Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Efficacy and safety of *de novo* or early everolimus with low cyclosporine in deceased-donor kidney transplant recipients at specified risk of delayed graft function: 12-month results of a randomized, multicenter trial

Jacques Dantal,[1] François Berthoux,[2] Marie-Christine Moal,[3] Lionel Rostaing,[4] Christophe Legendre,[5] Robert Genin,[6] Olivier Toupance,[7] Bruno Moulin,[8] Pierre Merville,[9] Jean-Philippe Rerolle,[10] François Bayle,[11] Pierre François Westeel,[12] Denis Glotz,[13] Niloufar Kossari,[14] Nicole Lefrançois,[15] Bernard Charpentier,[16] Stéphane Quéré,[17] Fabienne Di Giambattista[17] and Elisabeth Cassuto,[18] on behalf of the RAD A2420 Study Group*

1 Service de Néphrologie et Transplantation Rénale, Hôpital Hôtel Dieu, Nantes, France
2 Service de Néphrologie, Dialyse et Transplantation Rénale, Hôpital Nord – CHU, Saint Etienne, France
3 Service de Néphrologie et Transplantation Rénale, Hôpital La Cavale Blanche, 29609 Brest, France
4 Service de Néphrologie, Dialyse et Transplantation d'Organes, Hôpital de Rangueil, Toulouse, France
5 Université Paris Descartes & Hôpital Necker, Service de Néphrologie et Transplantation Rénale, Paris, France
6 Service de Néphrologie et Transplantation rénale, CHR La Réunion, Saint-Denis, La Réunion, France
7 Service de Néphrologie et Transplantation rénale, Hôpital Maison Blanche, Reims, France
8 Service de Néphrologie et Transplantation rénale, Hospices Civils, Strasbourg, France
9 Service de Néphrologie et Transplantation rénale, Hôpital Pellegrin, Bordeaux, France
10 Service de Néphrologie et Transplantation rénale, CHU Limoges, 87000 Limoges, France
11 Service de Néphrologie et Transplantation rénale, Hôpital Michallon, 38700 Grenoble, France
12 Service de Néphrologie et Transplantation rénale, Hôpital Sud, 80054 Amiens, France
13 Service de Néphrologie et Transplantation rénale, Hôpital Saint-Louis, Paris, France
14 Service de Néphrologie, Hôpital Bichat, Paris, France
15 Service de Néphrologie et Transplantation rénale, Hôpital Edouard Herriot, Lyon, France
16 Service de Néphrologie et Transplantation rénale, Hôpital Bicêtre, 94275 Kremlin-Bicêtre and Université Paris Sud 11, INSERM UMR542, Villejuif, France
17 Novartis Pharma SAS, Rueil-Malmaison, France
18 Service de Néphrologie et Transplantation Rénale, Hôpital Pasteur, Nice, France

Keywords
age, delayed graft function, everolimus, kidney transplantation, mycophenolate mofetil, wound healing.

Correspondence
Jacques Dantal MD, Service de Néphrologie et Transplantation Rénale, Hôpital Hôtel Dieu, 44093 Nantes, France. Tel.: +33 2 40 08 74 53; fax: +33 2 40 08 74 48; e-mail: jacques.dantal@chu-nantes.fr

(ClinicalTrials.gov number: NCT00154297)

*See Appendix.

Received: 11 December 2009
Revision requested: 25 January 2010
Accepted: 6 April 2010
Published online: 24 May 2010

doi:10.1111/j.1432-2277.2010.01094.x

Summary

Immediate or early use of proliferation signal inhibitor (PSI)/mammalian target of rapamycin (mTOR) inhibitor therapy can avoid high exposure to calcineurin inhibitors but concerns exist relating to the risk of delayed graft function (DGF) and impaired wound healing with the mTOR sirolimus. CALLISTO was a 12-month, prospective, multicenter, open-label study. Deceased-donor kidney transplant patients at protocol-specified risk of DGF were randomized to start everolimus on day 1 (immediate everolimus, IE; $n = 65$) or week 5 (delayed everolimus, DE; $n = 74$). Incidence of the primary endpoint (biopsy-proven acute rejection, BPAR; graft loss, death, DGF, wound healing complications related to transplant surgery or loss to follow-up) was 64.6% and 66.2% in the IE and DE groups, respectively, at month 12 ($P = 0.860$). The overall incidence of BPAR was 20.1%. Median estimated glomerular filtration rate was 48 ml/min/1.73 m^2 and 49 ml/min/1.73 m^2 in the IE and DE groups, respectively, at month 12. DGF and wound healing complications were similar between groups. Adverse events led to study drug discontinuation in 17 IE patients (26.2%) and 28 DE patients (37.8%) (NS). In conclusion, introduction of everolimus immediately or early posttransplant in DGF-risk patients is associated with good efficacy, renal function and safety profile. There seems no benefit in delaying initiation of everolimus.

Introduction

Everolimus, a proliferation signal inhibitor (PSI)/mammalian target of rapamycin (mTOR) inhibitor, offers potent immunosuppression coupled with an antiproliferative action that inhibits vascular smooth muscle cell proliferation [1], restricting vascular remodelling and neointimal growth in preclinical models [2–5]. The different mode of action of everolimus and calcineurin inhibitors (CNIs) [1] permits CNI exposure to be minimized in *de novo* kidney transplant without compromising efficacy [6–10]. Such an approach could be expected to ameliorate the CNI-related nephrotoxicity observed with CNI exposure in the early posttransplant phase and minimize irreversible histological damage [11].

However, PSI therapy is often reserved for CNI minimization in the maintenance phase (often in patients who have already developed symptoms of chronic allograft nephropathy) instead of being initiated as primary immunosuppression. This is partly due to reports from single-center studies that the mTOR inhibitor sirolimus is associated with an increased incidence or duration of delayed graft function (DGF) [12,13], and evidence that the rate of wound healing complications may be higher in kidney transplant patients receiving sirolimus [14–18]. However, introduction of PSIs in the maintenance phase after extended exposure to CNI treatment may be of only limited benefit. The CONVERT study, in which 830 kidney transplant patients were converted from CNI therapy to sirolimus at between 6 and 120 months posttransplant, led to an improvement in glomerular filtration rate (GFR) only in those patients with good function at the time of conversion (GFR > 40 m/min) [19]. When conversion from CNI to PSI takes place earlier (<6 months posttransplant), the benefit for renal function is more consistent and sizeable with no loss of efficacy [20–23]. In the recent ZEUS study, in which 300 kidney transplant patients were randomized to convert from cyclosporine (CsA) to everolimus at 4.5 months posttransplant or remain on CsA, calculated GFR was significantly higher in the everolimus cohort at one year posttransplant (72.3 ml/min/1.73^2 vs. 61.9 ml/min/1.73 m^2, $P < 0.001$) [21], with a similar rate of biopsy-proven acute rejection (BPAR) in both treatment arms [24]. Such results have re-ignited interest in use of *de novo* PSI therapy in an attempt to minimize early CNI exposure to an even greater extent. When PSI therapy is used from day 1 posttransplant with reduced-exposure CNI, studies have consistently reported good renal function and low rejection rates with everolimus [6,9,10,25–27] or sirolimus [28] but potential concerns about aggravation of renal ischemia-reperfusion injury or impaired wound healing still need to be addressed.

To date no controlled trial has assessed the use of immediate or early administration of everolimus in patients at risk of DGF. CALLISTO was a randomized, multicenter trial in which kidney transplant patients at protocol-specified DGF risk received everolimus from either day 1 or week 5 posttransplant. Three-month results relating to the primary analysis have been published previously [29]. Final results from this 12-month study are described here.

Methods

CALLISTO was a prospective, multicenter, open-label study undertaken at 17 transplant centers in France from June 2005 to June 2008.

Adult recipients of a kidney transplant from a deceased donor were eligible to take part in the study if they had one or more risk factor for DGF, defined as donor age >55 years, cold ischemia time ≥24 h but <40 h, and retransplantation. Additionally, while not a criterion for inclusion, recipient age >60 years was considered by the investigators to be a risk factor for DGF. Patients were excluded if they had received a multiorgan transplant or a previous nonkidney transplant, if the donor was non-heart-beating or was ABO incompatible or T-cell crossmatch positive, if panel reactive antibodies were ≥30%, body mass index >32 kg/m^2, if they had chronic active hepatitis C infection or were HIV or hepatitis B surface antigen positive, or if the donor was positive for hepatitis B surface antigen or hepatitis C.

Patients were randomized on day 1 posttransplant using an automated scratch-card system. The two randomized groups comprised immediate everolimus (IE) or delayed everolimus (DE). In the IE group, everolimus (Certican®, Novartis Pharma AG, Basel, Switzerland) was started on the first day after transplantation (day 1) at 0.75 mg b.i.d., adjusted to target everolimus C_0 in the range 3–8 ng/ml using locally measured values recorded with Innofluor® Certican® immunoassay (Seradyn, IN, USA) or HPLC. In the DE group, mycophenolic acid (MPA) was started on day 1 as enteric-coated mycophenolate sodium or mycophenolate mofetil (MMF), dosed according to local practice. MPA was discontinued at week 5 and everolimus was started at 0.75 mg b.i.d., again to target C_0 3–8 ng/ml. All patients were given CsA (Neoral®, Novartis Pharma SAS, Rueil-Malmaison, France) within 24 h posttransplant, targeting a locally-measured C_2 level of 500–700 ng/ml during weeks 0–8 and 350–450 ng/ml thereafter in the IE group, and 1100–1500 ng/ml during weeks 0–4, 500–700 ng/ml during weeks 5–8 and 350–450 ng/ml thereafter in the DE group. Thus, after week 4 all patients received the same immunosuppressive regimen.

Intravenous prednisone (or equivalent) was administered peri-operatively according to center practice, with oral corticosteroids started at ≥20 mg/day. Corticosteroid doses were tapered according to local practice but discontinuation was not permitted. Interleukin-2 receptor antibody induction was administered to both groups according to local practice.

Unless both recipient and donor were cytomegalovirus (CMV) negative, CMV prophylaxis was strongly recommended at least until the end of month 3. Prophylaxis for *Pneumocystis jiroveci* pneumonia was also strongly recommended throughout the study, to be administered according to local practice.

Glomerular filtration rate was estimated using the Nankivell formula [30] excluding creatinine values during dialysis or on the day after dialysis.

The primary efficacy endpoint was a composite of the following events: DGF, BPAR, graft loss, death, wound healing complications related to initial transplant surgery or loss to follow-up. DGF was defined as ≥1 dialysis session during days 2–7 posttransplant. The primary analysis for this endpoint was performed at month 3. The sample size calculation assumed that the primary efficacy endpoint would occur in 55% of IE patients by month 3, and in 25% fewer patients in the DE group. A minimum sample size of 122 randomized patients was estimated to have a power of 80% to detect a difference between groups (alpha level 0.05). Statistical comparisons between the two treatment groups were performed using the Fisher's exact test for categorical variables and unpaired Wilcoxon rank-sum tests for continuous variables. Unpaired Wilcoxon test was used to analyze the duration of DGF and time to nadir serum creatinine. Time-to-event analyses used Kaplan–Meier estimates and log-rank tests. Continuous variables are presented as mean ± standard deviation (SD) unless otherwise stated. Efficacy analyses were performed based on the intent-to-treat population (defined as randomized and treated patients, from whom at least one postbaseline measurement was obtained); safety analyses were performed based on the safety population (defined as randomized and treated patients who provided at least one safety/tolerability assessment).

Conduct of the study complied with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice and the Declaration of Helsinki and amendments. Written informed consent was obtained from all patients following approval from the Comité de Protection des Personnes in France.

Results

Study population

The study population comprised 139 randomized patients (IE 65, DE 74), all of whom received at least one dose of study drug, and were included in both the intent-to-treat and safety populations. Of these, 124 patients (89.2%) completed the 12-month study, 82 of whom (59.0%)

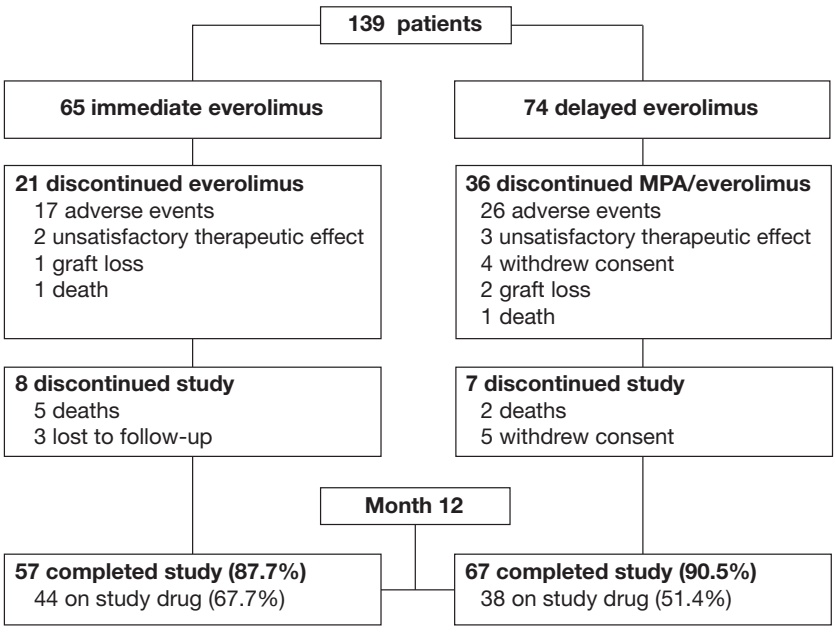

Figure 1 Patient disposition. Patients who discontinued study drug were followed to the last study visit where possible.

Table 1. Baseline characteristics.

	IE (n = 65)	DE (n = 74)	All patients (n = 139)
Recipients			
Recipient age (years)			
Mean ± SD	57.3 ± 10.5	58.4 ± 9.9	57.9 ± 10.1
>60	28 (43.1%)	41 (55.4%)	69 (49.6%)
Recipient >60 years and donor >55 years	23 (35.4%)	35 (47.3%)	58 (41.7%)
Male gender	46 (70.8%)	54 (73.0%)	100 (71.9%)
White race	59 (90.8%)	70 (94.6%)	129 (92.8%)
Body mass index (kg/m^2) (mean ± SD)	24.0 ± 3.4	24.6 ± 3.5	24.3 ± 3.5
Cause of end-stage renal disease			
Glomerulonephritis/glomerular disease/IgA nephropathy	23 (35.4%)	17 (23.0%)	40 (28.8%)
Polycystic disease	12 (18.5%)	14 (18.9%)	26 (18.7%)
Hypertension/nephrosclerosis	8 (12.3%)	14 (18.9%)	22 (15.8%)
Diabetes mellitus	6 (9.2%)	10 (13.5%)	16 (11.5%)
Interstitial nephritis	3 (4.6%)	6 (8.1%)	9 (6.5%)
Other/unknown	13 (20.0%)	13 (17.6%)	26 (18.7%)
Panel reactive antibodies >10%	3 (4.6%)	2 (2.7%)	5 (3.6%)
Retransplantation	7 (10.8%)	2 (2.7%)	9 (6.5%)
Donors			
Donor age (years)			
Mean ± SD	59.7 ± 12.7	62.8 ± 10.5	61.4 ± 11.7
>55 years	52 (80.0%)	67 (90.5%)	119 (85.6%)
Male gender	33 (50.8%)	44 (59.5%)	77 (55.4%)
Transplant			
HLA mismatches			
0	0 (0.0%)	3 (4.1%)	3 (2.2%)
1	2 (3.1%)	2 (2.7%)	4 (2.9%)
≥2	63 (96.9%)	69 (93.2%)	132 (95.0%)
CMV R–/D+	10 (15.4%)	13 (17.6%)	23 (16.5%)
Cold ischemia time (hours)			
Mean ± SD	21.5 ± 6.8	22.3 ± 6.9	21.9 ± 6.8
<24	45 (69.2%)	49 (66.2%)	94 (67.6%)
≥24	20 (30.8%)	25 (33.8%)	45 (32.4%)

remained on study drug (Fig. 1). In the DE group, 15/36 patients who discontinued study drug did so before conversion to everolimus, i.e. they stopped MPA during weeks 0–4. Demographics and baseline characteristics were similar between treatment groups, and there were no significant differences in risk factors for DGF (Table 1). In total, 67 patients (49.6%) had one risk factor for DGF, 56 patients (40.3%) had two risk factors and 14 (10.1%) had three risk factors, as described previously [29].

Immunosuppression

All but one patient in each group received IL-2 receptor antibody induction (131 basiliximab, 6 daclizumab). Mean everolimus C_0 remained within the target range 3–8 ng/ml at all timepoints in the IE group, with a mean value over the 12-month study of 6.7 ± 2.4 ng/ml. In the DE group, everolimus C_0 was within range except during month 2 (9.1 ± 4.4 ng/ml) and the mean value during the 12-month study was 6.5 ± 1.7 ng/ml. Mean CsA C_2 level was above target during months 0–6 in the IE group, and during months 2–6 in the DE group (i.e. after introduction of everolimus). At week 1, month 1, month 3, month 6 and month 12 the mean CsA C_2 level in the IE group was 909 ± 323, 815 ± 390, 535 ± 219, 468 ± 268 and 390 ± 152 ng/ml. Corresponding values in the DE group were 1181 ± 480, 1213 ± 608, 663 ± 292, 480 ± 214 and 368 ± 117 ng/ml. Except during month 1 in the DE arm, a high percentage of patients had a CsA C_2 level above target range: weeks 1–4, IE 78.1% and DE 10.8%; weeks 5–8, IE 48.6% and DE 86.1%; months 3–12, IE 49.0% and DE 57.4%. Both groups showed similar mean CsA C_2 levels at month 6 (IE 468 ± 268 ng/ml, DE 480 ± 214 ng/ml) and month 12 (IE 390 ± 152 ng/ml, DE 368 ± 117 ng/ml). Median steroid dose decreased from month 1 [IE 13.9 mg/day (range 5.0–178.6), DE 14.4 mg/day (6.2–99.6)] and month 3 (IE 8.4 mg/day [(5.0–62.3), DE 9.4 mg/day (5.0–875)] to month 6 [IE 7.1 mg/day (0.4–85.8), DE 6.8 mg/day (2.5–378)] and

month 12 [IE 5.0 mg/day (2.5–28.6), DE 5.0 mg/day (2.5–10.0)]. Eleven IE patients (16.9%) and 15 DE patients (20.3%) received steroids for treatment of rejection.

Efficacy and graft function

At month 12, patient survival was 92.3% and 97.3% in the IE and DE groups, respectively, and death-censored graft survival was 90.7% and 93.0%. There were six graft losses in the IE group [infection [2], antibody-mediated rejection, renal vein thrombosis, immunosuppression withdrawal due to septic shock (everolimus was withdrawn on the day before the event) and trauma to the graft following a fall] and five graft losses in the DE group (renal artery thrombosis [2], renal vein thrombosis, urological complications and vascular rejection). Five deaths (7.7%) were reported among IE patients, including two patients who had previously lost their graft (i.e. three deaths with a functioning graft), due to septic shock [2], disseminated aspergillosis infection, hemorrhage of the iliac artery and cardiac failure. There were two deaths (2.7%) in the DE group, caused by sudden death at home and cerebrovascular accident.

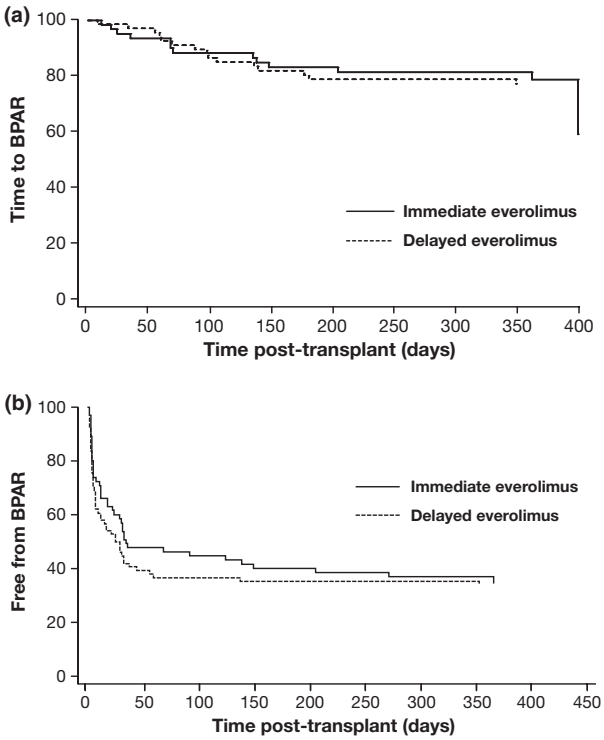

Figure 2 (a) Time to primary endpoint (log-rank test $P = 0.505$) and (b) freedom from first BPAR (log-rank test $P = 0.889$) (Kaplan–Meier estimates).

The primary endpoint, occurrence of BPAR, graft loss, death, DGF, wound healing complications related to transplant surgery or loss to follow-up, was similar between treatment groups at month 3 [IE 36 (55.4%), DE 47 (63.5%); $P = 0.387$] and month 12 [IE 42 (64.6%), DE 49 (66.2%), $P = 0.860$]. Kaplan–Meier estimates showed that the endpoint occurred earlier in the DE group than the IE cohort during the first 6 months of the study but thereafter the two curves are similar with no significant difference over the 12-month study (log-rank test, $P = 0.505$; Fig. 2a). Additionally, there were no significant differences in the incidence of the primary endpoint between the IE and DE groups at any time point from month 1 to 12 in the subpopulation of patients with at least two risk factors of DGF (as defined above; data not shown).

There were no significant differences between the IE and DE arms for any component of the primary endpoint (Table 2). Overall, DGF occurred in 24.5% of these high-risk patients, with no difference between groups (Table 2). The overall incidence of BPAR at 12 months was 20.1%, with no significant difference between groups (Fig. 2b) and no increase in the rate of BPAR after conversion from MPA to everolimus in the DE group. The

Table 2. Efficacy endpoints at month 12 posttransplant. All between-group differences were nonsignificant.

	IE (n = 65)	DE (n = 74)	All patients (n = 139)
Primary endpoint*	42 (64.6%)	49 (66.2%)	91 (65.5%)
DGF†	16 (24.6%)	18 (24.3%)	34 (24.5%)
BPAR	13 (20.0%)	15 (20.3%)	28 (20.1%)
Grade 1A	9	4	13
Grade 1B	6	4	10
Grade IIA	1	4	5
Grade IIB	0	3	3
Graft loss	6 (9.2%)	5 (6.8%)	11 (7.9%)
Death	5 (7.7%)	2 (2.7%)	7 (5.0%)
Wound healing complication related to initial transplant surgery	26 (40.0%)	28 (37.8%)	54 (38.8%)
Fluid collection‡	24 (36.9%)	25 (33.8%)	49 (35.2%)
Wound dehiscence	0	2 (2.7%)	2 (1.4%)
Incisional hernia	2 (3.1%)	0	2 (1.4%)
Urine leak	2 (3.1%)	2 (2.7%)	4 (2.9%)
Delayed scar cicatrisation	0	2 (2.7%)	2 (1.4%)
Loss to follow-up	0 (0.0%)	3 (4.1%)	3 (2.2%)

*DGF (defined as ≥1 dialysis session in the first week posttransplantation excluding day 1), BPAR, graft loss, death or loss to follow-up, or occurrence of wound healing complications related to transplant surgery.

†Defined as ≥1 dialysis session in the first week posttransplantation excluding day 1.

‡Lymphocele, hematoma, seroma.

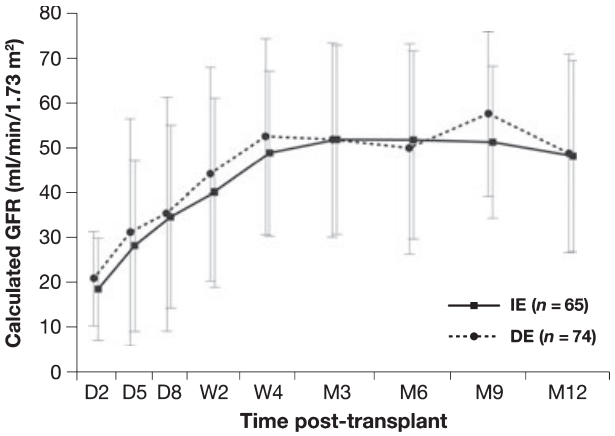

Figure 3 Estimated GFR (Nankivell). Values shown are mean ± SD.

incidence of BPAR was similar in both treatment arms after week 4 (i.e. the time of conversion). The majority of the episodes (28/31) were categorized as Grade I or IIA (Banff 1997 classification [30]) (Table 2).

Median (range) estimated GFR (eGFR) was similar between treatment groups at baseline [IE 17.0 (0–36.0) ml/min/1.73 m^2, DE 18.0 (0–33.5) ml/min/1.73 m^2, $P = 0.534$] and month 12 [IE 48.0 (0–106) ml/min/1.73 m^2, DE 49.0 (0–95) ml/min/1.73 m^2, $P = 0.724$]. The corresponding mean values were 17.6 ± 8.7 ml/min/1.73 m^2 and 18.3 ± 9.1 ml/min/1.73 m^2 at baseline and 48.4 ± 21.3 ml/min/1.73 m^2 and 49.0 ± 22.2 ml/min/1.73 m^2 at month 12 (Fig. 3). Median time from transplantation to serum creatinine <180 μmol/l was similar in both groups [IE 11 (2–187) days, DE 9 (2–193) days; $P = 0.50$], as was the median time from transplantation to eGFR > 50 ml/min/1.73 m^2 [IE 16 (2–269) days, DE 11 (3–193) days; $P = 0.17$]. Median values for nadir serum creatinine and time to nadir serum creatinine were similar between groups (Table 3).

In the DGF cohort, mean eGFR after month 3 (when renal function stabilized) ranged from 32–49 ml/min/1.73^2, compared to 51–59 ml/min/1.73^2 in the patients without DGF. As for the total study population, the median time from transplantation to serum creatinine <180 μmol/l was similar in both treatment groups [IE 20 (4–187) days; DE 28 (7–193) days; $P = 0.35$] among patients with DGF, as was the median time from transplantation to eGFR > 50 ml/min/1.73 m^2 [IE 91 (22–257) days, DE 91.5 (31–193) days; $P = 0.96$].

No marked between-group differences were observed for any other measured renal function parameter at month 12 including urine proteinuria/creatinine ratio, 24-h proteinuria and requirement for dialysis (Table 3). Proteinuria was reported as an adverse event in 15 patients (10.8%) (8 IE, 7 DE). At month 12, the incidence of dialysis (excluding dialysis after graft loss) was 24.6% in the IE group versus 32.4% in the DE group ($P = 0.35$). Other than six IE patients who required dialysis for DGF, no IE patient required dialysis while having a functioning graft. In the DE group, six patients required dialysis who had not experienced DGF: five during the first three months posttransplant, and one after month 3. The latter patient required one session due to fluid overload secondary to acute pulmonary edema during the period months 6–9. The median duration of dialysis (including dialysis for DGF) throughout the 12-month study was 11.5 days (range 1–28) and 5.5 days (1–29 days) in the IE and DE groups respectively ($P = 0.55$); the median number of dialysis sessions was 5 (1–26) and 3 (1–12). There were no significant differences in the mean duration of DGF (IE 10.2 ± 5.8 days, DE 7.6 ± 8.0 days; $P = 0.746$) between groups.

Table 3. Graft function parameters. All between-group differences were nonsignificant. Continuous variables are shown as median (range).

	IE (n = 65)	DE (n = 74)
Estimated GFR at month 12 (ml/min/1.73 m^2)*	48 (0–106)	49 (0–95)
Serum creatinine at month 12 (μmol/l)	159 (72–1044)	168 (70–920)
Nadir serum creatinine (μmol/l)	138 (57–637)	133 (51–695)
Time to nadir serum creatinine (days)	90 (2–378)	85 (1–421)
Proteinuria		
24-hour proteinuria at month 12 (mg/24 h)	280 (0–2340) (n = 22†)	265 (0–2340) (n = 20†)
Urine proteinuria/creatinine ratio at month 12 (g/mmol)	0.2 (0–1.3) (n = 33†)	0.3 (0–4.5) (n = 31†)
Incidence of posttransplant dialysis, n (%)‡	16 (24.6%)	24 (32.4%)
Number of dialysis sessions per patient	5 (1–26)	3 (1–12)
Duration of dialysis (days)	11.5 (1–28)	5.5 (1–29)

*Nankivell formula.
†Data missing in remaining patients.
‡During months 0–12, excluding day 1.

Adverse events

All patients experienced one or more adverse event during the study. The most frequent of these were peripheral edema, anemia, urinary tract infection and complications of the transplanted kidney (Table 4).

Thrombocytopenia and hyperlipidemia were reported as adverse events in fewer than 5% of patients in both treatment arms. In the IE and DE groups at month 12, mean total cholesterol was 6.2 ± 1.7 mmol/l and 6.0 ± 1.3

Table 4. Adverse events occurring in ≥15% of patients in either treatment group by month 12.

	IE (n = 65)	DE (n = 74)	All patients (n = 139)
Any adverse event	65 (100.0%)	74 (100.0%)	139 (100.0%)
Anemia	28 (43.1%)	34 (45.9%)	62 (44.6%)
Peripheral edema	26 (40.0%)	42 (56.8%)	68 (48.9%)
Urinary tract infection	25 (38.5%)	29 (39.2%)	54 (38.8%)
Complications of kidney graft*	20 (30.8%)	20 (27.0%)	40 (28.8%)
Hypertension	17 (26.2%)	21 (28.4%)	38 (27.3%)
Lymphocele*	13 (20.0%)	10 (13.5%)	23 (16.5%)
Dyslipidemia	12 (18.5%)	15 (20.3%)	27 (19.4%)
Constipation	12 (18.5%)	14 (18.9%)	26 (18.7%)
Renal impairment	10 (15.4%)	8 (10.8%)	18 (12.9%)
Hematoma†	7 (10.8%)	12 (16.2%)	19 (13.7%)
Hypokalemia	5 (7.7%)	13 (17.6%)	18 (12.9%)

*Mainly represented by DGF and slow graft function reported as adverse events.

†As reported by investigators as adverse events; these differ from the incidence as reported on specific wound healing case report forms (see text and Table 2).

mmol/l, mean triglyceride level was 2.3 ± 1.5 mmol/l and 2.1 ± 1.1, and lipid modifying agents were used in 34 patients (52.3%) and 37 patients (50.0%), respectively. Mean blood pressure at baseline and month 12 was 141/78 mmHg and 146/81 mmHg in the IE group, and 135/75 mmHg and 142/75 mmHg in the DE group, respectively.

Adverse events considered by the investigator to be related to study drug occurred in 46 IE patients (70.8%) and 44 DE patients (59.5%). One or more infection was reported in 44 IE patients (67.7%) and 44 DE patients (59.5%) by month 12. Bacterial infections occurred in 33 IE patients (50.8%) and 33 DE patients (44.6%), viral infections in 8 IE patients (12.3%) and 14 DE patients (18.9%), fungal infections in 6 IE patients (9.2%) and 4 DE patients (5.4%) and infections of unknown type in 12 IE patients (18.5%) and 15 DE patients (20.3%). CMV infection occurred in one patient (1.5%) in the IE group and in five patients (6.8%) in the DE cohort.

Serious adverse events were reported in 102 patients (73.4%), with a similar distribution in each group [IE 45 (69.2%,) DE 57 (77.0%)]. Serious adverse events included those related to the transplant (acute renal failure [11], increased serum creatinine [11], rejection [9], renal impairment [7] and complications of the transplanted kidney [6]), as well as pyelonephritis/acute pyelonephritis [11], urinary tract infection [12], sepsis/septic shock [9], hypertension [6], lymphocele [7] and pyrexia [6]. Malignancy was reported in one patient in the IE group (basal cell carcinoma diagnosed at 1 year posttransplant during everolimus and CsA therapy), and four patients in the DE group (basal cell carcinoma diagnosed on day 107 during everolimus/CsA therapy; Kaposi's sarcoma in a man aged 63 years of black race, diagnosed on day 59 posttransplant and 30 days after everolimus therapy initiation and which may have existed prior to transplant; leukemia diagnosed on day 15 which may have been present pretransplant; and prostate cancer diagnosed on day 11 which again may have been preexisting).

At month 12, adverse events or infections led to discontinuation of study medication in 17 IE patients (26.2%) and 26 DE patients (35.1%) (NS). Most study drug discontinuations occurred within the first three months [13 IE patients (20.0%) and 17 DE patients (23.0%); 12/17 of these DE patients discontinued before conversion to everolimus, while receiving MPA]. The most frequent of these were rejection [7 (2 IE, 5 DE)], infection [7 (5 IE, of whom a relation to study drug was suspected in 2 cases, 2 DE)] and renal impairment [4 (3 IE, 1 DE)]. Two patients in the DE cohort discontinued study medication due to proteinuria while receiving MPA. One patient in the IE arm discontinued due to thrombocytopenia; no patient discontinued due to dyslipidemia.

Wound healing

There was a similar incidence of wound healing complications in both treatment groups at month 12 for events related to initial transplant surgery [IE 26 (40.0%), DE 28 (37.8%), $P = 0.86$], and for all events [IE 28 (43.1%), DE 32 (43.2%), $P = 1.00$], and consistent with results reported previously at months 1 and 3 [29]. Very few new wound healing complications occurred after month 3 (2 in the IE group that were both reported as related to transplant surgery, and one in the DE group that was unrelated to the transplant procedure). Table 2 summarizes the type of wound healing complications occurring in relation to initial transplant surgery. For all wound healing events, regardless of relation to the transplant procedure, fluid collections (i.e. seroma, lymphocele or hematoma) also accounted for most complications (IE 26/28, DE 28/32), usually occurring within the first month posttransplant; in addition there were five cases of urine leak (IE 2, DE 3), two incisional hernias (both in the IE group) and two cases of wound dehiscence (both in the DE group).

Discussion

The use of immediate or early everolimus with reduced CsA resulted in good graft and patient survival and a BPAR rate of ∼20% with appropriate renal function in this population of kidney transplant patients preselected

for risk of DGF. These results are encouraging, indicating that the high CsA levels which are associated with the acute phase of nephrotoxicity in the first few months after transplantation [31] can be avoided by concomitant use of everolimus and low CsA without compromising efficacy.

In our population, *de novo* use of everolimus from the time of transplant was not associated with any disadvantage in terms of delaying graft recovery, even though patients were selected to have one or more risk factor for DGF. The rate and duration of DGF was similar with or without IE treatment from day 1 posttransplant. The observed DGF incidence of ~25% in this at-risk population was, as expected, somewhat higher than in standard-risk kidney transplant populations receiving everolimus in combination with low-exposure CNI therapy and corticosteroids, in which the rate of DGF has been reported to range from 8.7% [9] to 20% [6]. It is interesting to note, for comparison, that the large SYMPHONY study recently reported a DGF incidence of 32–36% in deceased-donor kidney transplant patients receiving CNI therapy [18]. Of note, deceased-donor patients receiving low-dose sirolimus with MPA in that study showed only a 21% rate of DGF, raising questions about the validity of findings from single-center studies suggesting that DGF is more likely in PSI-treated individuals [12,13,32–34]. Recently, it has been proposed that a BMI greater than 30–32 kg/m^2 is the most significant variable related to delayed wound healing in sirolimus-treated individuals and that a systematic program of wound care can produce wound healing complications comparable with that reported with other agents [35]. In the current trial, the discrepancy in CsA target levels means that no robust comparison of everolimus versus no everolimus can be performed since the difference in CNI exposure may have exerted an effect.

There was no evidence that IE incurred a penalty in terms of wound healing, since the occurrence of wound healing complications was virtually identical in both treatment groups. This is consistent with a recent pooled analysis of data from four studies in which no difference was observed for the incidence or severity of wound healing complications in kidney transplant patients receiving either MMF or everolimus as *de novo* immunosuppression [36]. Given the relatively robust indications in the literature that sirolimus may lead to an increased rate of wound healing complications following kidney transplantation [14–18,37], the intriguing question arises as to why a difference may potentially exist between these two PSIs. Contributing factors could be the known pharmacokinetic variations between everolimus and sirolimus [38] and the use of loading doses of sirolimus at some centers, but these possibilities are as yet unexplored.

Renal function stabilized between months 1 and 12 in both treatment groups. eGFR was similar between treatment groups at the end of the 12-month study. Renal function was less good than in standard-risk kidney transplant recipients, probably due to the high proportion of older recipients and donors in our population. Additionally, while everolimus levels remained within target, at least 45% of patients exceeded the target CsA C_2 range at all points other than in the DE group in month 1, and this overexposure would be expected to have impaired renal function. The higher-than-planned CsA exposure in both arms of the trial may also have influenced other endpoints, such as the BPAR rate, which should be borne in mind when interpreting the study results.

It is interesting to note that CMV infection was reported as an adverse event in only one patient who received everolimus immediately posttransplant, compared to five of the patients given MPA for the first four weeks. While these numbers are of course very low, and are not as robust as prospective collection of CMV data, they are consistent with larger-scale data from kidney [39] and heart [40] transplant populations.

The rate of study drug discontinuation due to adverse events was lower in the IE arm (26.2%) than in the DE arm (35.1%), for reasons that are not clear. During months 0–3, when the DE group were receiving MPA for the first four weeks and subsequently experiencing higher everolimus levels than in the IE group, there was little difference in the number of patients discontinuing because of adverse events [IE 12 (18.5%), DE 15 (20.3%)] and variations in discontinuation after month 3 cannot be attributed to the immunosuppression regimen since this was identical. The relatively high rate of discontinuation during the first four weeks of MPA treatment may have been influenced by the unexpectedly high CNI exposure during this period. The rate of drug discontinuation for adverse events in the IE group was not markedly higher than in other recent studies in PSI-free regimens [18,34]. Proteinuria, while reported as an adverse event in approximately 11% of patients, required study drug discontinuation in only two individuals.

In conclusion, introduction of everolimus either immediately or early after kidney transplantation in patients known to be at risk of DGF is associated with good efficacy, renal function and safety profile at 12 months when administered with a regimen of low-CsA, corticosteroids and IL-2R antibody induction. These results indicate that immediate introduction of everolimus is not associated with any disadvantage in terms of graft recovery or wound healing compared to initiation at week 4, even in patients at risk of DGF.

Authorship

JD, CL, LR, M-C M, FdG contributed to the design of the study. JD, FB, M-C M, LR, CL, RG, OT, BM, PM, J-P R, FB, PFW, DG, NK, NL, BC and EC performed the study and undertook data collection. SQ was the study statistician. JD, CL, LR, EC, SQ and FdG analyzed the data. All authors reviewed and approved the manuscript.

Funding support

The study was supported by Novartis Pharma AG (Basel, Switzerland) and Novartis Pharma SAS (Rueil-Malmaison, France).

Acknowledgements

The authors would like to thank the CALLISTO Study Group (see Appendix) and Professors Eric Rondeau (Paris) and Guy Touchard (Poitiers) for their participation in the Data Monitoring Committee. A medical writer funded by Novartis contributed to development of the manuscript.

Appendix. The CALLISTO Study Group

Prof Eric Alamartine, Saint Etienne; Dr Laetitia Albano, Nice; Dr Dany Anglicheau, Paris; Dr François Bayle, Grenoble; Dr Séverine Beaudreuil, Kremlin-Bicêtre; Prof François Berthoux, Saint Etienne; Prof Bernard Bourbigot, Brest; Dr Bruno Bourgeon, Saint-Denis-La Réunion; Dr Laura Braun-Parvez, Strasbourg; Dr Maria Brunet, Lyon; Dr Sophie Caillard, Strasbourg; Dr Elisabeth Cassuto, Nice; Prof Bernard Charpentier, Kremlin-Bicêtre; Prof Jacques Dantal, Nantes; Prof Antoine Durrbach, Kremlin-Bicêtre; Dr Jean-Pierre de Filippis, Saint-Etienne; Dr Hélène François, Kremlin-Bicêtre; Dr Carlos Frangie, Kremlin-Bicêtre; Dr Robert Genin, Saint-Denis-La Réunion; Prof Denis Glotz, Paris; Dr Bénédicte Janbon, Grenoble; Dr Nassim Kamar, Toulouse; Dr Niloufar Kossari, Paris; Dr Sylvie Lavaud, Reims; Dr Nicole Lefrançois, Lyon; Prof Christophe Legendre, Paris; Prof Yannick Le Meur, Brest; Prof Christophe Mariat, Saint-Etienne; Dr Hakim Mazouz, Amiens; Prof Pierre Merville, Bordeaux; Prof Françoise Mignon, Paris; Dr Marie-Christine Moal, Brest; Dr Delphine Morel, Bordeaux; Prof Emmanuel Morelon, Lyon; Prof Bruno Moulin, Strasbourg; Prof Marie Noëlle Peraldi, Paris; Dr Evangeline Pillebout, Paris; Dr Christine Randoux, Paris; Dr Jean-Phillipe Rerolle, Limoges; Prof Lionel Rostaing, Toulouse; Prof Jean-Paul Soulillou, Nantes; Dr Olivier Toupance, Reims; Dr Pierre François Westeel, Amiens. All participating centers were located in France.

References

1. Schuler W, Sedrani R, Cottens S, *et al.* SDZ RAD, a new rapamycin derivative: pharmacological properties *in vitro* and *in vivo*. *Transplantation* 1997; **64**: 36.
2. Schuurman HJ, Pall C, Weckbecker G, Schuler W, Bruns C. SDZ RAD inhibits cold ischemia-induced vascular remodelling. *Transplant Proc* 1999; **31**: 1024.
3. Farb A, John M, Acampado E, Kolodgie FD, Prescott MF, Virmani R. Oral everolimus inhibits in-stent neointimal growth. *Circulation* 2002; **106**: 2379.
4. Nishimura T, Faul JL, Berry GJ, Veve I, Pearl RG, Kao PN. 40-O-(2-hydroxyethyl)-rapamycin attenuates pulmonary arterial hypertension and neointimal formation in rats. *Am J Respi Crit Care Med* 2001; **163**: 498.
5. Waksman R, Pakala R, Baffour R, *et al.* Optimal dosing and duration of oral everolimus to inhibit in-stent neointimal growth in rabbit iliac arteries. *Cardiovasc Revasc Med* 2006; **7**: 179.
6. Vitko S, Tedesco H, Eris J, *et al.* Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. *Am J Transplant* 2004; **4**: 626.
7. Nashan B, Curtis J, Ponticelli C, Mourad G, Jaffe J, Haas T. On behalf of the 156 Study Group. Everolimus and reduced-exposure cyclosporine in *de novo* renal-transplant recipients: a three-year Phase II, randomized, multicenter, open-label study. *Transplantation* 2004; **78**: 1332.
8. Pascual J. Concentration-controlled everolimus (Certican): combination with reduced dose calcineurin inhibitors. *Transplantation* 2005; **79**(Suppl. 9): S76.
9. Chan L, Greenstein S, Hardy MA, *et al.* Multicenter, randomized study of the use of everolimus with tacrolimus after renal transplantation demonstrates its effectiveness. *Transplantation* 2008; **85**: 821.
10. Salvadori M, Scolari MP, Bertoni E, *et al.* for the EVEREST Study Group. Everolimus with very low-exposure cyclosporine a in *de novo* kidney transplantation: a multicenter, randomized, controlled trial. *Transplantation* 2009; **88**: 1194.
11. Nankivell BJ, Borrrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326.
12. Durrbach A, Rostaing L, Tricot L, *et al.* Prospective comparison of the use of sirolimus and cyclosporine in recipients of a kidney from an expanded criteria donor. *Transplantation* 2008; **85**: 486.
13. Stallone G, Di Paolo S, Schena A, *et al.* Addition of sirolimus to cyclosporine delays the recovery from delayed graft function but does not affect 1-year graft function. *J Am Soc Nephrol* 2004; **15**: 228.
14. Valente JF, Hricik D, Weigel K, *et al.* Comparison of sirolimus vs. mycophenolate mofetil on surgical complications and wound healing in adult kidney transplantation. *Am J Transplant* 2003; **3**: 1128.

15. Troppmann C, Pierce JL, Gandhi MM, Gallay BJ, McVicar JP, Perez RV. Higher surgical wound complication rates with sirolimus immunosuppression after kidney transplantation: a matched-pair pilot study. *Transplantation* 2003; **76**: 426.
16. Dean PG, Lund WJ, Larson TS, *et al.* Wound-healing complications after kidney transplantation: a prospective, randomized comparison of sirolimus and tacrolimus. *Transplantation* 2004; **77**: 1555.
17. Langer RM, Kahan BD. Incidence, therapy and consequences of lymphocele after sirolimus-cyclosporine-prednisone immunosuppression in renal transplant recipients. *Transplantation* 2002; **74**: 804.
18. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.*; for the ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
19. Schena FP, Pascoe MD, Alberu J, *et al.* Sirolimus CONVERT Trial Study Group. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation* 2009; **87**: 233.
20. Holdaas H, Bentdal Ø, Pfeffer P, Mjørnstedt L, Solbu D, Midtvedt K. Early, abrupt conversion of *de novo* renal transplant patients from cyclosporine to everolimus: results of a pilot study. *Clin Transplant* 2008; **22**: 366.
21. Budde K, Becker T, Sommerer C, *et al.* Analysis of renal function in everolimus/enteric-coated mycophenolate sodium treated *de novo* renal transplant recipients after calcineurin inhibitor withdrawal: the Zeus study. *Am J Transplant* 2009; **9**: 259. (Abstract 237).
22. Weir M, Mulgaonkar S, Pearson T, *et al.* Mycophenolate mofetil/sirolimus maintenance therapy after calcineurin inhibitor withdrawal in renal transplant recipients: 2-year outcomes of the Spare-the-Nephron (STN) trial. *Am J Transplant* 2009; **9**: 200. (Abstract 33).
23. 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.
24. Pietruck F, Klempnauer J, Arns W, *et al.* Efficacy and safety of everolimus (RAD)/enteric-coated mycophenolate sodium (EC-MPS) after calcineurin inhibitor (CNI) withdrawal in *de novo* renal transplant patients: final results of the ZEUS trial. *Am J Transplant* 2009; **9**: 259. (Abstract 1093).
25. Tedesco-Silva H Jr, Vitko S, Pascual J, *et al.* 12-month safety and efficacy of everolimus with reduced exposure cyclosporine in *de novo* renal transplant recipients. *Transpl Int* 2007; **20**: 27.
26. Tedesco-Silva H, Kim YS, Lackova E, *et al.* Everolimus with reduced-dose cyclosporine: results from a randomized study in 833 de-novo renal-transplant recipients. *Transpl Int* 2009; **22**: 186.
27. Bertoni E, Larti A, Farsetti S, *et al.* Cyclosporine (CyA) very low dose with everolimus (E) high dose is associated with better outcomes in renal transplant patients with respect to standard treatment with EC-MPS (M). *Transpl Int* 2009; **22**: 91.
28. Gaber AO, Kahan BD, Van Buren C, *et al.* Comparison of sirolimus plus tacrolimus versus sirolimus plus cyclosporine in high-risk renal allograft recipients: results from an open-label, randomized trial. *Transplantation* 2008; **86**: 1187.
29. Albano L, Berthoux F, Moal M-C, Rostaing L, Legendre C, Genin R. Incidence of delayed graft function and wound healing complications following deceased-donor kidney transplantation is not affected by *de novo* everolimus. *Transplantation* 2009; **88**: 69.
30. Nankivell BJ, Gruenewald SM, Allen R, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1995; **59**: 1683.
31. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Chapman JR, Allen RDM. Calcineurin nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004; **78**: 557.
32. Barsoum RS, Morsey AA, Iskander IR, *et al.* The Cairo kidney center protocol for rapamycin-based sequential immunosuppression in kidney transplant recipients: 2-year outcomes. *Exp Clin Transplant* 2007; **5**: 649.
33. Boratynska M, Banasik M, Patrzalek D, Klinger M. Impact of sirolimus treatment in kidney allograft recipients with prolonged cold ischemia times: 5-year outcomes. *Exp Clin Transplant* 2008; **6**: 59.
34. Chang GJ, Mahanty HD, Vincenti F, *et al.* A calcineurin inhibitor-sparing regimen with sirolimus, mycophenolate mofetil, and anti-CD25 mAb provides effective immunosuppression in kidney transplant recipients with delayed or impaired graft function. *Clin Transplant* 2000; **14**: 550.
35. Tiong HY, Flechner SM, Zhou L, *et al.* A systematic approach to minimizing wound problems for *de novo* sirolimus-treated kidney transplant recipients. *Transplantation* 2009; **87**: 296.
36. Margreiter R, Vitko S, Whelchel J, *et al.* Comparable incidence and severity of wound-healing-associated complications in the presence of MMF or everolimus following renal transplantation. *Am J Transplant* 2009; **9**: 201. (Abstract 34).
37. Goel M, Flechner SM, Zhou L, *et al.* The influence of various maintenance immunosuppressive drugs on lymphocele formation and treatment after kidney transplantation. *J Urol* 2004; **171**: 1788.
38. Kirchner GI, Meier-Wiedenbach I, Manns MP. Clinical pharmacokinetics of everolimus. *Clin Pharmacokinet* 2004; **43**: 83.
39. Vitko S, Margreiter R, Weimar W, *et al.* Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in *de novo* renal transplant patients. *Am J Transplant* 2005; **5**: 2521.
40. Eisen HJ, Tuzcu EM, Dorent R, *et al.* Everolimus for the prevention of allograft rejection and vasculopathy in cardiac transplant recipients. *N Engl J Med* 2003; **349**: 847.

