American Journal of Transplantation 2004; 4: 626–635
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2004.00389.x

Everolimus with Optimized Cyclosporine Dosing in Renal Transplant Recipients: 6-Month Safety and Efficacy Results of Two Randomized Studies

Stefan Vitko[a,*,†], Helio Tedesco[b,†], Josette Eris[c], Julio Pascual[d], John Whelchel[e], John C. Magee[f], Scott Campbell[g], Giovanni Civati[h], Bernard Bourbigot[i], Gentil Alves Filho[j], John Leone[k], Valter Duro Garcia[l], Paolo Rigotti[m], Ronaldo Esmeraldo[n], Vincenzo Cambi[o], Tomas Haas[p], Annette Jappe[p], Peter Bernhardt[p], Johanna Geissler[p] and Nathalie Cretin[p]

[a] *Transplant Centre IKEM, Prague, Czech Republic*
[b] *Division of Nephrology, Hospital do Rim e Hipertensao, Sao Paolo, Brazil*
[c] *Royal Prince Alfred Hospital, Sydney, Australia*
[d] *Servicio de Nefrologia, Hospital Ramon y Cajal, Madrid, Spain*
[e] *Organ Transplant Services, Piedmont Hospital, Atlanta, Georgia, USA*
[f] *Division of Transplant Surgery, University of Michigan Medical Center, Ann Arbor, MI, USA,*
[g] *Princess Alexandra Hospital, Brisbane, Queensland, Australia*
[h] *Divisione Nefrologia e Dialisi, Az. Osp. Niguarda Ca' Granda, Milano, Italy*
[i] *Service de Nephrologie, CHU, Cavalla Blanche, Brest, France*
[j] *Hospital das Clínicas – UNICAMP, Campinas, Sp, Brazil*
[k] *Lifelink Transplant Institute, Tampa General Hospital, Tampa, Florida, USA*
[l] *Santa Casa de Misericóridia de Porto Alegre, RS, Brazil*
[m] *Department of Medical and Surgical Sciences, Policlinico Hospital, Padua, Italy*
[n] *Hospital Geral de Fortaleza – Setor de Transplante Renal, Fortaleza, Ceará, Brazil*
[o] *Department of Medicine and Nephrology, Maggiore Hospital, Parma, Italy*
[p] *Business Unit Transplantation, Novartis Pharma AG, Basel, Switzerland*
[†] *To be listed as joint first authors.* [*] *Corresponding author: Stefan Vitko, stefan.vitko@medicon.cz*

Two prospective, randomized studies evaluated everolimus 1.5 vs. 3 mg/day with steroids and low-exposure cyclosporine (CsA) (C_2 monitoring) in *de novo* renal transplant patients. Everolimus dosing was adjusted to maintain a minimum trough level of 3 ng/mL. Study 1 (A2306; n = 237) had no induction therapy; in Study 2 (A2307; n = 256) basiliximab was administered (Days 0 and 4). The primary endpoint was renal function at 6 months. CsA C_2 target levels, initially 1200 ng/mL in Study 1 and 600 ng/mL in Study 2, were tapered over time post-transplant. Median creatinine levels in Study 1 were 133 and 132 μmol/L at 6 months in the 1.5 and 3 mg/day groups, respectively, and 130 μmol/L in both groups in Study 2. Biopsy-proven acute rejection (BPAR) occurred in 25.0% and 15.2% of patients in the 1.5 and 3 mg/day groups in Study 1, and 13.7% and 15.1% in Study 2. Incidence of BPAR was significantly higher in patients with an everolimus trough < 3 ng/mL. There were no significant between-group differences in the composite endpoint of BPAR, graft loss or death, nor any significant between-group differences in adverse events in either study. Concentration-controlled everolimus with low-exposure CsA provided effective protection against rejection with good renal function.

Key words: Cyclosporine, everolimus, immunosuppressive regimen, renal function, therapeutic drug monitoring, transplantation

Received 4 November 2003, revised and accepted for publication 15 December 2003

Introduction

With 1-year renal graft survival rates now exceeding 90% (1), the new clinical challenge is to develop immunosuppressive regimens that minimize the risk of long-term graft loss while preserving current low rates of acute rejection. Renal function is thought to be a predictor of long-term renal allograft survival (2), and the introduction of new immunosuppressive drugs offers the opportunity to assess regimens that may reduce renal toxicity without impairing efficacy. In view of the known nephrotoxic potential of calcineurin inhibitors (CNIs), CNI-sparing regimens are an attractive option. Proliferation signal inhibitors are potent immunosuppressants that appear to be non-nephrotoxic (3), suggesting that use of a proliferation signal inhibitor with a CNI may permit CNI dose reduction without loss of immunosuppressive potency or increased renal toxicity, and thus provide a valuable therapeutic option.

The novel immunosuppressant everolimus (Certican®, RAD, Novartis Pharma AG, Basel, Switzerland) inhibits the T-lymphocyte proliferative response to cytokine

signals (4), thus complementing the inhibitory effect of cyclosporine (CsA) on T-cell-dependent growth factors such as interleukin (IL)-2 (5,6). *In vitro* and preclinical evidence has demonstrated that everolimus enhances the immunosuppressive action of CsA-based regimens (7–10), and Phase III trials in which everolimus was used in combination with full-dose CsA have shown equivalent efficacy to mycophenolate mofetil (MMF) (11,12).

An open-label pilot study conducted in 111 de novo renal transplant patients receiving everolimus compared outcomes using full-dose or low-dose CsA (13). In patients receiving low-dose CsA, the incidence of biopsy-proven acute rejection (BPAR) at 12 months was 7%. Importantly, serum creatinine levels were consistently lower than in the full-dose CsA group, suggesting that use of a proliferation signal inhibitor with low-dose CsA may be an effective immunosuppressive strategy.

In the light of the findings from this pilot study, two prospective, multicenter, randomized studies were set up to evaluate the efficacy and safety of two doses of everolimus (1.5 mg/day vs. 3 mg/day) in combination with low-exposure CsA and corticosteroids in *de novo* renal transplant recipients. Given the equivalent efficacy demonstrated vs. MMF in Phase III trials, no comparator arm was included. An open-label design was adopted as therapeutic drug monitoring required investigators to adjust the dose of everolimus.

The two studies were undertaken concurrently, with similar protocols other than variations in CsA exposure levels and use of an IL-2 receptor antagonist in one of the trials. The methodologies and results of the two trials, A2306 (Study 1) and A2307 (Study 2) are presented here alongside one another.

Materials and Methods

Study design

Two prospective, parallel-group, open-label studies were undertaken to compare the safety and efficacy of two doses of everolimus used in combination with optimized CsA administration and corticosteroids. The designs were based on previous trials of everolimus in combination with CsA (11–13). In Study 1 [based on two Phase III studies with full-dose CsA comparing everolimus with MMF (11,12)], no induction therapy was employed. In Study 2 [based on a pilot Phase II study with everolimus in combination with an IL-2 receptor antagonist comparing full- and reduced-dose CsA (13)], the IL-2 receptor antagonist, basiliximab, was administered and CsA exposure targets were, accordingly, lower than in Study 1 (see 'Immunosuppression' below). In all other respects the two studies followed similar protocols. Local medical ethics committees approved the protocols and the studies were performed in accordance with the Declaration of Helsinki and US Food and Drug Administration guidelines for good clinical practice.

Inclusion and exclusion criteria

Adult male or nonpregnant female patients undergoing primary cadaveric, living-unrelated or human leukocyte antigen-mismatched living-related donor kidney transplantation were enrolled and received treatment for up to 1 year. In Study 1, but not in Study 2, patients had to have a functional graft within 24 h. All patients gave written informed consent.

Immunosuppression

Patients were randomized to either 1.5 mg/day or 3 mg/day everolimus, and treatment with all agents was initiated, within 24 h of transplantation. All nonblack patients were randomized to receive 1.5 or 3 mg/day everolimus. As black patients have a higher everolimus clearance rate than Caucasian patients (14), all black subjects received 3 mg/day everolimus to minimize the risk of graft loss. Everolimus was administered twice daily at 12-hourly intervals, simultaneously with CsA, at either 0.75 mg b.i.d. or 1.5 mg b.i.d. Everolimus trough concentrations were measured on Days 5, 7, 14 and 28 and at Months 2, 3, 4 and 6. A minimum target trough level of 3 ng/mL was adopted based on the results of a previous exposure-response analysis (15). Dose was adjusted by 0.5 mg or 0.75 mg b.i.d. if trough concentration was less than 3 ng/mL, and trough concentration was measured 5 days after dose adjustment to ensure the target was achieved. The dose was reduced if patients could not tolerate full-dose everolimus and discontinued if necessary.

CsA (Neoral®, Novartis Pharma AG, Basel, Switzerland) was given twice daily in equal divided doses at 12-h intervals, at an initial dose of 8 mg/kg/day in Study 1 and 4 mg/kg/day in Study 2. Adjustment of CsA dose to target levels, optimized on the basis of pharmacokinetic and pharmacodynamic, efficacy and safety data from previous studies (11–13), was achieved through monitoring of CsA concentration 2 h after dosing (C_2). C_2 is a superior marker of CsA exposure and hence predictor of acute rejection than trough concentration (16–18). Blood CsA (C_2) was measured in whole blood taken 2 h (±10 min) after the morning dose on Days 3, 5, 7, 14 and 28 and at Months 2, 3, 4 and 6. CsA dose was adjusted from Day 3 to target C_2 ranges that were tapered over time post-transplant. In Study 1, target C_2 was 1200 ng/mL (range 1000–1400 ng/mL) for Weeks 0–4; 800 ng/mL (range 700–900 ng/mL) for Weeks 5–8; 600 ng/mL (range 550–650 ng/mL) for Weeks 9–12; and 400 ng/mL (range 350–450 ng/mL) for Months 4–12. In Study 2, in which patients also received basiliximab, target C_2 was set lower: 600 ng/mL (500–700 ng/mL) for Weeks 0–8 and 400 ng/mL (range 350–450 ng/mL) from Week 9 to Month 12. CsA exposure could be reduced in the presence of delayed graft function, if patients received antibodies for steroid-resistant rejection episodes or vascular rejection, or for drug-induced kidney dysfunction. To provide additional information, CsA trough concentrations were measured in samples taken immediately before the morning dose on Days 5,7,14 and 28, and at study visits for Months 2, 3, 4 and 6; these values were not used to adjust CsA dose.

Intravenous corticosteroid was given according to local practice. Oral prednisone was initiated on Day 1 at a minimum dose of 20 mg/day and continued for at least 12 months. The dose was reduced according to local practice to a minimum of 5 mg/day. In Study 2, basiliximab was given according to the standard dose regimen, 20 mg on Day 0 (within 2 h before transplantation) and Day 4 as an i.v. bolus.

Primary endpoint

The primary endpoint was renal function, measured by calculated glomerular filtration rate (GFR) (Nankivell formula) (19) and calculated creatinine clearance (Cockroft-Gault) (20) or serum creatinine at 6 months.

Secondary endpoints

Efficacy endpoints included the incidence of efficacy failure (first occurrence of either BPAR, graft loss, death or lost to follow-up), graft loss, death and BPAR. All suspected acute rejection episodes were recorded. A graft core biopsy performed within 48 h of suspected rejection was graded according to the 1997 Banff criteria (21).

All adverse events, infections and serious adverse events were recorded. Laboratory values were determined on Days 7, 14 and 28 and Months 2, 3, 4 and 6 and all data assessed in a central laboratory.

Statistical methods

Data were analyzed separately for each study. All summary statistics are presented by treatment group. All statistical tests were two-sided and used the

0.05 level of significance. Safety and tolerability analyses were performed on the safety populations in each study, defined as all patients who received at least one dose of study medication and underwent at least one safety assessment. Safety evaluations were made to Day 225.

Renal function data to Month 6 were analysed using all data, including those observed after discontinuation of study medication [intent-to-treat (ITT) analysis], and were compared between treatment groups using a two-sided Wilcoxon rank-sum test. Patients with graft loss were excluded from the renal function analyses from the day on which the graft was lost.

Efficacy analyses were conducted on the ITT population using all efficacy data to Day 194. Patients were considered lost to follow-up if they had not experienced BPAR, graft loss or death and their last contact was on Day 154 or before. Comparisons between treatment groups of the proportion of patients experiencing composite efficacy failure and its individual components were made using Fisher's exact test. There was no p-value adjustment for multiple analyses.

Cox regression was used to model the effect of everolimus and CsA exposure on BPAR. The exposure was expressed as geometric mean until BPAR or censoring at Day 225.

Results

This paper reports separately the 6-month results from the two 12-month studies.

Table 1: Patient demographics and baseline characteristics

	Study 1 (without basiliximab)		Study 2 (with basiliximab)	
	Everolimus 1.5 mg/day (n = 112)	Everolimus 3 mg/day (n = 125)	Everolimus 1.5 mg/day (n = 117)	Everolimus 3 mg/day (n = 139)
Mean age ± SD (years) [range]	42.5 ± 12.3 [19–67]	42.8 ± 12.8 [19–67]	43.9 ±12.7 [18–68]	46.3 ± 11.8 [19–71]
Gender (% male)	70 (62.5%)	67 (53.6%)	81 (69.2%)	87 (62.6%)
Ethnicity				
Caucasian	88 (78.6%)	83 (66.4%)	106 (90.6%)	116 (83.5%)
Black	0	15 (12.0%)	0	13 (9.4%)
Hispanic	13 (11.6%)	14 (11.2%)	4 (3.4%)	4 (2.9%)
Oriental	0	5 (4.0%)	4 (3.4%)	3 (2.2%)
Other	11 (9.8%)	8 (6.4%)	3 (2.6%)	3 (2.2%)
BMI	24.2 ± 4.1	25.0 ± 4.7	25.3 ± 4.3	25.6 ± 5.0
Primary cause of end-stage renal disease				
Glomerular disease	30 (26.8%)	38 (30.4%)	32 (27.4%)	41 (29.5%)
Polycystic disease	16 (14.3%)	15 (12.0%)	14 (12.0%)	23 (16.5%)
Hypertension/ nephrosclerosis	12 (10.7%)	21 (16.8%)	4 (3.4%)	12 (8.6%)
Diabetes mellitus	6 (5.4%)	7 (5.6%)	10 (8.5%)	15 (10.8%)
Unknown	24 (21.4%)	19 (15.2%)	19 (16.2%)	10 (7.2%)
Other	13 (11.6%)	15 (12.0%)	24 (20.5%)	22 (15.8%)
Cadaveric donor	67 (59.8%)	82 (65.6%)	79 (67.5%)	107 (77.0%)
Patients with DGF	16 (14.3%)	21 (16.8%)	23 (19.7%)	28 (20.1%)
Mean HLA mismatches				
<3	27 (24.1%)	30 (24.0%)	22 (33.8%)	14 (20.6%)
≥3	84 (75.0%)	93 (74.4%)	43 (66.2%)	53 (77.9%)
Unknown	1 (0.9%)	2 (1.6%)	0	1 (1.5%)
%Patients with panel reactive antibodies >10%	10.8 (n = 93)	5.6 (n = 106)	13.7 (n = 111)	12.3 (n = 133)
Mean cold ischemia time ± SD (hours)				
Cadaveric donor	16.5 ± 5.8	17.6 ± 6.2	16.4 ± 6.5	16.3 ± 6.1
Living donor	1.4 ± 1.4	1.6 ± 2.1	1.3 ± 1.0	1.3 ± 1.3
Mean donor age ± SD (years)	42.4 ± 12.7	40.9 ± 13.9	40.6 ± 13.5	37.9 ± 14.2

DGF: delayed graft function.

Patient populations

All patients were included in the ITT and safety populations. Baseline demographics and background characteristics are shown in Table 1. There were no significant differences between the 1.5 and 3 mg/day treatment groups in either study, other than the inclusion of all black patients in the 3 mg/day everolimus cohorts (see Materials and Methods). Despite a functioning graft being an inclusion criterion in Study 1, 14% and 17% of patients in each treatment arm had delayed graft function.

Immunosuppression

Overall mean average daily doses of everolimus, including days without medication, were 1.8 and 2.6 mg in Study 1, and 2.2 and 3.0 mg in Study 2, in the 1.5 and 3 mg/day treatment groups, respectively (Table 2). The incidence of everolimus trough levels <3 ng/mL was significantly higher in the 1.5 mg/day group than the 3 mg/day group in both studies at Day 7 (Study 1: 21% vs. 2%, $p < 0.001$; Study 2: 42% vs. 9%, $p < 0.001$) and at Day 14 (Study 1: 23% vs. 6%, $p < 0.001$; Study 2: 34% vs. 15%, $p = 0.001$). At Month 6, few patients in either the 1.5 or 3 mg/day groups had trough concentrations of less than 3 ng/mL (Study 1: 3% and 2%; Study 2: 2% and 4%, respectively).

Overall mean average daily CsA dose was similar between the everolimus 1.5 and 3 mg/day treatment groups within each study (Study 1: 3.7 mg/kg vs. 3.4 mg/kg; Study 2: 2.5 mg/kg in both arms). Mean C_2 decreased over time, but were slightly above target after Week 4 in Study 1, remaining above target to Month 6. In Study 2, mean C_2 values slightly exceeded target range at all time points (Table 3). The overall mean daily dose of corticosteroids was the same in both treatment groups in each study (0.4 mg/kg). In Study 2, all patients received their first dose of basiliximab, and only four patients did not receive their second dose (two in each group).

Renal function

Renal function was good at Month 6 in patients receiving either everolimus 1.5 or 3 mg/day in both studies, as measured by calculated GFR or serum creatinine (Table 4). In Study 1, median calculated GFR values were 65 mL/min and 62 mL/min in the 1.5 and 3 mg/day groups, respectively (median serum creatinine 133 and 132 μmol/L); in Study 2 these values were 66 mL/min and 67 mL/min in (median serum creatinine 130 μmol/L in both treatment groups).

Serum creatinine concentration in Study 1 was ≤132 μmol/L (1.5 mg/dL) at Month 6 in 49% and 50% of patients in the 1.5 and 3 mg/day treatment groups, respectively, and in 53% and 58% of those in Study 2. Serum creatinine was ≤185 μmol/L (2.1 mg/dL) at Month 6 in 88% and 85% of patients in the 1.5 and 3 mg/day treatment groups in Study 1, respectively, and in 89% and 87% of patients in Study 2 (Table 4).

Efficacy

The incidence of death or graft loss was low in all patient groups (Table 5). Seven patients in Study 1 lost their graft due to acute rejection (n = 2), chronic rejection (n = 1), infection (n = 1), infarcted kidney (n = 1), renal artery thrombosis (n = 1) or other reasons (n = 1). In Study 2, nine grafts were lost; causes included urologic complications, acute rejection, infarcted kidney, malignancy in the allograft, primary nonfunction, technical failure and other reasons (sepsis and micotic aneurysm).

There were no significant differences in either study between the everolimus 1.5 and 3 mg/day groups for any efficacy parameter (Table 5), although there was a nonsignificant trend to reduced incidence of BPAR in Study 1 among patients in the 3 mg/day cohort compared with the 1.5 mg/day group ($p = 0.073$). Most cases of BPAR were mild or moderate in severity; only four cases of BPAR were Grade III in Study 1 (two in each treatment group) and two cases in Study 2 (both in the 3 mg/day group). In the 3 mg/day groups the proportion of patients experiencing BPAR was similar in the two studies, as was the proportion of patients in whom the first episode of BPAR occurred by day 14 post-transplant. In the 1.5 mg/day groups, more patients experienced BPAR in Study 1 than Study 2

Table 2: Everolimus dose and trough concentrations by visit window (mean ± standard deviation)

	Everolimus dose and trough concentrations							
	Study 1				Study 2			
	1.5 mg/day Everolimus (n = 112)		3 mg/day Everolimus (n = 125)		1.5 mg/day Everolimus (n = 117)		3 mg/day Everolimus (n = 139)	
Visit	Everolimus dose, mg/day	Everolimus C_0, ng/mL	Everolimus dose, mg/day	Everolimus C_0, ng/mL	Everolimus dose, mg/day	Everolimus C_0, ng/mL	Everolimus dose, mg/day	Everolimus C_0, ng/mL
Day 28	1.8 ± 0.8 (n = 106)	5.0 ± 2.2 (n = 102)	2.6 ± 0.7 (n = 119)	7.6 ± 4.4 (n = 113)	2.2 ± 0.9 (n = 112)	5.3 ± 2.0 (n = 109)	3.0 ± 0.9 (n = 133)	6.6 ± 3.2 (n = 128)
Month 3	1.8 ± 0.6 (n = 96)	5.4 ± 2.8 (n = 91)	2.5 ± 0.7 (n = 107)	7.5 ± 3.5 (n = 103)	2.4 ± 1.0 (n = 105)	6.3 ± 2.5 (n = 97)	3.0 ± 1.1 (n = 125)	7.9 ± 3.6 (n = 119)
Month 6	1.8 ± 0.6 (n = 86)	5.2 ± 1.8 (n = 82)	2.5 ± 0.7 (n = 103)	7.3 ± 3.0 (n = 98)	2.4 ± 0.9 (n = 99)	6.5 ± 3.8 (n = 97)	3.0 ± 0.9 (n = 118)	7.6 ± 3.2 (n = 118)

Table 3: Cyclosporine (CsA) target C_2 range and dose, trough (C_0), and peak (C_2) concentrations by visit window (mean ± standard deviation)

Target C_2 (range), ng/mL	Visit	Study 1: 1.5 mg/day Everolimus ($n = 112$) CsA dose, mg/kg	C_2, ng/mL	C_0, ng/mL	Study 1: 3 mg/day Everolimus ($n = 125$) CsA dose, mg/kg	C_2, ng/mL	C_0, ng/mL
Weeks 0–4 1200 (1000–1400)	Day 28	4.7 ± 1.7 (n = 106)	1121.2 ± 358.7 (n = 103)	239.0 ± 134.2 (n = 101)	4.6 ± 1.9 (n = 119)	1166.5 ± 410.0 (n = 119)	278.3 ± 206.8 (n = 110)
Weeks 5–8 800 (700–900)	Month 2	3.4 ± 1.4 (n = 99)	902.7 ± 400.7 (n = 98)	172.9 ± 119.0 (n = 93)	3.3 ± 1.2 (n = 112)	855.2 ± 347.2 (n = 112)	176.8 ± 108.6 (n = 111)
Weeks 9–12 600 (550–650)	Month 3	2.8 ± 1.1 (n = 96)	685.1 ± 283.9 (n = 95)	131.3 ± 85.3 (n = 91)	2.6 ± 1.0 (n = 107)	754.1 ± 362.0 (n = 103)	140.3 ± 99.1 (n = 101)
Month 4–end 400 (350–450)	Month 4	2.4 ± 0.9 (n = 91)	595.0 ± 258.3 (n = 89)	98.9 ± 58.8 (n = 84)	2.1 ± 0.8 (n = 105)	582.6 ± 298.2 (n = 102)	101.0 ± 78.6 (n = 99)
	Month 6	2.1 ± 0.7 (n = 86)	533.6 ± 264.8 (n = 84)	81.7 ± 59.5 (n = 80)	1.9 ± 0.7 (n = 103)	544.7 ± 318.1 (n = 99)	83.1 ± 67.0 (n = 96)
		Study 2: 1.5 mg/day Everolimus ($n = 112$)			Study 2: 3 mg/day Everolimus ($n = 125$)		
Weeks 0–8 600 (500–700)	Day 28	3.0 ± 1.0 (n = 112)	699.0 ± 265.4 (n = 111)	140.9 ± 127.4 (n = 106)	3.0 ± 1.2 (n = 133)	705.9 ± 280.5 (n = 128)	136.4 ± 121.3 (n = 128)
	Month 2	2.6 ± 0.9 (n = 110)	648.1 ± 247.2 (n = 107)	115.7 ± 89.7 (n = 105)	2.6 ± 1.2 (n = 127)	627.0 ± 276.3 (n = 124)	120.7 ± 92.3 (n = 122)
Week 9–study end 400 (350–450)	Month 3	2.3 ± 0.9 (n = 105)	555.2 ± 228.8 (n = 100)	86.7 ± 46.9 (n = 97)	2.2 ± 1.1 (n = 125)	557.5 ± 283.3 (n = 120)	95.4 ± 79.3 (n = 116)
	Month 4	2.1 ± 0.9 (n = 101)	507.0 ± 191.4 (n = 100)	72.3 ± 33.9 (n = 97)	2.1 ± 0.9 (n = 121)	506.0 ± 237.1 (n = 119)	79.1 ± 54.5 (n = 116)
	Month 6	2.0 ± 0.8 (n = 99)	447.6 ± 159.6 (n = 96)	63.7 ± 31.6 (n = 96)	1.9 ± 0.7 (n = 118)	459.6 ± 207.9 (n = 117)	67.7 ± 58.9 (n = 115)

Table 4: Serum creatinine and calculated clearance values for everolimus at 6 months (patients with at least one assessment in any visit window, including data obtained after discontinuation of study medication)

	Study 1 (without basiliximab) Everolimus 1.5 mg/day	Everolimus 3 mg/day	Study 2 (with basiliximab) Everolimus 1.5 mg/day	Everolimus 3 mg/day
Median calculated GFR[a] (mL/min) [mean ± SD]	65 [63 ± 19.5] (n = 102)	62 [62 ± 18.3] (n = 111)	66 [66 ± 18.8] (n = 107)	67 [65 ± 16.0] (n = 123)
Median serum creatinine (μmol/L) [mean ± SD]	133 [147 ± 104.7] (n = 105)	132 [140 ± 53.1] (n = 112)	130 [137 ± 49.8] (n = 113)	130 [136 ± 42.3] (n = 127)
Number of patients with serum creatinine ≤132 μmol/L at 6 months (%)	51 (49%) (n = 105)	56 (50%) (n = 112)	60 (53%) (n = 113)	74 (58%) (n = 127)
Number of patients with serum creatinine ≤185 μmol/L at 6 months (%)	92 (88%) (n = 105)	95 (85%) (n = 112)	101 (89%) (n = 113)	111 (87%) (n = 127)

[a]Calculated by the Nankivell formula (19).

Table 5: Efficacy-related events at 6 months (intent-to-treat analyses)

	Study 1 (without basiliximab)			Study 2 (with basiliximab)		
	Everolimus 1.5 mg/day (n = 112)	Everolimus 3 mg/day (n = 125)	p[a]	Everolimus 1.5 mg/day (n = 117)	Everolimus 3 mg/day (n = 139)	p[a]
Efficacy failure[b]	31 (27.7%)	24 (19.2%)	0.127	18 (15.4%)	27 (19.4%)	0.415
Biopsy-proven acute rejection	28 (25.0%)	19 (15.2%)	0.073	16 (13.7%)	21 (15.1%)	0.859
Graft loss/death	5 (4.5%)	6 (4.8%)	1.000	2 (1.7%)	7 (5.0%)	0.187
Graft loss	5 (4.5%)	2 (1.6%)	0.260	2 (1.7%)	7 (5.0%)	0.187
Death	0	4 (3.2%)	0.124	0	1 (0.7%)	1.000
Lost to follow-up	1 (0.9%)	0	0.473	0	3 (2.2%)	0.253

[a]Fisher's exact test.
[b]BPAR, graft loss, death or lost to follow-up.

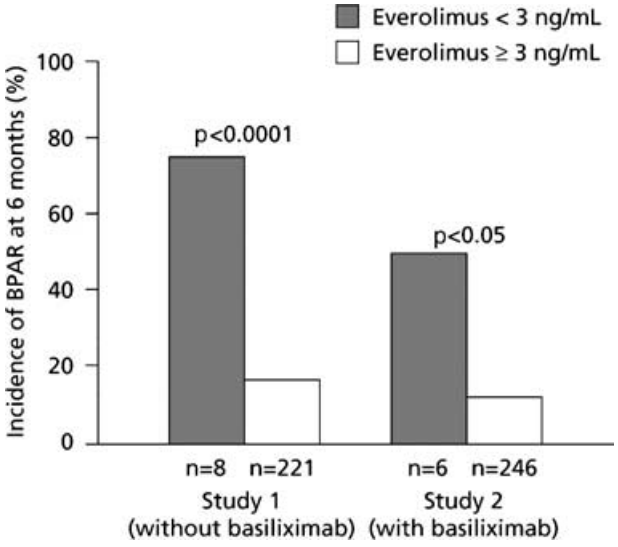

Figure 1: Incidence of biopsy-proven acute rejection (BPAR) at 6 months among patients with an everolimus trough level <3 ng/mL or ≥3 ng/mL; everolimus exposure calculated as arithmetic mean until BPAR or censoring at day 225.

(Table 5), and a higher proportion of patients with BPAR in Study 1 experienced their first episode within the first 14 days post-transplant (10/34, 29.4%) compared with Study 2 (2/17, 11.8%).

Effect of drug exposure

In both studies, BPAR was more common among patients with a mean everolimus trough concentration below 3 ng/mL compared with those with a trough concentration of 3 ng/mL or above (Figure 1) (Study 1: 6/8 vs. 38/221, $p < 0.0001$; Study 2: 3/6 vs. 31/246, $p < 0.05$). A Cox regression model demonstrated that risk of BPAR was affected by everolimus exposure; this relationship approached significance in Study 1 ($p = 0.054$) and was significant in Study 2 ($p = 0.001$). In patients with an everolimus trough concentration <3 ng/mL, use of basiliximab appeared to reduce the risk of BPAR: 6/8 patients with trough concentration <3 ng/mL in Study 1 experienced BPAR compared with 3/6 in Study 2. For patients with a trough concentration >3 ng/mL, addition of basiliximab had a less marked effect on risk of BPAR (Figure 1).

Discontinuations

At the 6-month analysis in Study 1, the incidence of discontinuation of study medication was higher in the 1.5 mg group compared with the 3 mg group. In Study 2, the incidence was similar in both groups. The most common reason for discontinuation of study medication in both studies was adverse events (Table 6). The only adverse event leading to discontinuation of study medication reported by greater than two patients was arthralgia in the Study 1 1.5 mg group. One patient in the 1.5 mg group in both studies discontinued due to thrombocytopenia, and 1 patient in the 3 mg group of Study 2 discontinued due to hyperlipidemia.

Safety

Almost all patients experienced adverse events (Table 7), with no significant between-group differences relating to the incidence or type of adverse events reported in either study. Infection was common, occurring in 50–60% of patients, with urinary tract infections being the most frequently reported, and with a low incidence of cytomegalovirus infection. The most frequent hematologic adverse event was anemia, with low incidences of leukopenia and thrombocytopenia. Neutropenia occurred in one patient receiving 1.5 mg/day in each study, and in three and two patients in the 3 mg/day group in Studies 1 and 2, respectively. At 6 months, in Study 1, values for mean hemoglobin, white blood cells (WBC), and platelets were 8.3 mmol/L, 8.0×10^9/L, and 244×10^9/L, respectively, in the 1.5 mg/day group and 8.1 mmol/L, 7.4×10^9/L, and 228×10^9/L in the 3 mg/day group. The corresponding values in Study 2 were 7.9 mmol/L, 7.4×10^9/L, and 240×10^9/L in the 1.5 mg/day group and 7.8 mmol/L, 7.0×10^9/L, and 246×10^9/L in the 3 mg/day group.

Table 6: Patient disposition (intent-to-treat population)

	Study 1 (without basiliximab)		Study 2 (with basiliximab)	
	Everolimus 1.5 mg/day (n = 112)	Everolimus 3 mg/day (n = 125)	Everolimus 1.5 mg/day (n = 117)	Everolimus 3 mg/day (n = 139)
Discontinued study medication	30 (26.8%)	25 (20.0%)	22 (18.8%)	22 (15.8%)
Adverse event	21 (18.8%)	13 (10.4%)	14 (12.0%)	13 (9.4%)
Abnormal laboratory value (s)	1 (0.9%)	1 (0.8%)	2 (1.7%)	0
Unsatisfactory therapeutic effect	3 (2.7%)	4 (3.2%)	3 (2.6%)	4 (2.9%)
Protocol violation	2 (1.8%)	0	1 (0.9%)	0
Withdrew consent	0	2 (1.6%)	0	2 (1.4%)
Death	0	3 (2.4%)	0	0
Graft loss	3 (2.7%)	2 (1.6%)	2 (1.7%)	3 (2.2%)
Still in study	111 (99.1%)	120 (96.0%)	116 (99.1%)	136 (97.8%)

The incidence of new-onset diabetes after transplantation was 4% in the 1.5 mg/day group and 5% in the 3 mg/day group in Study 1, and 4% and 3%, respectively, in Study 2. The incidence of major adverse cardiac events was low in both studies (3% in both cohorts in Study 1, 2% in both groups in Study 2). Hemolytic uremic syndrome was reported in three patients receiving 1.5 mg/day everolimus and one receiving 3 mg/day in each study.

In Study 1, mean total cholesterol concentration increased from 4.2 and 3.9 mmol/L at baseline to 6.6 and 6.5 mmol/L at Month 6 for the 1.5 mg/day and 3 mg/day cohorts, respectively. For patients in Study 2, the baseline value was 4.3 mmol/L in both cohorts, rising to 6.2 mmol/L and 6.1 mmol/L for the 1.5 and 3 mg/day groups, respectively. The pattern of increase in triglyceride levels was similar. Generally, increases in either total cholesterol or triglycerides were apparent by Month 2–3, after which they stabilized. Lipid-lowering agents, mostly statins, were used in 58.9% and 66.4% of patients in Study 1, and in 66.7% and 72.7% of patients in Study 2 receiving 1.5 or 3 mg/day, respectively.

Discussion

Previous strategies to exploit the synergistic effect of proliferation signal inhibitors and CsA have resulted in an imbalance between immunosuppressive efficacy and renal safety. Early trials, in which fixed-dose sirolimus was used in combination with full-dose CsA, reported low incidences of acute rejection but at the cost of impaired renal function (22–24), a finding that resulted from potentiation of CsA nephrotoxicity by sirolimus (25). Subsequently, early withdrawal of CsA with a maintenance regimen of sirolimus and steroids has been attempted, resulting in improved renal function but with a significantly higher risk of late acute rejection (26), which is known to be a predictor of chronic rejection (27) and graft loss (28). Moreover, the relatively high dose of sirolimus necessitated by CsA withdrawal led to an increase in sirolimus-related adverse events (26). Although everolimus does not increase CsA levels, similar renal findings were found using everolimus with full-dose CsA in Phase III trials (11,12,29).

In the two studies described here, C_2 adjusted dosing of CsA levels was used to achieve exposure lower than those in earlier studies of everolimus and full-dose CsA (11,12). After Month 1, the CsA C_2 level in Study 1 was approximately a third lower than typically targeted (30), and corresponding trough CsA levels were approximately half those seen in studies of everolimus with full-dose CsA (11,12). In Study 2, target C_2 levels were approximately half that typically used (30); trough levels were a little over a third of those reported in patients receiving everolimus with full-dose CsA (11,12). Low CNI exposure may be associated with long-term clinical benefits, such as reduced risk of chronic renal allograft dysfunction (31), hypertension or new-onset diabetes after transplantation (32), which in turn could contribute to improved patient and graft survival. Concentration-controlled everolimus with low-exposure CsA has the potential to minimize chronic CNI-related toxicity. Further data are required to assess the clinical effect of low-exposure CsA within this regimen over the longer term.

The studies presented here demonstrate that optimizing drug exposure for both everolimus and CsA achieves an effective balance of immunosuppression and renal function. Concentration-controlled everolimus and low-exposure CsA was effective in preventing graft rejection, while the effects on renal function were similar to those reported with current regimens (30,33,34). The optimized everolimus/CsA regimens appeared to be associated with better preservation of renal function than comparable high-exposure CsA regimens in Phase II and III everolimus trials (11–13). Addition of the IL-2 antagonist basiliximab

Table 7: Number (%) of patients reporting common adverse events (AEs) by 6 months (safety analyses)

	Study 1 (without basiliximab)		Study 2 (with basiliximab)	
	Everolimus 1.5 mg/day (n = 112)	Everolimus 3 mg/day (n = 125)	Everolimus 1.5 mg/day (n = 117)	Everolimus 3 mg/day (n = 139)
Any AE/infection	111 (99.1%)	123 (98.4%)	116 (99.1%)	139 (100%)
Any infection	69 (61.6%)	71 (56.8%)	68 (58.1%)	76 (54.7%)
Urinary tract	32 (28.6%)	34 (27.2%)	25 (21.4%)	30 (21.6%)
CMV	1 (0.9%)	4 (3.2%)	3 (2.6%)	3 (2.2%)
Herpes simplex	8 (7.1%)	1 (0.8%)	4 (3.4%)	11 (7.9%)
Pneumonia	4 (3.6%)	5 (4.0%)	2 (1.7%)	4 (2.9%)
Upper respiratory tract	5 (4.5%)	1 (0.8%)	9 (7.7%)	11 (7.9%)
Infection reported as serious AE	17 (15.2%)	22 (17.6%)	20 (17.1%)	19 (13.7%)
Malignancy	1 (0.9%)	2 (1.6%)	1 (0.9%)	2 (1.4%)
Blood and lymphatic system disorders	41 (36.6%)	50 (40.0%)	40 (34.2%)	64 (46.0%)
Anemia NOS	21 (18.8%)	28 (22.4%)	28 (23.9%)	41 (29.5%)
Leukopenia	5 (4.5%)	5 (4.0%)	4 (3.4%)	14 (10.1%)
Thrombocytopenia	4 (3.6%)	10 (8.0%)	4 (3.4%)	8 (5.8%)
Cardiac disorders	18 (16.1%)	22 (17.6%)	10 (8.5%)	19 (13.7%)
Hypertension NOS	17 (15.2%)	25 (20.0%)	30 (25.6%)	35 (25.2%)
Lymphocele	17 (15.2%)	8 (6.4%)	12 (10.3%)	10 (7.2%)
Gastrointestinal disorders	68 (60.7%)	73 (58.4%)	57 (48.7%)	83 (59.7%)
Constipation	26 (23.2%)	32 (25.6%)	32 (27.4%)	42 (30.2%)
Diarrhea NOS	18 (16.1%)	10 (8.0%)	15 (12.8%)	18 (12.9%)
Total cholesterol ≥6.2 mmol/L (239 mg/dL)	93 (83.0%)	105 (84.0%)	94 (81.0%)	122 (87.8%)
Total cholesterol ≥9.1 mmol/L (351 mg/dL)	25 (22.3%)	31 (24.8%)	22 (19.0%)	29 (20.9%)
Triglycerides ≥4.5 mmol/L (398 mg/dL)	33 (29.5%)	57 (45.6%)	48 (41.4%)	53 (38.1%)
Triglycerides ≥8.5 mmol/L (752 mg/dL)	4 (3.6%)	10 (8.0%)	6 (5.2%)	11 (7.9%)
Blood glucose >13.9 mmol/L (250 mg/dL)	12 (10.7%)	13 (10.4%)	10 (8.6%)	12 (8.6%)
Respiratory, thoracic and mediastinal disorders	38 (33.9%)	39 (31.2%)	30 (25.6%)	31 (22.3%)
Cough	11 (9.8%)	8 (6.4%)	3 (2.6%)	2 (1.4%)
Nasopharyngitis	8 (7.1%)	6 (4.8%)	2 (1.7%)	6 (4.3%)
Skin and subcutaneous disorders	32 (28.6%)	39 (31.2%)	25 (21.4%)	30 (21.6%)
Acne NOS	9 (8.0%)	11 (8.8%)	12 (10.3%)	9 (6.5%)

CMV: cytomegalovirus; NOS: not otherwise specified.

decreased the incidence of acute rejection among those who did not achieve a therapeutic level of everolimus exposure (3 ng/mL).

Hariharan and colleagues (2) have reported that 6-month serum creatinine >132 mmol/L (1.5 mg/dL) is associated with a decline in long-term graft function. Overall, in the studies reported here, over 50% of patients had a 6-month creatinine value ≤132 mmol/L, indicating excellent long-term graft prognosis. Creatinine values >185 mmol/L (2.1 mg/dL) are associated with less than half the projected graft half-life calculated for those with excellent renal function (2); less then 15% of patients in these studies had values >185 mmol/L.

In terms of efficacy, 6-month rejection rates in Study 1 were similar in the 3 mg/day arm to those seen with higher C_2 levels in combination with MMF or azathioprine and steroids (30). Overall, the incidence of efficacy failure compares favorably with that previously reported in multicenter efficacy trials. The incidence of rejection was higher with 1.5 mg/day (25%) in Study 1, but only slightly above that reported recently with full-dose tacrolimus-based triple therapy (20%) (33). When basiliximab was used in addition to everolimus and low-exposure CsA (Study 2), rejection rates were similar to those previously reported with full-dose CsA, MMF, steroids and basiliximab (35).

A comparison of results in the 1.5 and 3 mg/day cohorts showed no significant differences in efficacy between treatment groups within each trial. Safety differed only by a numerical trend towards increased prevalence of hematologic disturbances in the 3 mg/day groups. There was a higher incidence of rejection in the 1.5 mg/day group in Study 1. Although there was considerable overlap between patients in the different treatment groups in terms of everolimus trough levels, more patients in the 1.5 mg/day group fell below the minimum level of 3 ng/mL, which may account for the higher rate of rejection in this cohort. A minimum everolimus trough level of 3 ng/mL was selected on the basis of a retrospective analysis of data from a Phase III trial of everolimus which showed a strong relationship between an everolimus trough level >3 ng/mL and prevention of acute rejection, regardless of CsA exposure (15). Results presented here also indicate that an everolimus trough level >3 ng/mL is associated with a reduced risk of rejection. Few acute rejections occurred after month 3. Thus, therapeutic drug monitoring of everolimus can enhance both efficacy and safety of the regimen by allowing initial use of 1.5 mg/day with dose increments as required to ensure a trough concentration of at least 3 ng/mL. Black patients, in whom CsA and everolimus clearance rate is higher than nonblacks (14), still experienced a rate of acute rejection higher than nonBlacks, suggesting caution must still be observed in monitoring everolimus and CsA levels.

In conclusion, concentration-controlled everolimus in combination with low-exposure CsA results in effective protection against rejection with good renal function. Therapeutic drug monitoring to optimize exposure to both everolimus and CsA offers an innovative strategy that allows individualization of immunosuppression after transplantation. Long-term data are required to determine whether use of this regimen also helps to reduce risk of chronic allograft nephropathy.

Acknowledgments

We would like to acknowledge the collaboration and commitment of all A2306 (Study 1) and A2307 (Study 2) investigators and their staff, without whom the present paper would not have been possible. The studies were funded by a grant from Novartis Pharma AG.

References

1. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, Mcintosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 96. N Engl J Med 2000; 342: 605–612.
2. Hariharan S, Mcbride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP. Post-transplant renal function in the first year predicts long-term kidney transplant survival. Kidney Int 2002; 62: 311–318.
3. Kahan BD, Chang JY, Sehgal SN. Preclinical evaluation of a new potent immunosuppressive agent, rapamycin. Transplantation 1991; 52: 185–191.
4. Nashan B. Early clinical experience with a novel rapamycin derivative. Ther Drug Monit 2002; 24: 53–58.
5. Sehgal S. Rapamune (sirolimus, rapamycin): an overview and mechanism of action. Ther Drug Monit 1995; 17: 660–665.
6. Schuler W, Sedrani R, Cottens S et al. SDZ RAD, a new rapamycin derivative: pharmacologic properties in vitro and in vivo. Transplantation 1997; 64: 36–42.
7. Hausen B, Boeke K, Berry GJ, Segarra IT, Christians U, Morris RE. Suppression of acute rejection in allogenic rat lung transplantation: a study of the efficacy and pharmacokinetics of rapamycin derivative (SDZ RAD) used alone and in combination with a microemulsion formulation of cyclosporine. J Heart Lung Transplant 1999; 18: 150–159.
8. Schuurman H-J, Cottens S, Fuchs S et al. SDZ RAD, a new rapamycin derivative: synergism with cyclosporine. Transplantation 1997; 64: 32–35.
9. Serkova N, Hausen B, Berry GJ et al. Tissue distribution and clinical monitoring of the novel macrolide immunosuppressant SDZ-RAD and its metabolites in monkey lung transplant recipients: interaction with cyclosporine. J Pharmacol Exp Ther 2000; 294: 323–332.
10. Hausen B, Boeke K, Berry GJ et al. Coadministration of Neoral and the novel rapamycin analog, SDZ RAD, to rat lung allograft recipients: potentiation of immunosuppressive efficacy and improvement of tolerability by staggered simultaneous treatment. Transplantation 1999; 67: 956–962.
11. Vitko S, Margreiter R, Weimar W et al. and the RAD 201 Study Group. International, double-blind, parallel-group study of the safety and efficacy of Certican™ (RAD) versus mycophenolate mofetil (MMF) in combination with Neoral® and steroids. Am J Transplant 2001; 1 (Suppl. 1): 474, (Abstract) 1337.
12. Kaplan B, Tedesco-Silva H, Mendez R et al. and the RAD 251 Study Group. North/South American, double-blind, parallel group study of the safety and efficacy of Certican™ (RAD) versus mycophenolate mofetil (MMF) in combination with Neoral® and corticosteroids. Am J Transplant 2001; 1 (Suppl. 1): 475, (Abstract) 1339.
13. Curtis J, Nashan B, Ponticelli C et al. and the RAD 156 Study Group. One year results of a multicenter, open-label trial on safety, efficacy of Certican™ (RAD) used in combination with Simulect® corticosteroids and full or reduced dose Neoral® in renal transplantation. Am J Transplant 2001; 1 (Suppl. 1): 474, (Abstract) 1335.
14. Kovarik JM, Hsu C-H, Mcmahon L, Berthier S, Rordorf C. Population pharmacokinetics of everolimus in de novo renal transplant patients: impact of ethnicity and comedications. Clin Pharmacol Ther 2001; 70: 247–254.

15. Kovarik JM, Kaplan B, Tedesco-Silva H et al. Exposure-response relationships for everolimus in de novo kidney transplantation: defining a therapeutic range. Transplantation 2002; 73: 920–925.
16. Barama A, Perner F, Beauregard-Zollinger L, Prestele H. Absorption profiling of cyclosporine therapy for de novo kidney transplantation: a prospective, randomised study comparing sparse sampling to trough monitoring. Transplantation 2000; 69 (Suppl. 8): S162 (Abstract 19).
17. Halloran P, Helms LM, Kung L, Noujaim J. The temporal profile of calcineurin inhibition by cyclosporine in vivo. Transplantation 1999; 68: 1356–1361.
18. Sindhi R, Lavia MF, Paulling E et al. Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine + rapamycin regimen. Transplantation 2000; 69: 432–436.
19. Nankivell BJ, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. Transplantation 1995; 59: 1683–1689.
20. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31–41.
21. Racusen LC, Solez K, Colvin RB et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55: 713–723.
22. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y, Neylan J. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in Caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. Transplantation 1999; 68: 1526–1532.
23. 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: 194–202.
24. Macdonald AS, 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: 271–280.
25. Podder H, Stepkowski SM, Napoli KL et al. Pharmacokinetic interactions augment toxicities of sirolimus/cyclosporine combinations. J Am Soc Nephrol 2001; 12: 1059–1071.
26. Johnson RWG, Kreis H, Oberbauer R, Brattström 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: 777–786.
27. Basadonna GP, Matas AJ, Gillingham KJ et al. Early versus late acute renal allograft rejection: impact on chronic rejection. Transplantation 1993; 55: 993–995.
28. Leggat JE, Ojo AO, Leichtman AB et al. Long-term renal allograft survival: prognostic implication of the timing of acute rejection episodes. Transplantation 1997; 63: 1268–1272.
29. Oppenheimer F, Oyen O, Viljoen H, Vitko S, Falcone A, Cremer M. 36-month results of an international study with everolimus for the prevention of allograft rejection in de novo kidney transplant recipients. Am J Transplant 2003; 3 (Suppl. 5): 459 (Abstract).
30. .Thervet E, Pfeffer P, Scolari MP et al. Clinical outcomes during the first 3 months post-transplant in renal allograft recipients managed by C_2 monitoring of cyclosporine microemulsion (Neoral®). Transplantation 2003.
31. Libby P, Pober JS. Chronic rejection. Immunity 2001; 14: 387–397.
32. Jardine A. Assessing cardiovascular risk profile of immunosuppressive agents. Transplantation 2001; 72: S81–S88.
33. Margreiter R for the European Tacrolimus vs Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. Lancet 2002; 359: 741–746.
34. Lebranchu Y, Bridoux F, Buchler M et al. Immunoprophylaxis with basiliximab compared with antithymocyte globulin in renal transplant patients receiving MMF-containing triple therapy. Am J Transplant 2002; 2: 48–56.
35. Lawen JG, Davies EA, Mourad G et al. Randomized double-blind study of immunoprophylaxis with basiliximab, a chimeric anti-interleukin-2 receptor monoclonal antibody, in combination with mycophenolate mofetil-containing triple therapy in renal transplantation. Transplantation 2003; 75: 37–43.