function. Reductions in the various maintenance levels of cyclosporine used throughout Europe appear to improve renal allograft function without increasing acute rejection. No negative effects on blood lipids, blood pressure, infection rates, or malignancies were observed as a consequence of reduced maintenance levels of cyclosporine.

The current study has several limitations, including the lack of an adequate control group with mycophenolate mofetil and standard CsA, the omission of IL-2R antibody induction, and the short follow-up. In addition, the study

design may be biased toward advantageous outcomes, because patients with severe rejections, delayed graft function, and problematic outcomes may not undergo randomization at week 4. It is possible that this selection strategy resulted in a rather low incidence of delayed graft function, and better overall outcomes such as GFR and graft and patient survival. Nevertheless, overall acceptable outcomes with only nine deaths (2.1%) and 22 graft losses (5.2%) were observed in the enrolled population of 420 low-risk patients.

Additionally, high drug levels of CsA were permitted in the study, which was common at the time this study was performed. Even the reduced-dose CsA regimen in this study (75–100 ng/ml) utilized higher doses compared with doses used in clinical practice today, limiting conclusions on the long-term nephrotoxic potential of low-dose CsA with sirolimus. Although difficult to compare, renal function results for patients receiving low-dose CsA and sirolimus in the current study were inferior to results observed in the Symphony study with low-dose CsA alone [23] and in several other studies utilizing standard CsA-based regimens [24–26]. In addition, a retrospective analysis demonstrated inferior outcomes with sirolimus and standard doses of CsA, suggesting increased nephrotoxicity with this combination [27].

However, short-term results of prospective randomized trials suggest that the combination of mTOR inhibitors with low-dose CsA provides efficacy similar to that of patients receiving CsA and mycophenolic acid in *de novo* low-to-moderate immunological risk kidney transplant recipients. For instance, Tedesco-Silva *et al.* demonstrated similar efficacy and renal function in *de novo* transplant recipients with mycophenolic acid and standard CsA compared with everolimus and reduced-dose CsA (<100 ng/ml). However, these data must be interpreted with caution, as lower CsA doses may be nephrotoxic as well. Furthermore, due to the limitations inherent to open-label trials, reported rates of renal AEs (including increased serum creatinine levels and CsA toxicity) must also be interpreted with caution.

Because of the delay in publication (owing to a variety of reasons) and limited resources, the proper analysis of the initially planned primary end point was not possible. Nevertheless, in light of the responsibilities to the study participants and the recently published data by Tedesco-Silva *et al.*, we felt responsible for finally publishing the results. Although we would not recommend the doses utilized in the current study for today's practice, as drug levels have decreased over the years, we feel that this study highlights that sirolimus together with low-dose CsA is a valid alternative treatment, especially for those patients who do not tolerate MPA or who have significant problems (e.g., virology or tumors) with today's standard of care regimen. Further, despite the limitations, this study was, among other things, instrumental in providing the first safety data on lower CsA doses. It remains unclear whether patients who receive kidneys from elderly donors might benefit from such a low-dose CNI regimen, or whether the adverse long-term consequences outweigh the short-term benefits.

The results of this study suggest that cyclosporine may be safely reduced in a sirolimus-based treatment regimen. In this study, minimizing cyclosporine exposure in an immunosuppressive regimen with sirolimus and corticosteroids in *de novo* renal allograft recipients resulted in excellent 12-month patient and graft survival, was associated with a low incidence of acute rejection, and achieved these outcomes with an acceptable safety profile.

Authorship

FM, H-HN, DDC, SS, AJZ, and KB contributed to the conception and design, provision of study materials and/or patients, data analysis and interpretation, and manuscript development, and provided final approval of the manuscript. FM, H-HN, DC, SS, and KB were study investigators and contributed to the collection and assembly of data.

Funding

This research was sponsored by Wyeth Pharmaceuticals, which was acquired by Pfizer in October 2009.

Acknowledgements

Medical writing and editorial support were provided by Bina J. Patel, PharmD, of Peloton Advantage (Parsippany, NJ) and were funded by Pfizer.

References

1. Andoh TF, Burdmann EA, Bennett WM. Nephrotoxicity of immunosuppressive drugs: experimental and clinical observations. *Semin Nephrol* 1997; **17**: 34.
2. Manez R, Jain A, Marino IR, *et al.* Comparative evaluation of tacrolimus and cyclosporine as immunosuppressive agents. *Transplant Rev* 1995; **9**: 63.
3. Bennett WM. Insights into chronic cyclosporine nephrotoxicity. *Int J Clin Pharmacol Ther* 1996; **34**: 515.
4. Sehgal SN. Rapamune (sirolimus, rapamycin): an overview and mechanism of action. *Ther Drug Monit* 1995; **17**: 660.
5. Terada N, Lucas JJ, Szepesi A, Franklin RA, Domenico J, Gelfand EW. Rapamycin blocks cell cycle progression of activated T cells prior to events characteristic of middle to late G1 phase of the cycle. *J Cell Physiol* 1993; **154**: 7.

6. MacDonald AS, the Rapamune Global Study Group. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 2001; **71**: 271.
7. Kahan BD. for the Rapamune US Study Group: efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. *Lancet* 2000; **356**: 194.
8. Johnson RW, Kreis H, Oberbauer R, Brattstrom C, Claesson K, Eris J. Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. *Transplantation* 2001; **72**: 777.
9. Oberbauer R, Segoloni G, Campistol JM, *et al.* Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. *Transplant Int* 2005; **18**: 22.
10. Mota A, Arias M, Taskinen EI, *et al.* Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years. *Am J Transplant* 2004; **4**: 953.
11. Hariharan S, McBride MA, Cohen EP. Evolution of endpoints for renal transplant outcome. *Am J Transplant* 2003; **3**: 933.
12. Hariharan S, Kasiske B, Matas A, *et al.* Surrogate markers for long-term renal allograft survival. *Am J Transplant* 2004; **4**: 1179.
13. Tedesco-Silva H, Garcia VD, Contieri FL, *et al.* Comparison of the safety and efficacy of cyclosporine elimination in *de novo* renal allograft patients receiving sirolimus. *Transplant Proc* 2010; **42**: 1659.
14. Kahan BD, Benavides C, Schoenberg L, Podbielski J, Green C. Addition of induction therapy offsets the hazard of marked calcineurin minimization among renal transplant recipients treated with sirolimus. *Clin Nephrol* 2010; **73**: 344.
15. Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713.
16. Nankivell BJ, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1995; **59**: 1683.
17. Baboolal K. A phase III prospective, randomized study to evaluate concentration-controlled sirolimus (Rapamune) with cyclosporine dose minimization or elimination at six months in *de novo* renal allograft recipients. *Transplantation* 2003; **75**: 1404.
18. Kreis H, Oberbauer R, Campistol JM, *et al.* Long-term benefits with sirolimus-based therapy following early cyclosporine withdrawal. *J Am Soc Nephrol* 2004; **15**: 809.
19. Touchard G, Hauet T, Cogny Van Weydevelt F, *et al*. Maintenance cyclosporin monotherapy after renal transplantation—clinical predictors of long-term outcome. *Nephrol Dial Transplant* 1997; **12**: 1956.
20. Groth CG, Backman L, Morales JM, *et al.* Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation* 1999; **67**: 1036.
21. Kreis H, Cisterne JM, Land W, *et al.* Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 2000; **69**: 1252.
22. Aalamian Z. Reducing adverse effects of immunosuppressive agents in kidney transplant recipients. *Prog Transplant* 2001; **11**: 271.
23. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
24. Weir MR, Mulgaonkar S, Chan L, *et al.* Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. *Kidney Int* 2011; **79**: 897.
25. Lebranchu Y, Thierry A, Toupance O, *et al.* Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant* 2009; **9**: 1115.
26. Budde K, Becker T, Arns W, *et al.* Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 2011; **377**: 837.
27. Meier-Kriesche HU, Steffen BJ, Chu AH, *et al.* Sirolimus with neoral versus mycophenolate mofetil with neoral is associated with decreased renal allograft survival. *Am J Transplant* 2004; **4**: 2058.

Appendix

The following investigators participated in this trial as part of the European Rapamune Cyclosporine Minimization Study Group: Daniel Abramowicz (ULB Hôpital Erasme, Brussels, Belgium); Alberto Albertazzi (Azienda Policlinico di Modena, Modena, Italy); Antonis Antoniadis MD (Surgical Transplantation Clinic, "Ippokratio" General Hospital of Thessaloniki, Thessaloniki, Greece); Wolfgang Arns (Kliniken der Stadt Köln Krankenhaus Köln-Merheim, Köln, Germany); Klemens Budde (Charité Universitätsmedizin, Berlin, Germany); Gilberto Calconi (Ospedale "Cà Foncello", Divisione di Nefrologia e Dialisi, Treviso, Italy); Carlo Casciani (HOSPITAL "S. Eugenio", Rome, Italy); Giovanni Civati (Niguarda Cà Granda,Milan, Italy); Stephan Conrad (Universitätskrankenhaus Eppendorf, Hamburg, Germany); Domingo Del Castillo (Hospital Reina Sofía, Córdoba, Spain); Luigi Gastaldi (Azienda Ospedaliera Macchi, Varese, Italy); Manuel González-Molina (Hospital Carlos Haya, Málaga, Spain); Evangelos Hadzigianakis (1st Surgical Clinic, "Evangelismos" General Hospital of Athens, Athens, Greece); Herwig Holzer (University of Graz, Graz, Austria); Michele Kessler (Hôpital Brabois, Vandoeuvre les Nancy, France); Günter Kirste (Klinikum der Albert-Ludwigs-Universität, Freiburg,

Germany); Franciszek Kokot (Klinic of Nephrology and Transplantation, Katowice, Poland); Guiseppe La Greca (Ospedale Civile, Vicenza, Italy); Alkiviadis Kostakis (Renal Transplantation Unit, "Laiko" General Hospital of Athens, Athens, Greece); Ali Al Lehbi (King Faisal Specialist Hospital–KFSH&RC, Riyadh, Kingdom of Saudi Arabia); Josep Lloveras (Hospital del Mar, Barcelona, Spain); Benito Maceira (Hospital Universitario de Canarias, Sta. Cruz de Tenerife); Rosario Maiorca (Azienda Ospedaliera Spedali Civili, Brescia, Italy); Ivo Matl (Institute for Clinical and Experimental Medicine, Prague, Czech Republic); Ferdinand Müehlbacher (University of Vienna, Vienna, Austria); Pavel Navrátil (Faculty Hospital, Transplant Center, Hradec Kralove, Czech Republic); Hans-Hellmut Neumayer (Universitätsklinikum Charité Campus Mitte, Berlin, Germany); Leszek Paczek (Transplantation Institute, Warsaw, Poland); Luis Pallardó (Hospital Dr. Peset, Valencia, Spain); Dr Porfirio Pereira (Hospital Virgen del Rocío, Sevilla, Spain); Ferenc Perner (Semmelweis Medical University, Budapest, Hungary); José Reimão Pinto (Unidade de Transplante, Serviço de Nefrologia do Hospital Curry Cabral, Lisbon, Portugal); Manuel Rengel (Hospital Gregorio Marañón, Madrid, Spain); Manuel Ribero (Hospital Puerta del Mar, Cádiz, Spain); B Ringe (Universitätsklinikum Göttingen, Göttingen, Germany); Gaetano Rizzo (Azienda Ospedaliera Pisana, Pisa, Italy); Maurizio Salvadori (Az. Ospedaliera "Careggi", Firenze, Italy); Ricardo Solá (Fundación Puigvert, Barcelona, Spain); Vito Sparacino (Ospedale Civico - Unità Medica di Trapianto Renale, Palermo, Italy); Sergio Stefoni (Azienda ospedaliera S. Orsola Malpighi, Bologna, Italy); Tegzess (The Netherlands); Fernando Teixeira e Costa (Unidade de Transplante, Serviço de Nefrologia do Hospital Curry Cabral, Lisbon, Portugal); Zbigniew Wlodarczyk (Department of Transplantation, Szpital Wojewodzki, Poznan, Poland); Prof Wolf (Hôpital de Hautepierre, Strasbourg, France); Sofía Zarraga (Hospital de Cruces, Bilbao, Spain).

