R E V I E W

The use of everolimus in renal-transplant patients

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Abstract: Despite advances in immunosuppressive therapy, long-term renal-transplantation outcomes have not significantly improved over the last decade. The nephrotoxicity of calcineurin inhibitors (CNIs) is an important cause of chronic allograft nephropathy (CAN), the major driver of long-term graft loss. Everolimus is a proliferation signal inhibitor with a mechanism of action that is distinct from CNIs. The efficacy and tolerability of everolimus in renal-transplant recipients have been established in a wide range of clinical trials. Importantly, synergism between everolimus and the CNI cyclosporine (CsA) permits CsA dose reduction, enabling nephrotoxicity to be minimized without compromising efficacy. Currently, everolimus is being investigated in regimens where reduced exposure CNIs are used from the initial post-transplant period to improve renal function and prevent CAN. By inhibiting the proliferation of smooth muscle cells, everolimus may itself delay the progression or development of CAN. Although everolimus is associated with specific side effects, these can generally be managed. By targeting the main causes of short- and long-term graft loss, everolimus has a key role to play in renal transplantation, which is being explored further in a number of ongoing Phase III–IV trials.

Keywords: calcineurin inhibitors, chronic allograft nephropathy, cyclosporine, everolimus, renal function, renal transplantation

Introduction

Although advances in immunosuppressive therapy have improved the control of acute allograft rejection, long-term renal-transplantation outcomes have not significantly improved over the last decade.[1] In renal-transplant patients, chronic allograft nephropathy (CAN; specifically interstitial fibrosis and tubular atrophy) is the main cause of graft failure. A number of factors have been implicated in the development of CAN, including donor age, acute rejection, vascular remodeling and calcineurin inhibitor (CNI)-induced nephrotoxicity.[2] The CNIs cyclosporine (CsA) and tacrolimus have been the cornerstone of immunosuppressive therapy for many years, due to their efficacy in preventing acute rejection. However, CNIs have nephrotoxic side effects that can directly contribute to renal dysfunction and compromise long-term outcomes.[3] Consequently, there has been strong interest in developing immunosuppressive regimens that maintain efficacy for the prevention of acute rejection, whilst minimizing risk factors for chronic allograft dysfunction and late graft loss.

Everolimus (Certican®; Novartis Pharma AG, Basel, Switzerland) is a proliferation signal inhibitor (PSI) with potent immunosuppressant effects.[4] In the setting of renal transplantation, everolimus has displayed comparable efficacy to mycophenolate mofetil (MMF) when used with corticosteroids and standard-dose CsA for prevention

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of acute rejection.[5,6] Moreover, Phase III studies in *de novo* renal-transplant patients have shown that everolimus allows for the early halving of CNI treatment whilst maintaining renal function, compared with full-dose CsA studies.[7]

In addition to its immunosuppressive efficacy, everolimus possesses other desirable attributes.[4] For example, the antiproliferative mechanism of action of everolimus may help to prevent the main causes of long-term graft loss by inhibiting the underlying processes that contribute to chronic allograft dysfunction.

This review will summarize the clinical trial data for everolimus and its role in renal transplantation.

Everolimus in renal transplantation – efficacy
Mechanism of action
Everolimus belongs to a class of immunosuppressive agents, the PSIs (also known as mammalian target of rapamycin [mTOR] inhibitors), that inhibit the progression of T cells from G1 into the S phase of the cell cycle.[8] By interfering with DNA replication at an early stage, PSIs exert an antiproliferative effect. The immunosuppressive action of everolimus has been demonstrated in preclinical studies in animal models of renal transplantation.[9] Importantly, everolimus has a mechanism of action that is distinct from CNIs.[3] Whereas CNIs prevent T-cell proliferation by blocking transcriptional activation of early T-cell-specific genes, inhibiting the production of T-cell growth factors (eg, IL-2), everolimus acts on a later stage of the T-cell response, by blocking the transduction of signals generated by such growth factors.[4] A synergistic immunosuppressive effect has been demonstrated between everolimus and CsA in preclinical studies, which could be due to their complementary modes of action.[10] These studies showed that, when used concomitantly, the equivalent efficacy of either agent alone could be achieved using 10% to 20% of the everolimus dose and 20% to 40% of the CsA dose,[10] providing a rationale for investigating whether everolimus could allow CsA dose reduction in patients receiving organ transplants.

Since everolimus inhibits growth factor-driven cell proliferation in general, its antiproliferative effects are not limited to the immune system.[4] PSIs have been shown to inhibit smooth muscle cell proliferation and prevent vascular remodeling.[11,12] Animal studies have demonstrated that the antiproliferative effects of everolimus reduce long-term graft-specific histological changes, delaying the progression of CAN, even when already at an advanced stage.[13]

Therefore, the mechanism of action of everolimus appears to target the key cause of CAN.

Clinical efficacy studies
Everolimus versus MMF with full-dose CsA
Two similarly designed Phase III studies (B201 and B251) compared the efficacy of everolimus *versus* MMF in *de novo* renal-transplant recipients (Table 1).[5,6] Both were 36-month, parallel-group studies in which patients were randomized to fixed everolimus doses (1.5 or 3 mg/day) or MMF (2 g/day) as part of a triple immunosuppressive therapy regimen with full-dose CsA and corticosteroids.[5,6] Treatment was blinded for the first year, followed by 2 years of open-label therapy. The primary endpoint was efficacy failure, a composite endpoint defined as the incidence of biopsy-proven acute refection (BPAR), graft loss, death, or loss to follow-up. In both studies, incidences of composite efficacy failure were similar between the MMF and everolimus 1.5 or 3.0 mg/day cohorts, with therapeutic equivalence maintained over 36 months.[5,6] In study B201, the incidence of graft loss at 36 months was higher in the everolimus 3 mg/day group (16.7%) compared with the everolimus 1.5 mg/day group (7.2%, p = 0.0048) and the MMF group (10.7%, p = 0.1067).[6] In Study B251, the rate of antibody-treated acute rejection was significantly lower with everolimus 1.5 mg than with MMF at 12 months (7.8% vs 16.3%; p = 0.01) and at 36 months (9.8% vs 18.4%; p = 0.014).[5]

Subsequent analysis of data from these studies demonstrated that patients with everolimus trough blood levels ≥3 ng/mL had a significantly reduced incidence of BPAR after 6 months of treatment, compared with those with trough blood levels <3 ng/mL (p < 0.0001).[14] In addition, patients receiving everolimus had higher mean serum creatinine levels than those receiving MMF.[15] After 12 months, protocol amendments were introduced, permitting lower CsA trough levels (50 to 75 ng/mL) in the everolimus groups, provided that everolimus blood trough levels were maintained above 3 ng/mL. After the protocol amendments, mean serum creatinine levels decreased slightly, or remained stable, with no increase in BPAR.[15] The finding that everolimus trough blood levels ≥3 ng/mL were necessary to gain the most clinical benefit highlighted that therapeutic drug monitoring might be useful in optimizing dosing for patients receiving everolimus and CsA.

Everolimus with full- or reduced-exposure CNIs
CNI therapy is associated with nephrotoxicity, which can complicate otherwise successful therapy.[16] Therefore,

Table 1 Summary of clinical studies of everolimus in renal-transplant patients

Study	Design	Number of patients	Treatments	Summary of main findings
B201[6]	36-month, Phase III, multicenter, randomized, parallel-group, double-blind (12 months), then open-label (24 months)	588 de novo Renal-amendment population: 236 patients	Everolimus (1.5 or 3 mg/day) vs MMF (2 g/day), in addition to CsA and steroids	• At 36 months, efficacy failure rates were similar for all groups (p = NS) • At 36 months, patient survival, graft survival and rejection rates were similar for everolimus 1.5 mg/day vs MMF; everolimus 3 mg/day demonstrated inferior graft survival (p = 0.0048 for everolimus 1.5 vs 3 mg/day)
B251[5]	36-month, Phase III, multicenter, randomized, parallel-group, double-blind for ≥12 months, then open-label	583 de novo	Everolimus (1.5 or 3 mg/day) vs MMF (2 g/day), in addition to CsA and steroids	• At 36 months, efficacy failure rates were similar for all groups (p = NS) • At 36 months, antibody-treated acute rejection was significantly lower for everolimus 1.5 mg/day vs MMF (p = 0.014)
B156[17]	36-month, Phase II, multicenter, randomized, open-label, parallel-group	111 de novo	Everolimus 3 mg/day in combination with basiliximab, steroids and either full-dose or reduced-dose CsA	• Efficacy failure was significantly lower in the reduced-dose vs full-dose CsA group at 6, 12 and 36 months (p < 0.05 for all) • Mean creatinine clearance was higher in the reduced-dose vs full-dose CsA group at 6 months (p = 0.009), 12 months (p = 0.007) and 36 months (p = 0.436)
US09[18]	6-month, exploratory, multicenter, randomized, open-label	92 de novo	Everolimus in combination with steroids, basiliximab and either low- or standard-exposure tacrolimus	• Efficacy was similar between groups, with BPAR occurring in 14% of patients in each group • Renal function (mean serum creatinine level and estimated GFR) was similar between groups (p = NS)
A2306[7]	12-month, Phase III, randomized, open-label, parallel-group	237 de novo	Everolimus 1.5 vs 3 mg/day, in combination with steroids and low-exposure CsA (C2 monitoring)	• After 6 months, median serum creatinine levels were 133 and 132 μmol/L in the everolimus 1.5 and 3 mg/day groups, respectively • After 6 months, there were no significant differences between groups for any efficacy parameter
A2307[7]	12-month, Phase III, randomized, open-label, parallel-group	256 de novo	Everolimus 1.5 vs 3 mg/day, in combination with steroids, low-exposure CsA (C2 monitoring) and basiliximab induction therapy (Days 0 and 4)	• After 6 months, median serum creatinine levels were 130 μmol/L in both everolimus groups • After 6 months, there were no significant differences between groups for any efficacy parameter
CENTRAL[27]	6-month, single-center, pilot	20 recipients of a first or second single renal transplant from a deceased or living donor	Patients were converted from CsA to everolimus 7 weeks post-transplant; all received basiliximab induction therapy with maintenance EC-MPS and steroids	• Calculated GFR improved significantly following conversion from CsA to everolimus (p = 0.001) • BPAR occurred in 3/20 (15.0%) patients during the 7 weeks post-conversion to everolimus, but all episodes were mild and reversible, with subsequent recovery of renal function • Abrupt conversion from CsA to everolimus was well tolerated

Abbreviations: BPAR, biopsy-proven acute refection; CsA, cyclosporine; CNI, calcineurin inhibitor;; EC-MPS, enteric-coated mycophenolate sodium; GFR, glomerular filtration rate; IL-2, interleukin-2; MMF, mycophenolate mofetil; NS, not significant.

exploring drug combinations that allow for a reduction in CNI exposure might help to improve long-term outcomes.

Study B156 was a Phase II, 3-year, multicenter, randomized, open-label, parallel-group, CsA dose-finding study of everolimus in *de novo* renal-transplant recipients (Table 1).[17] After transplantation, patients were randomized to either full-dose (trough blood level 125 to 250 ng/mL from 3 to 36 months) or reduced-dose (trough blood level 50 to 100 ng/mL from 3 to 36 months) CsA, in addition to identical dose regimens of everolimus (3 mg/day), basiliximab (20 mg prior to transplantation and on Day 4) and corticosteroids.[17] Following a protocol amendment, CsA dosing was adjusted to achieve trough blood levels of 50 to 75 ng/mL and everolimus dosing was adjusted to ensure trough blood levels ≥3 ng/mL in all patients continuing treatment from 12 months onwards.[17] The incidence of efficacy failure (BPAR, graft loss, death, or loss to follow-up) was significantly lower in the reduced-dose CsA group compared with the full-dose CsA group at 6 months (p = 0.046), 12 months (p = 0.012) and 36 months (p = 0.032), mainly as a result of the lower incidence of BPAR in the reduced-dose CsA group, compared with the full-dose group (3.4% vs 15.1% at 6 months; 6.9% vs 17.0% at 12 months; 12.1% vs 18.9% at 36 months).[17] In addition, mean serum creatinine levels were numerically lower in patients receiving reduced-dose CsA compared with full-dose CsA, and mean creatinine clearance rates were significantly higher in reduced-dose *versus* full-dose patients at 6 months (p = 0.009) and 12 months (p = 0.007).[17] Following transition to the amended protocol after 12 months, mean serum creatinine levels fell in the full-dose CsA group, whilst mean serum creatinine and creatinine clearance values remained stable in the reduced-dose CsA group, reflecting the smaller reduction in CsA dose in these patients.[17] Study B156 therefore demonstrated that using everolimus with reduced-dose CsA resulted in preserved renal function without loss of efficacy, when compared with standard-dose CsA regimens.

Similar results were found with low-exposure tacrolimus and everolimus in Study US09, which was a prospective, 6-month, multicenter, open-label, exploratory study. *De novo* renal-transplant recipients (n = 92) were randomized to everolimus, steroids and basiliximab with low or standard tacrolimus exposure (Table 1).[18] Lower tacrolimus exposure was not associated with loss of efficacy compared with a standard tacrolimus regimen, with BPAR occurring in 14% of patients in both the low and standard tacrolimus exposure groups at 6 months. Moreover, there were no significant differences in renal function between groups at 6 months: mean serum

creatinine levels were 112 ± 31 and 127 ± 50 µmol/L, and mean estimated glomerular filtration rates (GFRs) were 75.3 ± 16.6 and 72.5 ± 15.2 mL/min, in the low and standard tacrolimus exposure groups, respectively.[18] Overall, the study found that treatment with everolimus, in combination with low-exposure tacrolimus, steroids and basiliximab, was effective and well tolerated, resulting in good efficacy with excellent renal function at 6 months.[18]

Given that clinical data are lacking concerning therapeutic action and systemic exposure of a combined regimen of tacrolimus and everolimus in renal transplantation, EVEROTAC, an investigator-driven, prospective, open-label, randomized Phase II pharmacokinetic (PK) study was undertaken in five Spanish centers randomly comparing two fixed everolimus dosages (0.75 mg bid, Group A, or 1.5 mg bid, Group B) in combination with tacrolimus (Pascual unpublished data). Antibody induction was not permitted and all patients received steroid therapy. Complete 12-hour PK curves of both drugs (high performance liquid chromatography) were performed at Days 4, 14 and 42 post-transplant. After Day 42, everolimus trough levels were adjusted to 3 to 8 ng/mL and tacrolimus to 5 to 8 ng/mL. Higher tacrolimus trough blood levels were observed with everolimus dose of 0.75 mg bid. Accordingly, the exposure to tacrolimus was lower in the group receiving 3 mg/day everolimus despite this combination requiring higher tacrolimus doses to maintain target concentrations. Everolimus minimum concentration (C_{min}), maximum concentration (C_{max}) and area under the curve (AUC) were very low with the initial dose of 0.75 mg bid when combined with tacrolimus and everolimus 1.5 mg bid seems to be the minimal initial advisable dose for Phase III trials. Higher doses would probably be needed for tacrolimus minimization strategies, as 3 mg/day appears insufficient to achieve >3 ng/mL during the first 2 weeks. Acute rejection incidence was 17%, good graft function was consistently achieved, wound healing was uneventful in all patients and lymphocele was diagnosed in only two cases (6%) (Pascual, unpublished data).

Everolimus with reduced-exposure CsA

A2306 and A2307 were similarly designed Phase III, 1-year, parallel-group studies in which *de novo* renal-transplant patients were randomized to everolimus at an initial dose of 1.5 or 3 mg/day (with subsequent dosing adjusted to maintain trough levels of ≥3 ng/mL for both groups), in combination with reduced-exposure CsA and steroids; patients in A2307 also received induction therapy with basiliximab on the day of

transplantation and after 4 days (Table 1).[7] In Study A2306, CsA C2 (the 2-hour post-dose blood CsA concentration) target ranges were 1000 to 1400 ng/mL for Weeks 0 to 4, 700 to 900 ng/mL for Weeks 5 to 8, 550 to 650 ng/mL for Weeks 9 to 12 and 350 to 450 ng/mL thereafter, but in Study A2307, the ranges were lower, owing to the use of basiliximab induction therapy: 500 to 700 ng/mL for Weeks 0 to 8 and 350 to 450 ng/mL thereafter.[7] The primary efficacy endpoint in both studies was renal function at 12 months. Secondary endpoints included the incidence of efficacy failure and its individual components at 12 months. Serum creatinine levels were stable from Month 2 or 3 onwards. When data from Study A2306 were compared with data from the B251 and B201 studies, concentration-controlled everolimus with reduced-exposure CsA was shown to result in an improvement in serum creatinine, creatinine clearance and GFR, compared with everolimus plus full-exposure CsA.[7,14] There were no significant differences between the everolimus 1.5 and 3 mg/day groups in either study for any efficacy parameter, and the incidences of efficacy failure and BPAR were comparable to those observed in the B251 and B201 studies.[7] However, BPAR occurred more frequently with everolimus 1.5 mg/day in Study A2306 (25.0%) than in Study A2307 (13.7%), suggesting that anti-IL-2 receptor induction therapy is probably beneficial in reducing the risk of early BPAR when used with a lower dose of everolimus.[7] Importantly, a comparison of data from Studies B201 (full-exposure CsA) and A2306 (reduced-exposure CsA) demonstrated that CsA blood levels can be reduced by at least 57% at 12 months when used in combination with everolimus, without adversely affecting either efficacy or safety.[19] Consistent with data from studies B201 and B251, in which full-dose CsA was used,[14] a *post hoc* analysis of data from Study A2306 demonstrated that optimal efficacy and safety are achieved in patients receiving reduced-exposure CsA if everolimus trough blood levels are between 3 and 8 ng/mL.[20] Ongoing studies are continuing to investigate the use of therapeutic drug monitoring to optimize everolimus levels in combination with reduced-exposure CsA.[21–26]

CNI elimination

The use of CNIs during the initial post-transplant period to prevent acute rejection and the subsequent elimination of CNIs from the treatment regimen may provide a means of preventing long-term nephrotoxicity.

The CENTRAL (CErtican Nordic Trial in RenAL transplantation) study evaluated whether early conversion to everolimus from CsA might improve long-term renal function and slow down the progression of CAN (Table 1).[27] In this single-center pilot study, 20 renal-transplant patients without prior rejection were converted from CsA to everolimus at Week 7 post-transplantation. All patients received basiliximab induction therapy with maintenance enteric-coated mycophenolate sodium (EC-MPS) and corticosteroids. Patients were monitored for 7 weeks, with a follow-up visit after 6 months.[27] After conversion to everolimus and CsA elimination, calculated GFR improved significantly, from 51 ± 11 mL/min at the time of conversion to 58 ± 12 mL/min at Week 7 post-conversion and 57 ± 17 mL/min at the 6-month follow-up visit (p = 0.001).[27] BPAR occurred in 3/20 (15.0%) patients during the 7 weeks post-conversion, but all episodes were mild and reversible, with subsequent recovery of renal function.[27] In this pilot study, abrupt conversion from CsA to everolimus at Week 7 post-transplant was well tolerated. Consequently the trial has been extended and is currently ongoing with planned enrollment of 300 patients and a follow-up of 3 years.

Additional benefits and clinical considerations
Multifaceted benefits
Antiproliferative effects

As described earlier, the antiproliferative effects of everolimus are not limited to the immune system.[4] PSIs have been shown to inhibit smooth muscle cell proliferation and prevent vascular remodeling. This attribute may represent an additional benefit of everolimus as these proliferative processes are implicated in the development of CAN in renal-transplant recipients and cardiac allograft vasculopathy in cardiac-transplant recipients, which are key causes of allograft dysfunction[11,12] Furthermore, animal studies have demonstrated that the antiproliferative effects of everolimus reduce long-term graft-specific histological changes, delaying the progression of CAN, even when already at an advanced stage.[13] Studies of sirolimus and everolimus drug-eluting stents further support the ability of this class of drugs to inhibit pathological vascular remodeling.[28,29] Taken together, these data suggest that the mechanism of action of everolimus appears to target the key cause of CAN.

Reduced CMV infection

A number of other factors aside from vascular remodelling have also been implicated in the development of CAN, including acute rejection episodes, CNI-induced nephrotoxicity, and complications of immunodeficiency such as opportunistic CMV infection.[30] CMV is a leading

cause of infectious complications in patients who have undergone solid organ transplantation. CMV infection is associated with allograft rejection, decreased graft and patient survival, and predisposition to malignancies.[31] In the B201 study, the incidence of viral infection, particularly CMV infection, was significantly higher after treatment with MMF compared with everolimus.[6] Similarly, earlier studies have suggested a reduced CMV infection rate with sirolimus.[32]

Anti-neoplastic effects

PSIs have been associated with anti-neoplastic effects as a result of their inhibition of cellular signaling pathways involved in critical functions such as cell division, T-cell activation, invasion and growth factor production.[33] A lower incidence of malignancies has been observed in patients receiving PSIs in clinical trials, compared with those receiving CNI-based immunosuppression.[33] In renal carcinoma, everolimus has been shown to significantly prolong progression-free survival after failure of the approved therapies sunitinib or sorafenib in patients with advanced renal cell carcinoma and is currently being investigated in multiple tumor types.[34]

Adverse events

Renal-transplant recipients frequently experience adverse events as a result of surgery, immunosuppressant side effects and over-immunosuppression. The adverse events most frequently associated with everolimus treatment are similar to those associated with other immunosuppressive therapies, but PSIs, as a class, are associated with a number of specific adverse events.

Proteinuria

Many studies have confirmed that patients with CAN and, to a certain extent, patients without pre-existing CAN, are at risk of high-range urinary protein excretion after conversion to sirolimus.[35,36] Moreover, proteinuria may occur in patients who receive *de novo* sirolimus.[37] Less data are available about everolimus, but in the A2306 and A2307 studies, conducted in *de novo* renal-transplant recipients, proteinuria (determined by a spot urine protein/creatinine ratio) was detected in <5% of patients.[38] The onset of abundant urinary protein excretion is of importance because proteinuria is a marker for the risk of progressive decline in renal function[39] and is an important predictor of renal dysfunction following conversion from a CNI- to a PSI-based regimen.[40] However, the mechanisms of PSI-induced proteinuria continues to be debated.[41]

Patients with pre-existing proteinuria at levels >800 mg/day should not undergo CNI elimination with conversion to a PSI.[40] Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers may be used for the management of both hypertension and proteinuria in patients receiving everolimus.[39,42] If massive proteinuria occurs after conversion, (re)introduction of CNI may partially reverse urinary protein excretion and seems a reasonable option.[41]

Dyslipidemia

Dyslipidemia is common in solid organ transplant recipients. PSIs have been linked to hyperlipidemia, with increased serum cholesterol and triglyceride levels occurring in 30% to 50% of patients.[35,43]

In renal-transplant recipients, sirolimus induces dose-dependent hyperlipidemia, including hypertriglyceridemia, increased low-density lipoprotein (LDL)-cholesterol and increased apolipoprotein B-100 and apolipoprotein C-III circulating levels. A similar increase in serum cholesterol and triglyceride levels has also been reported in renal transplant recipients receiving everolimus.[44] However, when compared with MMF in *de novo* cardiac transplantation, everolimus did not induce clinically meaningful changes in triglyceride, LDL-cholesterol, or high-density lipoprotein-cholesterol levels.[45] Dyslipidemia should be managed in accordance with guidelines, using lifestyle changes and drug therapy (eg, statins, fibrates).[46] A crossover study conducted in healthy individuals found that single-dose administrations of everolimus with either atorvastatin or pravastatin did not influence the pharmacokinetics of everolimus or the statins to a clinically relevant extent.[47]

Hypercholesterolemia and hypertriglyceridemia are major risk factors for atherosclerosis and associated cardiovascular disease. Recent pre-clinical studies with sirolimus have demonstrated protection from atheroma progression in hyperlipidemic apolipoprotein E-deficient mice.[48–50] As this may be a class effect of PSIs, studies are required to investigate if everolimus has any beneficial effects on the development on atherosclerosis.[44]

Wound healing

Due to the antiproliferative action of PSIs, concerns have been raised over possible effects on tissue-regeneration processes. For example, the antiproliferative action of everolimus can reduce the healing of lymphatic channels that are divided during transplant surgery, which may lead to lymphatic leakage and the formation of a lymphocele.[51] The potential impact on wound healing is most relevant in the immediate

post-transplant period. Increased incidence of wound-healing complications associated with sirolimus treatment has been observed in renal transplantation.[52] However, data pooled from the B201, B251, A2306 and A2307 everolimus studies showed that the overall incidence and severity of wound-healing-associated complications following renal transplantation were comparable for MMF- and everolimus-based immunosuppressive regimens.[53]

Edema

Limb edema and bilateral eyelid edema have been observed in transplant recipients receiving sirolimus and everolimus.[36,51,54] Although edema appears to be a class effect, in a study of 56 cardiac-transplant patients undergoing CNI reduction or elimination, fewer patients experienced edema with everolimus (14.3%) than with sirolimus (64.3%; $p = 0.001$).[55] When edema does occur with everolimus treatment, dose reduction may be required, but it is generally still possible to maintain everolimus trough blood levels within the optimal therapeutic window (3 to 8 ng/mL).[51]

Pharmacokinetics: safety considerations

Although everolimus and sirolimus are PSIs with similar chemical structures (everolimus is a derivative of rapamycin bearing a hydroxyethyl chain at position 40), there are pharmacokinetic and pharmacodynamic differences between the molecules.[15] For example, the half-life of everolimus (28 hours) is shorter than that of sirolimus (62 hours). Consequently steady-state is achieved more quickly with everolimus (4 days) than with sirolimus (6 days), due to differences in their treatment regimens.[35] These differences may explain certain variations in the safety profiles of the two agents. For example, sirolimus has been associated with the development of pneumonitis following renal transplantation,[56–58] which may be a cause of pulmonary fibrosis in later stages of the disease. By contrast, no cases of pneumonitis have been reported in renal-transplant patients receiving everolimus with low-dose CsA. Indeed, there have been case reports of the successful resolution of sirolimus-associated pneumonitis following switching from sirolimus to everolimus in renal-transplant patients[59] and recipients of other solid-organ transplants.[60]

Ongoing Phase III–IV studies with everolimus

A number of Phase III and IV studies are underway to investigate the use of everolimus in renal transplantation and these studies are described here and in Table 2.

De novo renal transplantation

The open-label Mycophenolate sodium vs Everolimus or Cyclosporine with Allograft Nephropathy as Outcome (MECANO) study is investigating an initial 6-month regimen of basiliximab, CsA, EC-MPS and prednisolone, followed by randomization to 18 months of treatment with either CsA plus prednisolone, EC-MPS plus prednisolone, or everolimus plus prednisolone (Table 2).[61] The aim of the study is to achieve optimal immune suppression with maximal reduction of side effects, especially of vascular injury. The primary outcome is the degree of inflammation, fibrosis and arteriolar hyalinosis in renal biopsies taken 6 and 24 months post-transplantation.[61]

Immediate (de novo) versus delayed everolimus administration

Delaying the administration of everolimus in *de novo* renal-transplant patients allows a shift of the anti-proliferative effect at the early post-transplantation period. CALLISTO is a multicenter, open-label, 12-month study, being conducted in patients who are deceased-donor renal-transplant recipients at risk of delayed graft function (DGF) (Table 2).[62] Patients are randomized to receive immediate everolimus (within 48 hours post-transplantation) or delayed everolimus after 4 weeks of EC-MPS treatment. All patients received anti-IL-2 receptor induction therapy and steroids. The primary endpoint is a composite of BPAR, graft loss, death, DGF, wound-healing events, or loss to follow-up.

CNI reduction or elimination

The use of therapeutic drug monitoring to optimize everolimus levels in combination with reduced-exposure CsA is being investigated further in the EVEREST (the upper target EVErolimus RandomisEd STudy) AIT02 study (Table 2).[21] This is a 6-month, multicenter, randomized, open-label study that is comparing two immunosuppressive regimens in *de novo* renal-transplant recipients: (a) higher everolimus target trough levels (C0 8 to 12 ng/mL) with very low-dose CsA (C2 600 ng/mL, tapered to 300 ng/mL at Month 3) and (b) standard everolimus target trough levels (C0 3 to 8 ng/mL) with low-dose CsA (C2 600 ng/mL, tapered to 500 ng/mL at Month 3). The primary objectives are to assess if the optimized new regimen with higher everolimus target trough levels and very low-dose CsA allows improvement in 6-month creatinine clearance, in comparison with the standard everolimus regimen with low-dose CsA and to assess if the optimized new regimen is equally effective in preventing acute rejection, in comparison with the standard regimen.

Table 2 Summary of ongoing Phase III–IV studies with everolimus in renal-transplant patients

Study	Design	Patient population	Treatments	Primary outcome	Secondary outcomes
MECANO[61]	24-month, prospective, multicenter, randomized, open-label	255 patients undergoing first or second renal transplant	6 months treatment with basiliximab, CsA, EC-MPS and prednisolone, followed by randomization to 18 months treatment with CsA + prednisolone, EC-MPS + prednisolone, or everolimus + prednisolone	Degree of inflammation, fibrosis and arteriolar hyalinosis in renal biopsies taken at Months 6 and 24	• Vascular assessments by IMT and M-mode of carotis interna • Blood pressure and number of anti-hypertensive drugs • Lipid profile • Renal allograft survival and function • Patient survival • Incidence of malignancies • Infectious complications
CALLISTO A2420[62]	12-month, Phase III, multicenter, open-label	139 de novo with risk of developing DGF	Immediate vs delayed everolimus after 1 month of EC-MPS treatment. All patients also received anti-IL-2 receptor induction therapy and steroids	To compare the incidence of the composite of BPAR, graft loss, death, DGF and wound healing complications with immediate vs delayed administration of everolimus at 3 months	• Renal function at 3 months (creatinine clearance; Nankivell) at 6 and 12 months (serum creatinine, creatinine clearance [Nankivell and Cockcroft Gault]) and proteinuria • Wound healing complications • To assess efficacy (BPAR, graft loss/ re-transplantation, death or lost to follow-up) at 6 and 12 months post transplantation • Safety based on adverse event reporting
EVEREST AIT02[21]	6-month, Phase III, multicenter, randomized, open-label	285 de novo	Higher everolimus target trough levels (C0 8 to 12 ng/mL) with very low-dose CsA (C2 600 ng/mL, tapered to 300 ng/mL at Month 3) or standard everolimus target trough levels (C0 3 to 8 ng/mL) with low-dose CsA (C2 600 ng/mL, tapered to 500 ng/mL at Month 3)	• To assess if higher target everolimus trough levels and very-low-dose CsA improves the 6-month creatinine clearance, in comparison with the standard everolimus regimen with low-dose CsA • To assess if the optimized new regimen is equally effective in preventing acute rejection, compared with the standard regimen	• Incidence of BPAR, graft loss, death or lost to follow-up • Efficacy parameters: BPAR, antibody-treated acute rejection and clinically-confirmed acute rejection • Evaluate the percentage of patients with a stable serum creatinine increase of more than 30% from the previous nadir after transplantation • Incidence of graft loss or death • Safety and tolerability
A2309[22]	24-month, Phase III, multicenter, randomized, parallel-group, open-label	833 de novo	Everolimus (1.5 or 3 mg/day) + reduced-exposure CsA vs EC-MPS + standard-exposure CsA	Treated biopsy acute rejection, graft loss and survival within 12 months	• Graft loss, survival and renal function at 12 months

Study	Design	N	Treatment	Renal function assessment	Outcomes
ZEUS A2418[63]	12-month, Phase IV, multicenter, randomized, open-label study with additional 4-year follow-up	300 de novo renal transplant patients	Following basiliximab induction therapy, all patients were treated with CsA, EC-MPS and steroids for 4.5 months, then randomized to either continue the same treatment or switch from CsA to everolimus	Renal function assessed as GFR (Nankivell) 12 months after transplantation	• To assess renal function by GFR (Cockcroft-Gault and MDRD) at Month 12 post transplant • To assess efficacy (BPAR, graft loss, death) at Month 6 and 12 • Occurrence of treatment failures up to or at Month 12 • To assess evolution of renal function between Month 4.5 and 12 (creatinine slope) • To assess safety and tolerability at Month 4.5 and 12 • Changes in cardiovascular risk (Framingham Score) between Month 4.5 and 12
A2426[23]	12-month, Phase IV, multicenter, randomized, open-label, parallel-group	230 de novo	Everolimus + IL-2 receptor antagonist + steroids, in combination with one of two tacrolimus doses	Renal function, assessed as GFR (MDRD), at Month 12	• BPAR incidence from Month 4 to Month 12 • Efficacy (BPAR, graft loss, death) • Renal function • Incidence of AEs and SAEs and new onset diabetes mellitus
HERAKLES (ADE13)[24]	12-month, Phase III, multicenter, randomized, open-label, parallel-group	450 de novo	Everolimus in combination with low-dose CNI vs CNI-free vs EC-MPS with standard-dose CNI	Renal function, assessed as GFR (Nankivell), at Month 12	• Renal function (GFR) assessed by Cockcroft Gault and MDRD at Month 12 • Efficacy (BPAR, graft loss, death) at Months 6 and 12 • Treatment failure up to Month 12 • To assess efficacy (BPAR, graft loss, death, renal function [creatinine, GFR]) safety and tolerability (CMV, tumor incidence, cardiovascular risk, proteinuria) at follow up visits at Month 18, 24, 36, 48, and 60
SOCRATES (A2421)[64]	12-month, Phase IV, multicenter, randomized, open-label, parallel-group	177 de novo	Initial treatment with CsA, EC-MPS and steroids, followed after 2 weeks by everolimus and EC-MPS with either CsA or steroids (ie, either steroid withdrawal or CsA withdrawal). These two groups will be compared with a third control group that will receive CsA, EC-MPS and steroids	Renal function, assessed as estimated GFR (Nankivell), at Month 12	• BPAR incidence at Month 12 • Histology (CAN, subclinical acute rejection) at Month 12 • Proteinuria at Month 12 • Patient graft survival at Month 12 • Incidence of wound problems • Prevalence of post-transplant diabetes mellitus at Month 12 • Effect on cardiovascular health • Incidence of anemia, leucopenia, thrombocytopenia and erythropoietin usage

(Continued)

Table 2 (*Continued*)

Study	Design	Patient population	Treatments	Primary outcome	Secondary outcomes
					• Quality of life at Month 12 • Effect on healthcare resource utilization (hospital events) and employment status • Influence of parameters on safety and efficacy outcomes
A2423[65]	12-month, Phase IV, multicenter, randomized, open-label, parallel-group	51 *de novo*	Everolimus in combination with basiliximab and steroids, in a maintained vs discontinued CsA regimen	Renal function, assessed as calculated GFR, at Month 12	• Serum creatinine and calculated serum creatinine at Months 6 and 12 • Incidence of composite of BPAR, graft loss, death, or loss to follow-up at Months 6 and 12 • Incidence of graft loss, death, BPAR, antibody-treated acute rejection, clinically confirmed acute rejection and clinically confirmed chronic rejection at Months 6 and 12 • Safety based on adverse event reporting
A2419[25]	12-month, Phase IV, multicenter, randomized, open-label, parallel-group	119 *de novo*	Everolimus + basiliximab, in combination with CsA, either discontinued after 3 months or minimized	Renal function, assessed as calculated GFR, serum creatinine and calculated creatinine clearance at Month 12	• Incidence of composite of BPAR, graft loss, death, or loss to follow-up at Month 12 • Incidence of graft loss, death, BPAR, antibody-treated acute rejection, clinically confirmed acute rejection and clinically confirmed chronic rejection at Month 12 • Safety based on adverse event reporting
ASCERTAIN (A2413)[26]	Phase IV, randomized, open-label, parallel-group	398 maintenance patients	Patients are randomized to one of three groups: • Continuation of current immunosuppressive regimen without everolimus • Initiation of everolimus with discontinuation of CNI, or • Initiation of everolimus with reduction of CNI blood levels by 70% to 90%	Renal function evaluated by measured GFR at Month 24	• Patient and graft survival and BPAR at 24 months • Other renal function data • Progression of CAN • Progression of atherosclerosis • Safety based on adverse event reporting

Abbreviations: BPAR, biopsy-proven acute rejection; CAN, chronic allograft nephropathy; CsA, cyclosporine; CNI, calcineurin inhibitor; DGF, delayed graft function; EC-MPS, enteric-coated mycophenolate sodium; GFR, glomerular filtration rate; IL-2, interleukin-2; IMT, intima-media thickness; MDRD, modification of diet in renal disease.

A2309 is a Phase III, 24-month, multicenter, randomized, open-label, non-inferiority study that will assess two doses of everolimus in combination with reduced-exposure CsA, compared with everolimus/EC-MPS administered with standard-exposure CsA (Table 2).[22] A2309 has enrolled 833 *de novo* renal-transplant patients at 83 centers worldwide. The primary objective of the study is to demonstrate that at least one of the everolimus treatment regimens is not inferior to the EC-MPS treatment regimen within 12 months of the initial dose of study medication with respect to primary efficacy failure, namely, the composite efficacy endpoint of treated BPAR episodes, graft loss, death or loss to follow-up.[22]

The ERIC study is a Spanish multicenter, randomized, open-label trial, to assess the effect of CNI withdrawal and early (at 3 months) introduction of everolimus on renal allograft function. The primary end-point will be calculated GFR at 2 years, and the first functional and histological results will be available in 2010.

The ZEUS A2418 study has been conducted in *de novo* renal-transplant patients in order to assess whether an EC-MPS plus everolimus regimen after CNI withdrawal was as safe and well-tolerated as the CsA plus EC-MPS regimen, and to determine whether this regimen resulted in improved renal function (Table 2).[63] After induction therapy with basiliximab, all patients were treated with CsA, EC-MPS and corticosteroids for the first 4.5 months post-transplantation. Subsequently, patients were randomized 1:1 to either continue the current regimen of CsA and EC-MPS or to convert from CsA to everolimus. The primary objective of this trial was to show superiority of a CNI-free regimen with respect to the renal function at Month 12 post transplant assessed by GFR (Nankivell method) compared with the standard CNI-based regimen. The results have recently been submitted for publication.

Several other studies are investigating the use of everolimus treatment as a means of reducing or eliminating CNI therapy in *de novo* renal-transplant patients (Table 2).[23–26,64,65]

Maintenance renal-transplant recipients

The Assessment of everolimuS in addition to Calcineurin inhibitor reduction in the maintEnance of Renal TrAnsplant RecipIeNts (ASCERTAIN; A2413) study is a pivotal Phase IV trial that will assess the feasibility of CNI reduction/elimination in maintenance renal-transplant patients suffering from renal impairment, and its impact on renal function and cardiovascular risk (Table 2).[26] Patients are randomized to one of three parallel treatment groups: continuation of the current immunosuppressive regimen without everolimus;

initiation of everolimus with discontinuation of CNI; or initiation of everolimus with reduction of CNI blood levels by 70% to 90%.[26] The study is designed to evaluate whether the initiation of everolimus, together with the reduction or discontinuation of CNIs, will improve graft function and reduce the progression of CAN in maintenance renal-transplant recipients.[26] The development of atherosclerosis in the native arteries of the patients will also be explored.[26]

It is noteworthy that the effect of conversion from sirolimus to everolimus has been assessed in a 6-month, pilot study. Eleven maintenance renal-transplant patients receiving sirolimus, mycophenolic acid and corticosteroids without CNI therapy were converted to everolimus 8 mg/day (8 to 15 ng/mL).[66] Mean GFR and mean renal-phosphate threshold remained stable throughout the study and no patient died, lost their graft or experienced BPAR after conversion.[66]

Conclusions

Evidence from clinical trials supports the efficacy and tolerability of everolimus in renal-transplant recipients. Notably, clinical trial data indicate that everolimus can facilitate CNI minimization/halving without compromising efficacy. By facilitating CNI minimization, and inhibiting smooth-muscle proliferation, everolimus may prevent the progression or development of CAN, hypotheses which are currently being investigated in the A2309,[22] MECANO[61] and ASCERTAIN (A2413)[26] trials. There are several class-specific side effects associated with everolimus, but experience to date suggests that these can be managed. Everolimus has a key role to play in addressing current unmet needs in transplantation by targeting the causes of short and long-term graft loss. Ongoing clinical studies will provide further information to refine the therapeutic role of everolimus in renal transplantation.

Acknowledgments

References

1. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant*. 2004; 4:378–383.
2. Chapman JR, O'Connell PJ, Nankivell BJ. Chronic renal allograft dysfunction. *J Am Soc Nephrol*. 2005;16:3015–3026.
3. Nankivell BJ, Borrows RJ, Fung CL, et al. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation*. 2004;78:557–565.

4. Schuler W, Sedrani R, Cottens S, et al. SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. *Transplantation*. 1997;64:36–42.

5. Lorber MI, Mulgaonkar S, Butt KM, et al. Everolimus versus mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: a 3-year randomized, multicenter, phase III study. *Transplantation*. 2005;80:244–252.

6. 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;5:2521–2530.

7. 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;4:626–635.

8. Abraham RT, Wiederrecht GJ. Immunopharmacology of rapamycin. *Annu Rev Immunol*. 1996;14:483–510.

9. Viklicky O, Zou H, Muller V, et al. SDZ RAD prevents manifestation of chronic rejection in rat renal allografts. *Transplantation*. 2000;69: 497–502.

10. Schuurman HJ, Cottens S, Fuchs S, et al. SDZ RAD, a new rapamycin derivative: synergism with cyclosporine. *Transplantation*. 1997;64:32–35.

11. Cao W, Mohacsi P, Shorthouse R, Pratt R, Morris RE. Effects of rapamycin on growth factor-stimulated vascular smooth muscle cell DNA synthesis. Inhibition of basic fibroblast growth factor and platelet-derived growth factor action and antagonism of rapamycin by FK506. *Transplantation*. 1995;59:390–395.

12. Schuurman HJ, Pally C, Weckbecker G, Schuler W, Bruns C. SDZ RAD inhibits cold ischemia-induced vascular remodeling. *Transplant Proc*. 1999;31:1024–1025.

13. Lutz J, Zou H, Liu S, Antus B, Heemann U. Apoptosis and treatment of chronic allograft nephropathy with everolimus. *Transplantation*. 2003;76:508–515.

14. Lorber MI, Ponticelli C, Whelchel J, et al. Therapeutic drug monitoring for everolimus in kidney transplantation using 12-month exposure, efficacy, and safety data. *Clin Transplant*. 2005;19:145–152.

15. Pascual J. Everolimus in clinical practice – renal transplantation. *Nephrol Dial Transplant*. 2006;21 Suppl 3:iii18–iii23.

16. Bennett WM. The nephrotoxicity of immunosuppressive drugs. *Clin Nephrol*. 1995;43 Suppl 1:S3–S7.

17. Nashan B, Curtis J, Ponticelli C, et al. Everolimus and reduced-exposure cyclosporine in de novo renal-transplant recipients: a three-year phase II, randomized, multicenter, open-label study. *Transplantation*. 2004;78:1332–1340.

18. 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;85:821–826.

19. Pascual J. Concentration-controlled everolimus (Certican): combination with reduced dose calcineurin inhibitors. *Transplantation*. 2005;79: S76–S79.

20. Kovarik JM, Tedesco H, Pascual J, et al. Everolimus therapeutic concentration range defined from a prospective trial with reduced-exposure cyclosporine in de novo kidney transplantation. *Ther Drug Monit*. 2004;26:499–505.

21. EVEREST.ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT00170885?term=CRAD001AIT02&rank=1. Accessed 05 January 2009.

22. Study A2309.ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT00251004?term=crad001a2309&rank=1. Accessed 05 January 2009.

23. Study A2426.ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT00369161?term=CRAD001A2426&rank=1. Accessed 05 January 2009.

24. HERAKLES Study.ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00514514?term=crad001ade13&rank=1. Accessed 05 January 2009.

25. Study A2419.ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00154284?term=crad001a2419&rank=1. Accessed 05 January 2009.

26. Assessment of Everolimus in Addition to Calcineurin Inhibitor Reduction in the Maintenance of Renal Transplant Recipients (ASCERTAIN) study (A2413).ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00170846?term=crad001 a2413&rank=1. Accessed 05 January 2009.

27. Holdaas H, Bentdal O, Pfeffer P, et al. Early, abrupt conversion of de novo renal transplant patients from cyclosporine to everolimus: results of a pilot study. *Clin Transplant*. 2008;22:366–371.

28. Morice MC, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med*. 2002;346:1773–1780.

29. Grube E, Sonoda S, Ikeno F, et al. Six- and twelve-month results from first human experience using everolimus-eluting stents with bioabsorbable polymer. *Circulation*. 2004;109:2168–2171.

30. Nashan B. The role of Certican (everolimus, rad) in the many pathways of chronic rejection. *Transplant Proc*. 2001;33:3215–3220.

31. Fishman JA, Emery V, Freeman R, et al. Cytomegalovirus in transplantation – challenging the status quo. *Clin Transplant*. 2007;21: 149–158.

32. Demopoulos L, Polinsky M, Steele G, et al. Reduced risk of cytomegalovirus infection in solid organ transplant recipients treated with sirolimus: a pooled analysis of clinical trials. *Transplant Proc*. 2008;40:1407–1410.

33. Gutierrez-Dalmau A, Campistol JM. The role of proliferation signal inhibitors in post-transplant malignancies. *Nephrol Dial Transplant*. 2007;22 Suppl 1:i11–i16.

34. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet*. 2008;372:449–456.

35. Pascual J, Boletis IN, Campistol JM. Everolimus (Certican) in renal transplantation: a review of clinical trial data, current usage, and future directions. *Transplant Rev*. 2006;20:1–18.

36. Kuypers DR. Benefit-risk assessment of sirolimus in renal transplantation. *Drug Saf*. 2005;28:153–181.

37. Stephany BR, Augustine JJ, Krishnamurthi V, et al. Differences in proteinuria and graft function in de novo sirolimus-based vs calcineurin inhibitor-based immunosuppression in live donor kidney transplantation. *Transplantation*. 2006;82:368–374.

38. 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;20:27–36.

39. Keane WF. Proteinuria: its clinical importance and role in progressive renal disease. *Am J Kidney. Dis* 2000;35:S97–S105.

40. Diekmann F, Budde K, Oppenheimer F, et al. Predictors of success in conversion from calcineurin inhibitor to sirolimus in chronic allograft dysfunction. *Am J Transplant*. 2004;4:1869–1875.

41. Letavernier E, Legendre C. mToR inhibitors-induced proteinuria: mechanisms, significance, and management. *Transplant Rev (Orlando)*. 2008;22:125–130.

42. Muirhead N, House A, Hollomby DJ, Jevnikar AM. Effect of valsartan on urinary protein excretion and renal function in patients with chronic renal allograft nephropathy. *Transplant Proc*. 2003;35:2412–2414.

43. Augustine JJ, Bodziak KA, Hricik DE. Use of sirolimus in solid organ transplantation. *Drugs*. 2007;67:369–391.

44. Andres V, Castro C, Campistol JM. Potential role of proliferation signal inhibitors on atherosclerosis in renal transplant patients. *Nephrol Dial Transplant*. 2006;21 Suppl 3:iii14–iii17.

45. Magnani G, Vigano M, Maccherini M, et al. Everolimus- vs MMF-based immunosuppression: comparable lipid profiles at one year after cardiac transplantation [poster]. American Transplant Congress, Toronto, Canada, 30 May – 4 June. *Am J Transplant*. 2008; 8(S2):347.

46. Kasiske BL, de MA, Flechner SM, et al. Mammalian target of rapamycin inhibitor dyslipidemia in kidney transplant recipients. *Am J Transplant*. 2008;8:1384–1392.

47. Kovarik JM, Hartmann S, Hubert M, et al. Pharmacokinetic and pharmacodynamic assessments of HMG-CoA reductase inhibitors when coadministered with everolimus. *J Clin Pharmacol*. 2002;42:222–228.

48. Elloso MM, Azrolan N, Sehgal SN, et al. Protective effect of the immunosuppressant sirolimus against aortic atherosclerosis in apo E-deficient mice. *Am J Transplant*. 2003;3:562–569.

49. Pakala R, Stabile E, Jang GJ, Clavijo L, Waksman R. Rapamycin attenuates atherosclerotic plaque progression in apolipoprotein E knockout mice: inhibitory effect on monocyte chemotaxis. *J Cardiovasc Pharmacol*. 2005;46:481–486.

50. Waksman R, Pakala R, Burnett MS, et al. Oral rapamycin inhibits growth of atherosclerotic plaque in apoE knock-out mice. *Cardiovasc Radiat Med*. 2003;4:34–38.

51. Pascual J, Marcen R, Ortuno J. Clinical experience with everolimus (Certican): optimizing dose and tolerability. *Transplantation*. 2005;79: S80–S84.

52. Troppmann C, Pierce JL, Gandhi MM, et al. Higher surgical wound complication rates with sirolimus immunosuppression after kidney transplantation: a matched-pair pilot study. *Transplantation*. 2003;76:426–429.

53. Margreiter R, Vitko S, Whelchel J, et al. Post-operative tissue regeneration in renal transplantation: comparable outcome with everolimus or MMF. Transplantation Society XXII Annual Congress, August 10–14, Sydney, Australia. *Transplantation*. 2008;86(S2):188.

54. Mohaupt MG, Vogt B, Frey FJ. Sirolimus-associated eyelid edema in kidney transplant recipients. *Transplantation*. 2001;72:162–164.

55. Moro JA, Almenar L, Martinez-Dolz L, et al. Tolerance profile of the proliferation signal inhibitors everolimus and sirolimus in heart transplantation. *Transplant Proc*. 2008;40:3034–3036.

56. Champion L, Stern M, Israel-Biet D, et al. Brief communication: sirolimus-associated pneumonitis: 24 cases in renal transplant recipients. *Ann Intern Med*. 2006;144:505–509.

57. Garrean S, Massad MG, Tshibaka M, et al. Sirolimus-associated interstitial pneumonitis in solid organ transplant recipients. *Clin Transplant*. 2005;19:698–703.

58. Haydar AA, Denton M, West A, Rees J, Goldsmith DJ. Sirolimus-induced pneumonitis: three cases and a review of the literature. *Am J Transplant*. 2004;4:137–139.

59. Rehm B, Keller F, Mayer J, Stracke S. Resolution of sirolimus-induced pneumonitis after conversion to everolimus. *Transplant Proc*. 2006;38:711–713.

60. De SP, Petruccelli S, Precisi A, et al. Switch to everolimus for sirolimus-induced pneumonitis in a liver transplant recipient – not all proliferation signal inhibitors are the same: a case report. *Transplant Proc*. 2007;39:3500–3501.

61. Mycophenolate sodium vs Everolimus or Cyclosporine with Allograft Nephropathy as Outcome (MECANO) study. Netherlands Trial Register. Available from: http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=567. Accessed 05 January 2009.

62. CALLISTO.ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT00154297?term=CRAD001A2420&rank=2. Accessed 05 January 2009.

63. ZEUS.ClinicalTrials.gov. Available from:http://clinicaltrials.gov/ct2/show/NCT00154310?term=CRAD001A2418&rank=1. Accessed 05 January 2009.

64. Steroid Or Cyclosporine Removal After Transplantation using EverolimuS (SOCRATES) study (A2421).ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00371826?term=crad001a2421&rank=1. Accessed 05 January 2009.

65. Study A2423.ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00170807?term=crad001a2423&rank=1. Accessed 05 January 2009.

66. Kamar N, Jaafar A, Esposito L, et al. Conversion from sirolimus to everolimus in maintenance renal transplant recipients within a calcineurin inhibitor-free regimen: results of a 6-month pilot study. *Clin Nephrol*. 2008;70:118–125.