Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Calcineurin inhibitor sparing regimens using m-target of rapamycin inhibitors: an opportunity to improve cardiovascular risk following kidney transplantation?

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Keywords
calcineurin inhibitors, cardiovascular risk, cardiovascular, everolimus, m-TOR inhibitors, sirolimus.

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Received: 20 February 2010
Revision requested: 7 April 2010
Accepted: 22 June 2010
Published online: 19 July 2010

doi:10.1111/j.1432-2277.2010.01140.x

Summary

Maintenance therapy with calcineurin inhibitors (CNIs) increases cardiovascular risk. Use of the m-TOR inhibitors everolimus or sirolimus to minimize CNI exposure is usually undertaken to preserve renal function following kidney transplantation, but may also improve cardiovascular risk status. Recent studies of early conversion from CNI to m-TOR inhibitors have shown a numerical improvement in the incidence of hypertension, but results are not clear-cut. Dyslipidaemia, in contrast, is more frequent under m-TORs than with CNI-based immunosuppression. New-onset diabetes is rare (≤5%) using modern m-TOR regimens, for example, everolimus and reduced-exposure CNI. Renal function improvement with m-TOR inhibitor regimens versus CNIs would also be expected to improve cardiovascular risk. Moreover, m-TOR-based CNI-minimization regimens are not associated with proteinuria, a known cardiovascular risk factor, with the possible exception of late conversion in patients with poor renal function. Interestingly, m-TOR inhibitors may also exert cardioprotective effects. Animal data suggest that m-TORs may restrict the pathogenesis of atherosclerosis, consistent with preliminary clinical data that conversion from CNIs to everolimus can stabilize markers for arterial stiffness. In conclusion, use of m-TORs has the potential to lessen the toll of cardiovascular disease following kidney transplantation – an opportunity that merits further exploration.

Introduction

Cardiovascular disease (CVD) continues to exert a high burden in terms of morbidity and mortality following kidney transplantation. The incidence of cardiovascular risk is already high in patients with end-stage kidney disease awaiting transplantation, and although renal function improves post-transplant, several other important risk factors worsen [1,2] such that the risk of death because of CVD is only slightly reduced following kidney transplantation compared with dialysis [3]. The most frequent fatal CVD events in the kidney transplant population are ischaemic heart disease (IHD) and cerebrovascular accident [2,4], but as with other states of kidney disease, the incidence of congestive heart failure (CHF) and left ventricular hypertrophy is also high [5,6]. Overall, the annual risk of a cardiovascular event is up to 50-fold higher for a kidney transplant patient then for the general population [7,8] and cardiovascular disease accounts for over a third of all deaths following kidney transplantation [2,9]. With up to 5% of recipients experiencing a cardiovascular event each year [7,8], reducing cardiovascular risk is a priority.

Kidney transplant patients face three main categories of cardiovascular risk factors. First, conventional factors such as age, gender, family history, obesity, hypertension, hyperlipidaemia and smoking. Of these, obesity [10] and hypertension [11,12] are more frequent than in the general population. As in the nontransplant population, diabetes, hyperlipidaemia, hypertension and smoking

increase the risk of IHD, although diabetes, increased serum cholesterol and smoking are associated with a greater relative risk for IHD following kidney transplantation [4] than in nontransplant patients. Second, declining renal function and proteinuria pre and post-transplant, often associated with progressive anaemia, further hypertensive pressure, and chronic micro-inflammation, contribute a further level of risk [1,8,12–15]. Third, there are adverse influences which are specific to transplantation. These include the effect of acute rejection and opportunistic viral infections such as cytomegalovirus [16], but the most notable are complications associated with maintenance immunosuppressive drugs.

Calcineurin inhibitor therapy and cardiovascular risk

The introduction of calcineurin inhibitors (CNIs) resulted in a dramatic reduction in acute rejection and short-term graft survival rates [17,18]. Additionally, their use permitted a welcome decrease in corticosteroid doses, reducing the impact of steroid-related cardiovascular complications such as diabetes [19], hypertension [20], hyperlipidaemia [21] and obesity [22]. Disappointingly, however, it became apparent that maintenance CNI therapy is also associated with certain important cardiovascular risk factors. In animal studies and in humans there is clear evidence that CNIs interact negatively with the endothelium [23,24], the critical defence line for protection against the initiation and progression of atherosclerotic and arteriosclerotic changes. In the clinical setting, the use of CNIs is associated with the onset of *de novo* diabetes, hypertension and hyperlipidaemia. Of these, diabetes confers the greatest IHD risk of any risk factor following kidney transplantation, estimated to increase the relative risk of IHD by almost threefold in men and over fivefold in women more than 1 year post-transplant [3]. Calcineurin inhibitor therapy, particularly tacrolimus [25–27], adversely affects glucose metabolism [28] and increases the risk of new-onset diabetes [29,30], although the question of whether this effect is dose-dependent remains unclear [31,32]. The presence of diabetes, in turn, is associated with higher rates of hypercholesterolaemia and hypertension. Hypertension remains widespread in the kidney transplant population, despite extensive antihypertensive therapy [11], with 25–50% of patients exhibiting systolic blood pressure (SBP) >140 mmHg [11,12]. While hypertensive mechanisms in the transplant setting are complex [33,34], introduction of CNIs was associated with a marked increase in the prevalence of hypertension [35] and CNIs are a well-recognized contributor to hypertension post-transplant [34,36]. Equally, hyperlipidaemia is a known complication of CNI maintenance therapy [21], although multiple other factors contribute. Despite intervention, raised cholesterol and triglyceride levels remain common in kidney transplant recipients [2]. Lastly, the contribution of CNI-related nephrotoxicty to chronic allograft nephropathy [37] would be expected to exacerbate the adverse cardiovascular effects of deteriorating graft function.

The role for proliferation signal inhibitors

Attempts to achieve entirely CNI-free immunosuppression have generally been associated with an unacceptable rate of acute rejection [38,39] or a high rate of discontinuation because of adverse events [40–42]. Strategies to minimize CNI exposure, rather than replace CNIs completely, now form the focus of most research. One of the most best-researched and most successful approaches is to employ the m-TOR inhibitor agents everolimus or sirolimus, with the aim of withdrawing CNI after the high-risk period immediately post-transplant or facilitating low-exposure CNI maintenance therapy [43–45]. The driving force for most trials investigating CNI minimization with m-TOR inhibitor therapy has been to preserve or improve renal function [46–49]. However, CNI discontinuation or reduced-exposure CNI therapy achieved by the use of m-TOR inhibitors may also offer an opportunity to improve cardiovascular risk following kidney transplantation, as the CNI and mTOR inhibitor classes are associated with different safety profiles. Importantly, any benefit from variations in cardiovascular risk between the two classes would not be compromised by higher rejection rates or deteriorating renal function in mTOR inhibitor-treated patients. Efficacy appears similar using modern m-TOR inhibitor-based CNI withdrawal or reduction strategies versus standard CNI regimens [49–55], and there is convincing evidence that graft function is superior or at least stabilized under m-TOR inhibitor therapy versus standard CNI maintenance regimens [47,49–52,54–56].

The potential advantage of m-TOR inhibitor-based regimens in terms of improving cardiovascular risk following kidney transplantation falls under two broad categories: first, reduction of CNI-related complications and second, possible cardioprotective effects of the m-TOR inhibitor class of drugs. Each of these is discussed here, based on the available evidence. To date, most studies have investigated cardiovascular effects only in terms of incidence, and only rarely as a primary or main secondary endpoint. Generally, onset of diabetes, hypertension or lipid disturbances is recorded or data captured. These data are presented and have sometimes been used to argue in favour of a certain immunosuppressant but in most studies, the focus is on immunological details, graft survival and renal

function and not primarily on cardiovascular parameters. In addition, most studies record cardiovascular parameters but definitions and measurement techniques are not precisely defined and sometimes vary considerably from those used in pure cardiovascular studies, such that cardiovascular information from these studies is limited. As a consequence, most of the data discussed here are from observational studies with sometimes questionable impact. Undertaking clinical trials using cardiovascular endpoints in renal transplant recipients is a priority. Observational studies, however, have the advantage of generating new hypotheses which have to be proved.

CNI minimization with m-TOR inhibition: effect on cardiovascular risk factors

Blood pressure

Kidney transplant patients are subjected to a wide range of hypertensive influences, including donor factors (age, graft quality) and recipient factors (e.g. male gender, age, diabetes, body mass, pretransplant hypertension, primary kidney disease) as well as post-transplant effects including acute rejection, delayed graft function, renal artery stenosis, chronic allograft nephropathy and immunosuppression [1,57]. Effective blood pressure control is challenging in this setting and estimates of the proportion of patients with SBP greater than 140 mmHg range from more than 50% [11,12] up to 75% [2] or even 80% [1]. Against this background, it can be difficult to determine the relative hypertensive effect of individual immunosuppressive agents. Nevertheless, maintenance treatment with cyclosporine (CsA) and tacrolimus is believed to represent one of the most potent etiologic factors for post-transplant hypertension [1]. CNIs exert their hypertensive effect via increased oxidative stress and sympathetic activation resulting in afferent arteriolar vasoconstriction [1,58–60], effects that are not observed with m-TOR inhibitors [61]. CNI-related nephrotoxicity and its contribution to chronic allograft nephropathy could also be expected to contribute to the long-term progression of hypertension.

Mulay *et al.* [62] undertook a systematic review of randomized trials of CNI withdrawal published prior to 2005. Among 1047 patients in six trials, conversion from CNI to sirolimus led to a significant reduction in the incidence of hypertension at one year (relative risk 0.56, 95% CI 0.40–0.78, $P < 0.001$), although in these early trials the rate of rejection increased after conversion from CNI. Results from more recent studies, in which CNI discontinuation has not been associated with greater rejection, have all shown a numerical or statistically significant benefit in terms of blood pressure, occurrence of hypertension and/or use of antihypertensive medication [47,63–68] as summarized in Table 1. The exception is the CONVERT study, in which conversion from CNI therapy only took place at 6–12 months post-transplant; at month 24, mean SBP was identical in both the CNI continuation and discontinuation groups [46]. At 4 years post-transplant, the Rapamune Maintenance Study [66] showed significantly lower mean arterial pressure in the CNI-free patients, whereas 2-year follow-up data in a pilot study of tacrolimus withdrawal from an m-TOR inhibitor regimen also reported a benefit for CNI withdrawal that reached significance for mean diastolic pressure that was sustained at year 2 (74 vs. 80 mmHg, $P = 0.009$) [50].

With evidence from nontransplant indications giving conflicting results as to whether the hypertensive effect of CNIs is dose-dependent [69,70], it is interesting to observe whether CNI dose reductions in the presence of m-TOR inhibitors confers an advantage. Unfortunately, few randomized trials have reported data on blood pressure. In the large, randomized A2309 study [55], hypertension was reported as an adverse event for a similar proportion of patients in the everolimus-low CsA and mycophenolic acid (MPA)-standard CsA groups. Bertoni *et al.* [54], however, used higher exposure targets for everolimus with very low CsA and found mean systolic pressure to be significantly lower in the everolimus arm although this has not been shown elsewhere [59] (Table 1).

Dyslipidaemia

Hyperlipidaemia was recognized as a complication of sirolimus early in its development [71]. At high doses, dyslipidaemia is more frequent under sirolimus than CNI therapy [72], a finding confirmed in a meta-analysis of randomized trials performed prior to 2005 [73]. The dyslipidaemic effect appears to be dose-related, with improvement after reductions in sirolimus trough level [72] or dose [74]. Of note, the recent SYMPHONY trial reported similar rates of hypercholesterolaemia and hyperlipidaemia with CNI-free and low-dose mTOR inhibitor therapy or with standard-dose CsA, both in combination with MPA [39]. Nevertheless, trials of conversion to an m-TOR inhibitor or withdrawal of CNI from an m-TOR inhibitor-containing regimen have consistently shown higher levels of total cholesterol, triglycerides and lipid abnormalities as adverse events in the m-TOR inhibitor arms [46,47,63–66] (Table 2). For concomitant m-TOR inhibitor and CNI therapy, early trials using standard-exposure CNI showed high rates of dyslipidaemia [75–77], as might be expected. A subsequent randomized trial of 111 *de novo* kidney transplant patients randomized to everolimus with reduced-exposure or standard-exposure CsA showed a more favourable lipid profile in the reduced-exposure cohort [48]. At

Table 1. Blood pressure parameters in randomized trials of m-TOR inhibitor-based CNI minimization trials.

Study	*n*	Randomization protocol	Adjunctive maintenance immunosuppression	Follow-up (months post-transplant)	Parameter	Treatment group	Outcome	*P*-value
Conversion from CNI to m-TOR								
CONVERT [46]	830	Month 6–12: Convert to sirolimus or continue CsA/tacrolimus	MPA or azathioprine Corticosteroids	24	Mean systolic pressure	Sirolimus CsA or tacrolimus	132 mmHg 132 mmHg	n.s.
CONCEPT [47]	192	Month 3: Convert to sirolimus or continue CsA	MPA Corticosteroids to month 8	12	Mean systolic pressure Antihypertensive medication	Sirolimus CsA Sirolimus CsA	135 mmHg 137 mmHg 49% 62%	n.s. n.s.
ZEUS [49,65]	300	Month 4.5: Convert to everolimus or continue CsA	MPA Corticosteroids	12	Mean systolic/diastolic pressure	Everolimus CsA	132/79 133/81	–
Withdrawal of CNI								
Rapamune Maintenance Study [66]	430	Month 3: Discontinue CsA with increased sirolimus exposure or continue CsA with sirolimus	Corticosteroids	48	Mean arterial pressure	Sirolimus CsA	97.1 mmHg 101.3 mmHg	0.047
Grinyo 2004 [63]	87	Month 3: Discontinue tacrolimus + increased-exposure sirolimus or continue tacrolimus + sirolimus	Corticosteroids	12	Mean systolic pressure Mean diastolic pressure	No tacrolimus Tacrolimus No tacrolimus Tacrolimus	132 mmHg 141 mmHg 75.6 mmHg 80.4 mmHg	0.06 0.03
Baloobal 2003 [67]	133	Month 3: Discontinue CsA + increased-exposure sirolimus or continue sirolimus with reduced CsA	Low-dose corticosteroids	6	Hypertension reported as adverse event	No CsA CsA	26.2% 40.0%	n.s.
Stallone 2004 [64]	40	Month 3: Discontinue CsA + increased exposure sirolimus or continue CsA + sirolimus	Corticosteroids	12	Mean arterial pressure Arterial hypertension	No CsA CsA No CsA CsA	98 mmHg 104 mmHg 60% 80%	– –
De novo low-exposure CNI								
RAD 2309 [55]	833	*De novo*: Everolimus 1.5 or 3 mg/day + reduced CsA or MPA + standard CsA	Corticosteroids	12	Hypertension as adverse event	Everolimus 1.5 mg/day Everolimus 3 mg/day MPA	29.6% 27.3% 30.0%	–
Bertoni 2009 [54]	106	*De novo*: Everolimus + very low CsA or MPA + standard CsA	Corticosteroids	12	Mean systolic pressure	Everolimus CsA	125 mmHg 131 mmHg	0.03
Spagnoletti 2009 [68]	60	*De novo*: Everolimus + low CsA or tacrolimus + MMF	Corticosteroids	6	Mean systolic/diastolic pressure	Everolimus/low-CsA MPA/tacrolimus	77/129 78/128	n.s.

CsA, cyclosporine; MPA, mycophenolic acid; MMF, mycophenolate mofetil.

Table 2. Lipid parameters in randomized trials of m-TOR inhibitor-based CNI minimization trials.

Study	*n*	Randomization protocol	Adjunctive maintenance immunosuppression	Follow-up (months post-transplant)	Parameter	Treatment group	Outcome	*P*-value
Conversion from CNI to m-TOR								
CONVERT [46]	830	Month 6–12: Convert to sirolimus or continue CsA/tacrolimus	MPA or azathioprine Corticosteroids	24	Hypercholesterolaemia as adverse event	Sirolimus CsA or tacrolimus	41.9% 12.1%	<0.001
					Use of statin therapy	Sirolimus CsA or tacrolimus	77.7% 54.6%	<0.001
CONCEPT [47]	192	Month 3: Convert to sirolimus or continue CsA	MPA Corticosteroids to month 8	12	Mean total cholesterol	Sirolimus CsA	2.1 mmol/l 1.9 mmol/l	n.s.
					Mean triglycerides	Sirolimus CsA	1.9 mmol/l 1.4 mmol/l	<0.01
ZEUS [65]	300	Month 4.5: Convert to everolimus or continue CsA	MPA Corticosteroids	12	Hypercholesterolaemia as adverse event	Everolimus CsA	46% 40%	–
					Mean total cholesterol	Everolimus CsA	6.61 mmol/l 6.34 mmol/l	–
Withdrawal of CNI								
Rapamune Maintenance Study [66]	430	Month 3: Discontinue CsA with increased sirolimus exposure or continue CsA with sirolimus	Corticosteroids	48	Mean total cholesterol	Sirolimus CsA	6.3 mmol/l 5.7 mmol/l	0.022
Grinyo 2004 [63]	87	Month 3: Discontinue tacrolimus + increased-exposure sirolimus or continue tacrolimus + sirolimus	Corticosteroids	12	Mean total cholesterol	No tacrolimus Tacrolimus	6.2 mmol/l 5.4 mmol/l	<0.05
					Mean triglycerides	Tacrolimus No tacrolimus	2.1 mmol/l 1.9 mmol/l	n.s.
Stallone 2004 [64]	40	Month 3: Discontinue CsA + increased exposure sirolimus or continue CsA + sirolimus	Corticosteroids	12	Mean total cholesterol	No CsA CsA	5.6 mmol/l 4.4 mmol/l	–
					Mean triglycerides	No CsA CsA	2.7 mmol/l 2.1 mmol/l	–
De novo low-exposure CNI								
B156 [48]	111	*De novo*: Everolimus 3 mg/day with reduced-or full-exposure CsA	Corticosteroids	12	Mean total cholesterol	Reduced CsA Standard CsA	6.4 mmol/l 7.1 mmol/l	0.021
					Mean triglycerides	Reduced CsA Standard CsA	3.1 mmol/l 3.5 mmol/l	n.s.
RAD 2309 [55]	833	*De novo*: Everolimus 1.5 or 3 mg/day + reduced CsA or MPA + standard CsA	Corticosteroids	12	Hypercholesterolaemia as an adverse event	Everolimus 1.5 mg/day Everolimus 3 mg/day MPA	17.2% 18.0% 12.5%	–

Table 2. continued

Study	*n*	Randomization protocol	Adjunctive maintenance immunosuppression	Follow-up (months post-transplant)	Parameter	Treatment group	Outcome	*P*-value
Bertoni 2009 [54]	106	*De novo*: Everolimus + very low CsA or MPA + standard CsA	Corticosteroids	12	Mean total cholesterol	Everolimus CsA	5.7 mmol/l 5.4 mmol/l	n.s.
Spagnoletti [68]	60	*De novo*: Everolimus + low CsA or tacrolimus + MMF	Corticosteroids	6	Mean total cholesterol	Everolimus Tacrolimus	6.5 mmol/l 5.3 mmol/l	0.003
					Mean triglycerides	Everolimus Tacrolimus	3.0 mmol/l 1.9 mmol/l	0.027

CsA, cyclosporine; MPA, mycophenolic acid; MMF, mycophenolate mofetil.

3 years post-transplant, the incidence of hypercholesterolaemia (≥9.1 mol/l) was 32.8% in the reduced-exposure cohort compared with 56.6% in the standard-dose group. Two subsequent trials of concentration-controlled everolimus with a greater reduction in CsA exposure (target C_2 level 350–450 ng/ml at month 6) yielded a lower rate of hypercholesterolaemia using the same definition (22.3% and 20.7% at 12 months post-transplant) [78]. Since then, further trials exploring even lower target levels for CsA exposure (target C_2 level 250–300 ng/ml at month 6), with a corresponding increase in everolimus exposure, have not revealed any further benefit in terms of lipid profiles [53,54]. Data on aggressive reduction of tacrolimus exposure are sparse [79]. It appears that concomitant m-TOR inhibitor therapy with reduced-exposure CNI achieves a pronounced improvement in hyperlipidaemia versus standard-exposure CNI [48,78], but that incremental further reductions in CNI levels, balanced by higher m-TOR inhibitor exposure, offer no additional benefit [53,54].

Diabetogenicity

The presence of diabetes carries the greatest relative risk for IHD following kidney transplantation, exceeding dyslipidaemia, age, hypertension or smoking [3]. A prospective study of 201 patients followed for 8 years after kidney transplantation has shown new-onset diabetes to be associated with a threefold increase in the risk of major cardiac events and a twofold increase in the risk of death [80], consistent with previous results from an analysis of United States Renal Data System (USRDS) data [26]. Among the modifiable risk factors for new-onset diabetes after transplant (obesity, hepatitis C virus, cytomegalovirus infection and immunosuppression), choice of immunosuppressive therapy is believed to account for approximately 75% of the variation in risk [81]. CNI therapy is well-known to increase the rate of new-onset diabetes after kidney transplantation [82], as confirmed in two recent analyses [28,29], an effect believed to be due to increased insulin resistance [25,83] and, particularly with tacrolimus, decreased insulin secretion [25,84]. Thus, discontinuation of CNI would be expected to ameliorate CNI-induced insulin metabolism abnormalities and clinical diabetes.

A few small, single-centre studies [85,86] have suggested that sirolimus may exert a diabetogenic effect, including one retrospective analysis that indicated sirolimus to be associated with a similar risk of diabetes to tacrolimus [87]. A retrospective analysis of USRDS data from over 20 000 patients receiving a kidney transplant during the early period of sirolimus use (1995–2003) has reported sirolimus-treated patients to be at increased risk for new-onset diabetes either in combination with

CsA or with mycophenolate mofetil (MMF) [88]. These findings, however, do not appear to have been borne out in randomized trials. Although regrettably few randomized studies of m-TOR-based, CNI-minimization regimens have described blood glucose levels or the rate of diabetes, the available data suggest no pattern of difference between treatment arms following CNI withdrawal [63,64] or with low-exposure CNI [66]. In two large trials of everolimus with low-exposure CsA, each involving over 250 *de novo* kidney transplant patents, the 6-month incidence of new-onset diabetes (defined as ≥1 fasting glucose measurement of 126 mg/dL) was low, at ≤5% [89]. A further trial with a similar design also reported rates of 3–4% at month 6 [90]. Each of these three trials included patients randomized to 1.5, 3 mg everolimus, and there was no difference in the rate of new-onset diabetes between doses [89,90]. Long-term analyses of the relative risk of diabetes in mTOR inibitor-treated patients – particularly versus CsA – would be helpful.

Renal function and other cardiovascular risk factors

Declining allograft function is an independent risk factor for cardiovascular events in the general population [91] and in kidney transplant patients [13]. Since preservation of renal function is the primary reason for conversion to m-TOR inhibitor-based CNI-free or low-CNI regimens, the contribution of diminishing glomerular filtration rate to cardiovascular risk could be expected to decrease. The beneficial effects of m-TOR inhibitor-based, CNI-sparing regimens on renal function have been well documented [43,73]. Related to this, proteinuria is now another well-established risk factor for IHD following kidney transplantation [92,93]. There have been single-centre reports in the literature describing an increase in the incidence of proteinuria with sirolimus [94–97].

In the CONVERT study, in which maintenance kidney transplant patients were randomized at 6–120 months post-transplant to covert to sirolimus or remain on CNI, *de novo* proteinuria and progression of pre-existing proteinuria was observed in the sirolimus arm [46], which was more pronounced in patients with high proteinuria and poor renal function at baseline [46]. In contrast, when 300 patients were converted at an earlier time-point (4.5 months post-transplant) to everolimus in the ZEUS study, the rate of proteinuria at 1 year was similar in the everolimus and CNI continuation arms (16% and 17%, respectively) [49]. Results from the CONCEPT study also showed no difference in 1-year proteinuria with switch to sirolimus or CsA at month 3 or CsA continuation [47], as has been reported elsewhere following withdrawal of tacrolimus at month 3 [50]. For everolimus with low-exposure CsA from day 1 post-transplant, the RAD2309 study reported a comparable one-year incidence of mild and severe proteinuria to MPA with standard CsA (mild proteinuria: 71.3%, 68.5% and 77.1% in the everolimus 1.5, everolimus 3 mg and MPA groups, respectively; severe proteinuria 0.7%, 1.4% and 0.4%) [55] in this population of 833 *de novo* transplant patients. In terms of long-term data, Ciancio *et al.* [98] randomized *de novo* kidney transplant patients to m-TOR inhibitor therapy with low-exposure CsA or tacrolimus versus MMF and standard-exposure tacrolimus, and observed no difference in the rate of proteinuria between treatment arms at year 3 post-transplant. Based on recent evidence, m-TOR-based CNI-minimization regimens do not appear to place kidney transplant patients at increased risk of proteinuria when administered with low-exposure CNI [55,97] or following conversion with CNI discontinuation [47,49,50], with the possible exception of late conversion in patients with poor renal function [46].

As in nontransplant patients with chronic kidney disease, anaemia is common in the kidney transplant population, with mild anaemia in up to 40% of individuals and severe anaemia in 9–22% of patients [99], and observational studies have suggested a causal association between anaemia and cardiovascular outcomes [99]. The early Rapamune Maintenance Regimen group reported a significantly higher mean haemoglobin level at month 48 in kidney transplant patients following CsA withdrawal from a sirolimus-containing regimen [66]. However, this has not been observed in more recent studies of CNI withdrawal [63,65,67] or conversion to m-TOR inhibitor therapy with CNI discontinuation [46,47] and there does not appear to be a causal relationship between use of mTOR inhibitors and haemoglobin level.

Weight gain following kidney transplantation is widespread after transplantation [1,100] even with steroid avoidance protocols [101,102], and is associated with inferior graft survival [103] and heightened risk of IHD [3]. In a single-centre study of weight gain after kidney transplantation, mean BMI was significantly lower in the m-TOR inhibitor treatment arm compared with patients given CsA (24.2 vs. 26.0 mg/m^2, $P = 0.031$) [103]; experimental data from a rat model reported from the same centre also showed significantly lower weight under m-TOR inhibitor treatment versus CsA [104].

A cardioprotective role for m-TOR inhibitors?

Coronary stents coated with m-TOR inhibitors are already widely used in revascularization procedures following evidence that m-TOR-eluting stents reduce binary restenosis, late lumen loss and repeat revascularization compared with standard stents [105–107]. The development of such stents was based on animal data that everolimus and

sirolimus attenuate neointimal thickening and transplant atherosclerosis [108,109]. There is currently intense interest in the extent to which m-TORs may restrict the pathogenesis of atherosclerosis. Animal models have indicated that everolimus and sirolimus prevent lipid accumulation in tissues [110,111] and help to stabilize atherosclerotic plaques by selective clearance of macrophages [112–115] and inhibit the local inflammatory response in arterial smooth muscle cells [116,117]. Importantly, these effects may counteract the increase in hypercholesterolaemia and hypertriglyceridaemia associated with m-TOR inhibitor therapy. There are convincing data that m-TOR inhibitors limit atherosclerotic plaque size and progression in animal models [113,117,118]. Mueller *et al.* [113] observed a dose-dependent reduction in atherosclerotic lesions following administration of everolimus to cholesterol-induced atherosclerosis in mice. Compared with controls, everolimus 0.05 or 1.5 mg/kg reduced lesions by 44% and 85%, respectively, and significantly reduced the complexity of lesions ($P < 0.001$) – results that were obtained despite a 40% increase in plasma cholesterol. Indeed, it has been postulated that m-TOR inhibitors may increase lipid concentration because of lipolysis from plaques [110], rather than by lipogenesis.

Clinically, data on a cardioprotective effect of everolimus and sirolimus remain preliminary. Pulse wave velocity is a well-established marker for arterial stiffness, which occurs as a consequence of aging and atherosclerosis and represents an independent risk factor for cardiovascular events in kidney transplant patients [119]. Seckinger *et al.* [120] undertook a prospective trial in which 27 kidney transplant patients with stable function at month 6 post-transplant were converted from CsA to everolimus or remained on CsA. Nine months after conversion, pulse wave velocity had stabilized in the everolimus group (9.50 m/s at baseline, 9.13 m/s after conversion; $P = 0.16$) but increased in the CsA continuation patients (9.93–10.8 m/s; $P = 0.03$). It has been clearly established that an increase in pulse wave velocity is associated with higher cardiovascular risk [121], because the heart becomes overcharged by the rapid reflection of pulse waves. Increased pulse wave velocity reflects ongoing progressive arteriosclerotic and atherosclerotic disease. Separately, in a nonrandomized study in 13 nondiabetic kidney transplant recipients converted from CNI to sirolimus, left ventricular mass at 1 year post-conversion showed a significant decrease in the sirolimus group compared with controls, despite similar changes in blood pressure [122], consistent with data from animal models [123–125]. The authors speculated that the regression of left ventricular hypertrophy may have been induced by a decrease in myocardial fibrosis, but it is also possible that a reduced or stable pulse wave velocity in these patients may have reduced the cardiac burden of early reflected pulse waves. The ongoing A2429 study, in which kidney transplant patients are randomized at week 8 to continue CNI or convert to everolimus, includes left ventricular hypertrophy and arterial stiffness (using pulse wave velocity) as 12- and 24-month endpoints, and will provide more robust data to address these questions. The finding in heart transplantation that *de novo* use of everolimus leads to a significant reduction in cardiac allograft vasculopathy at 1-year post-transplant [126] and in the rate of major cardiac events at 4 years [127] highlights the potential for a cardioprotective effect of m-TOR inhibitors in kidney transplantation and the need for further research.

Conclusion

It seems likely that m-TOR inhibitors will be employed more widely following kidney transplantation as clinical trials continue to define prescribing strategies. In terms of cardiovascular risk, there are indications that m-TOR inhibitor therapy with CNI elimination may reduce the toll of hypertension in addition to the benefits associated with preservation of renal function, although this remains to be confirmed conclusively. There is also the enticing possibility of inherent cardioprotective actions. It seems feasible that future use of m-TOR inhibitors may partly be driven by the desire to alleviate the cardiovascular burden. Clinical trials of m-TOR inhibitor-based regimens could henceforth include markers of cardiovascular risk as study endpoints to help understand the potential for m-TOR inhibitors to reduce long-term mortality in the kidney transplant population.

Authorship

MZ and MvdG both considered the available data and key topics to include and together finalized the manuscript.

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