TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

Calcineurin inhibitor-sparing regimens based on mycophenolic acid after kidney transplantation

Nassim Kamar,[1,2,3] Arnaud Del Bello,[1,2] Julie Belliere[1] and Lionel Rostaing[1,2,3]

1 Department of Nephrology and Organ Transplantation, CHU Rangueil, Toulouse, France
2 Université Paul Sabatier, Toulouse, France
3 INSERM U1043, IFR–BMT, CHU Purpan, Toulouse, France

Keywords
calcineurin inhibitors, kidney transplantation, minimization, mycophenolic acid, withdrawal.

Correspondence
Nassim Kamar MD, PhD, Department of Nephrology and Organ Transplantation, CHU Rangueil,
TSA 50032, 31059 Toulouse Cedex 9, France.
Tel.: +33 5 61 32 23 35;
fax: +33 5 61 32 39 89;
e-mail: kamar.n@chu-toulouse.fr

Conflicts of interest
No conflicts of interest exist.

Received: 23 September 2014
Revision requested: 16 November 2014
Accepted: 24 December 2014
Published online: 29 January 2015

doi:10.1111/tri.12515

Summary

The use of calcineurin inhibitors (CNIs) has dramatically reduced the number of acute rejections and improved kidney allograft survival. However, CNIs can also cause kidney damage and several adverse events. This has prompted transplant physicians to use CNI-sparing regimens. CNI withdrawal, minimization, or avoidance protocols have been conducted using mycophenolic acid (MPA), and/or mammalian-target-of-rapamycin inhibitors, and/or belatacept. Herein, we review the outcomes of minimizing, withdrawing, or avoiding CNIs when giving mycophenolic acid to *de novo* and maintenance kidney transplant patients. Protocols on CNI withdrawal, when based on MPA without mammalian-target-of-rapamycin inhibitors (mTORi) or belatacept, in *de novo* and maintenance kidney transplant patients, are associated with an increased risk of acute rejection. Consequently, these strategies have been abandoned and are not recommended. Protocols on CNI minimization show a beneficial impact of kidney function and acceptable acute rejection rates mainly in patients who have been recipients of a graft for >3–5 years. However, no significant improvement to graft survival has been observed.

Introduction

Since the early 1980s, the use of cyclosporine A (CsA) has dramatically reduced acute rejection rates and, consequently, improved the survival of kidney allografts [1]. Similarly, the use of tacrolimus, since the mid-1990s, as a part of a triple immunosuppressive regimen, has been associated with a low acute rejection rate and excellent kidney allograft survival [1,2]. However, within the last few years, although the acute rejection rate has been significantly reduced and the survival of kidney allografts has improved, chronic rejection and death with a functioning graft remain the leading causes of late loss of renal allografts [3]. Chronic allograft nephropathy (CAN) [also termed chronic allograft dysfunction (CAD), interstitial fibrosis, and tubular atrophy (IFTA)], as well as glomerular disease, has become the main cause of late graft loss [4].

Several causes of CAN have been identified [3]. These include immune factors, such as acute rejection, the presence of donor-specific antibodies, and noncompliance, but also nonimmune factors, such as hypertension and calcineurin-inhibitor (CNIs)-induced nephrotoxicity [3]. In a large number of patients who received a simultaneous kidney–pancreas transplant and were receiving CNI-based immunosuppression, Nankivell *et al.* [5] reported that nearly all patients developed features of CNI-induced nephrotoxicity at 10 years post-transplantation. Although these data should be considered with caution, because at that time patients were not screened for donor-specific anti-HLA antibodies (DSAs) using sensitive assays and the

© 2015 Steunstichting ESOT

impact of DSAs on the microcirculation had not then been established, they reported that this therapeutic class of immunosuppressive drugs had long-term nephrotoxic effects [5]. The harmful effects of CNIs on kidney function have been also observed in nonkidney solid organ transplant patients [6]. For instance, at 1 year post-transplant, glomerular filtration rate (GFR) was significantly higher in liver transplant patients given CNI-free belatacept-based immunosuppression compared to those receiving tacrolimus-based immunosuppression [7]. Apart from nephrotoxicity, CNIs can cause several other side effects, such as increased post-transplant malignancy, hyperlipidemia, hypertension, impaired glucose metabolism, cosmetic problems, and neurological toxicity [1].

Several CNI-sparing strategies have been used after kidney transplantation, especially since the introduction of newer agents, such as MPA, mTORi, and, more recently, belatacept. The main CNI-sparing strategies are (i) reduction of CNI dose, which is compensated for by the use of MPA or mTORi; (ii) CNI-free MPA-based immunosuppression; and (iii) CNI-free mTORi-based or belatacept-based immunosuppression. Herein, we specifically review MPA-based CNI minimization, withdrawal, or avoidance protocols. CNI-sparing protocols, including mTORi and/or belatacept, are discussed elsewhere [8].

CNI-sparing protocols in maintenance kidney transplant patients

CNI-sparing protocols were first given to maintenance kidney transplant patients with deteriorating kidney function and/or evidence of histological features of CAN, especially those with histological signs of CNI nephrotoxicity. Thereafter, these protocols were used in maintenance kidney transplant patients with stable kidney function to avoid possible CNI-induced nephrotoxicity.

In this comprehensive review, we focus on studies that included ≥30 patients and that have been published since the year 2000.

CNI-sparing protocols in maintenance patients beyond 3 years post-transplant but with deteriorating kidney function

CNI withdrawal

Several studies have assessed the efficacy and safety of CNI-sparing protocols in maintenance kidney transplant patients with deteriorating kidney function and with or without biopsy-proven CAN [9–13]. The investigators compared CNIs given at standard doses or at reduced doses for CNI withdrawal, where CNIs were replaced by MPA. These studies have mainly included patients that have been graft recipients for several years and have poor kidney function, that is, they had a mean serum creatinine level of approximately 250 μmol/l or an estimated/measured GFR of approximately 20–30 ml/min. The follow-up period after intervention ranged between 6 months and 2 years. In nearly all the studies, kidney function improved after CNI withdrawal. The kidney function declined slower in these patients compared to those that were maintained on CNI-based immunosuppression. In the short term, very few cases of graft loss were observed (Table 1).

Weir *et al.* [10,11] reported on a 4-year follow-up CNI withdrawal study in which patients that received reduced doses of cyclosporine A suffered 37.5% graft loss, whereas there was 32% graft loss in patients receiving reduced doses of tacrolimus and only 7.7% graft loss in patients that received a MPA-based CNI-free regimen. In parallel with the improvement in kidney function, in most studies, blood pressure and lipid levels were also improved after CNI withdrawal.

The largest of these CNI withdrawal studies was a randomized, controlled, multicenter study that included 143 patients who had been recipients of a graft for a minimum of 6 months and had documented deteriorating kidney function [13]. The median time between transplantation and randomization was 5 years. Serum creatinine level ranged from 100 to 400 μmol/l, and creatinine clearance was >20 ml/min. The patients were randomized to either have CsA withdrawn and replaced with mycophenolate mofetil (MMF) plus steroids (n = 73) or to be maintained on their CsA-based immunosuppressive regimen (n = 70). In this latter group, CsA trough level was maintained at >80 ng/ml. At 6 months after the intervention, in the intent-to-treat (ITT) population, kidney function had improved in 58% of patients receiving MMF but in only 32% of patients receiving CsA (P = 0.006). In the per protocol population, the difference was greater, 60% vs. 26%, respectively (P = 0.0008). However, in the ITT population, there was no difference between the groups at 1 year (48% vs. 35%, P = 0.18). Patient survival rates were similar in both groups. There were six graft losses: two within the MMF group and four within the CsA group. No acute rejection episodes were observed in the MMF group. The main adverse event observed in the MMF group was diarrhea.

Addition of MPA to CNIs

A few studies have assessed the impact of reducing CNI dose on kidney function under the umbrella of introducing MPA [14–16]. One of the largest studies conducted has been a French randomized prospective study that included 101 kidney transplant patients who had been recipients of a graft for at least 1 year and had presented with a negative slope of 1/serum creatinine [15]. At inclusion, serum creatinine level ranged from 1.7 to 3.4 mg/dl. The patients were randomized to receive either MMF plus half-dose CsA

Table 1. Minimization and withdrawal of calcineurin inhibitors in maintenance kidney transplant patients beyond 3 years post-transplant but with deteriorating kidney function.

Study	No.	Study design	Study type	Kidney function at inclusion	Time since transplantation	Follow-up, month	Kidney function	Graft survival	Acute rejection
McGrath *et al.* [9]	30	CsA + S → MMF + S (n = 15) CsA + S → Tac + S (n = 15)	RCT	GFR ≈ 25 ml/min	7 ± 1.1 years 7.5 ± 0.8 years	6	Improved sCr and isotope GFR in the MMF group	100% vs. 87% P = NS	None
Weir *et al.* [11]	118	Reduction of CsA dose (n = 67) Reduction of Tac dose (n = 33) CNIs → MMF (n = 13)	NRCT	sCr ≈ 270 μmol/l	2.41 ± 0.22 years	22	Improved 1/sCr in the MMF group 51.6% vs. 59.3% vs. 91.7% P = 0.001	Not available*	One in the Tac arm
Suwelack *et al.* [12]	39	CNIs + S → CNIs + MMF + S (n = 20) CNIs + S → MMF + S (n = 19)	RCT	sCr <350 μmol/l	7.5 ± 5.1 years 6.4 ± 2.6 years	8	Improved 1/sCr in the CsA withdrawal group 58% vs. 32% P = 0.021	100% vs. 84% P = NS	None
Dudley *et al.* [13]	143	CsA + S → MMF + S (n = 73) Standard CsA dose (n = 70)	RCT	sCr: 100–400 μmol/l and CCl ≥20 ml/min	0.6–24.2 years Median: 5 years	6	Improved sCr in the MMF group 58% vs. 32% P = 0.0006	93.2% vs. 94.3% P = NS	None
Stoves *et al.* [14]	42	CsA + S → Reduced CsA + MMF + S (n = 11) CsA + S → Tac + S (n = 12) Standard CsA (n = 16)	NRCT	SCr <400 μmol/l	>6 months	6	Improved 1/sCr and isotopic GFR	91% vs. 92% vs. 100%	None
Frimat *et al.* [15]	101	CsA + S → CsA (50%) + MMF + S (n = 70) Standard-dose CsA (n = 31)	RCT	sCr: 150–300 μmol/l	6.7 ± 2.3 years	24	Improved 1/sCr	98.5% vs. 96.7% P = NS	None

CsA, cyclosporine A; Tac, tacrolimus; MMF, mycophenolate mofetil; CNI, calcineurin inhibitors; S, steroids; RCT, randomized controlled trial; NR, nonrandomized controlled trial; sCr, serum creatinine; GFR, glomerular filtration rate; CCl, creatinine clearance.

*The same group also later reported on the impact of these immunosuppressive strategies on graft survival. They observed 37.5% graft loss in patients that received reduced doses of cyclosporine A, 32% loss in those with reduced doses of tacrolimus, but only 7.7% loss in patients receiving a MPA-based CNI-free regimen.

© 2015 Steunstichting ESOT

($n = 70$) or to continue their maintenance CsA therapy ($n = 31$). In the MMF group, CsA trough level was 131 ± 39 ng/ml at baseline and 71 ± 71 ng/ml at 2 years. In the CsA group, CsA trough level was 140 ± 48 ng/ml at baseline and 117 ± 39 ng/ml at 2 years. At 24 months, kidney function, as assessed by regression line analysis of 1/serum creatinine, had improved in the MMF group ($+4.2 \times 10^{-4}$) versus the CsA group (-3×10^{-4}; $P < 0.001$) [15]. No acute rejection episodes were observed, and only one patient in each group lost a graft. At 5 years, kidney function had improved in the MMF group, whereas it was impaired in the CsA group [16]. At 5 years, graft survival was 95.8% in the MMF group and 90.9% in the CsA group [16]. The lack of improvement in graft survival despite the reduction of exposure to CsA could have been caused by the irreversible CsA-induced histological lesions that occurred before CsA dose reduction. However, the improvement in kidney function could have been also related to a hemodynamic effect, that is, the reduction of vasoconstriction induced by CsA.

In summary, MPA-based CNI-sparing strategies for patients with deteriorating kidney function and histological features of CAN have shown a beneficial impact on kidney function without significantly impacting on graft survival. The low risk of acute rejection observed in this setting can be related to the fact that most patients included in these studies had been recipients of a graft for longer than 5 years.

CNI-sparing protocols for maintenance patients who were recipients of a graft for <30 months and had stable kidney function

CNI withdrawal

Several studies have assessed the effect of CNI withdrawal in maintenance kidney transplant patients with stable kidney function [17–20]. In all of these studies, conversion from CNIs to mycophenolate was performed at 1 year post-transplant or later [17–20]. Within the short and mid term, although kidney function improved significantly in all these studies, there was also a significant increase in the rate of acute rejections [17–20]. However, the risk factors for cardiovascular disease, such as blood pressure and lipid levels, were improved in most studies (Table 2).

In a multicenter prospective study, patients that had received a first or second, deceased or living donor, kidney allograft between 12 and 30 months previously, and who had stable kidney function, and were receiving CsA, MMF, plus steroids, were randomized to either have CsA withdrawn and to continue a dual therapy of MMF plus steroids ($n = 85$), or to continue the same CsA immunosuppressive regimen ($n = 85$) [19]. At 6 months after randomization, kidney function was significantly better in the MMF group. However, the acute rejection rate was significantly higher in the MMF group (10.6% in the MMF group vs. 2.4% in the CsA group, $P = 0.03$). At 5 years, the patient and death-censored graft survival rates were similar in both arms: 93% and 88% in the MMF arm, and 95% and 92% in the CsA arm, respectively [20]. However, the acute rejection rate was significantly higher in the MMF arm (18.8% vs. 3.5%, $P = 0.003$): All graft losses were related to rejection episodes. In the MMF arm, two patients lost graft function because of acute rejection and seven other patients experienced graft loss because of chronic rejection without having had an acute rejection episode within the first year post-transplant. However, kidney function was significantly better in the MMF arm (67 vs. 61.7 ml/min, $P = 0.05$).

Smak Gregoor *et al.* [17] reported that the incidence of acute rejection was significantly higher in patients who had CsA withdrawn and were then maintained on azathioprine plus steroids when compared to patients given MMF plus steroids.

CNI dose reduction

Because of the increased risk of acute rejection after withdrawal of CNIs, several studies have assessed the effect of reducing doses of CNIs under the umbrella of introducing MPA or giving high doses of MPA [21–24]. In addition, tapering CsA dose contributes to increased MPA exposure [25] (Table 3).

In a large prospective multicenter study, stable kidney transplant patients that were receiving CsA and MMF were randomized to either continue the same therapy ($n = 102$) or to have CsA dose reduced by 50% ($n = 106$) [23]. The outcomes from greater exposure to CsA were determined by measuring CsA $AUC_{0-12\ h}$. The standard exposure was 4.3 (range: 3.5–4.8) mg h/l, and the low exposure was 2 (range: 2–2.6) mg h/l. At 24 months, treatment failure, defined as graft loss, biopsy-proven acute rejection, biopsy-proven CsA nephrotoxicity, and/or a >15% increase in mean serum creatinine level from baseline, occurred more often in the standard CsA exposure arm (37%) compared to the low-exposure arm (18%), $P = 0.003$. Standard CsA exposure was an independent predictive factor for treatment failure after adjustment for donor age, dialysis technique, and exposure to MMF (assessed by measuring an $AUC_{0-12\ h}$) [23]. By 24 months, acute rejection rates (2.94% in the standard-exposure arm and 5.66% in the low-exposure arm) and graft survival rates (98% in the standard-exposure arm and 100% in the low-exposure arm) were similar in both arms. However, there was significant improvement in eGFR in the low-exposure arm (+0.57 ± 8.8 ml/min) compared to the standard-exposure arm (−4.27 ± 8.06 ml/min; $P < 0.001$) [23].

Another prospective multicenter study evaluated a strategy to reduce tacrolimus exposure while increasing doses of

Table 2. Withdrawal of calcineurin inhibitors in maintenance kidney transplant patients at <30 months post-transplant and with stable kidney function.

Study	No.	Study design	Study type	Kidney function at inclusion	Time since transplantation	Follow-up	Kidney function	Graft survival	Acute rejection
Smak Gregoor *et al.* [17]	64	CsA + S → MMF + S (n = 34) CsA + S → AZA + S (n = 30)	RCT	Stable kidney function Median sCr:125 μmol/l	1 year	18 months	Improved in both arms but no difference between the arms	Not available	11.8% on MMF vs. 36.6% on AZA, P = 0.04
Keunecke *et al.* [18]	46	CsA or Tac → MMF monotherapy	NRCT	Stable kidney function	1 year	10 weeks	Improved sCr 1.74 ± 0.57 (before) vs. 1.9 ± 0.64 mg/l (after), P = 0.006	100%	11%
Abramowicz *et al.* [19]	170	CsA +MMF +S→ MMF + S (n = 85) Continue CsA +MMF + S (n = 85)	RCT	sCr <300 μmol/l stable for at least 3 months	12–30 months	1 year	+2.3 ml/min (P = NS)	Not available	10.6% (MMF group) vs. 2.4% (CsA group) P = 0.03
Abramowicz *et al.* [20]	151	CsA + MMF + S → MMF + S (n = 74) Continue CsA + MMF + S (n = 77)	RCT	sCr <300 μmol/l stable for at least 3 months	12–30 months	5 years	Improved CCl 67.1 ml/min (MMF group) vs. 61.7 ml/min (CsA group), P = 0.05	88% vs. 92% P = NS	18.8% (MMF group) vs. 3.5% (CsA group) P = 0.003
Schnuelle *et al.* [41]	84	CsA + MMF + S → MMF + S (n = 40) CsA + MMF + S → CsA + S (n = 44)	RCT	sCr <200 μmol/l Stable since transplantation and PRA <50%	3 months	1 year	Improved calculated GFR 71.7 ± 20.6 ml/min (MMF group) vs. 60.9 ± 16.7 ml/min (CsA group), P = 0.01	97.7% in the MMF group vs. 100% in the CsA group	11.3% (MMF group) vs. 5% (CsA group) P = 0.01

CsA, cyclosporine A; Tac, tacrolimus; MMF, mycophenolate mofetil; CNI, calcineurin inhibitors; S, steroids; RCT, randomized controlled trial; NR, nonrandomized controlled trial; sCr, serum creatinine; GFR, glomerular filtration rate; CCl, creatinine clearance; PRA, panel-reactive antibodies.

© 2015 Steunstichting ESOT

Table 3. Minimization of calcineurin inhibitors in maintenance kidney transplant patients at <30 months post-transplant and with stable kidney function.

Study	No.	Study design	Study type	CNI target trough levels	Kidney function at inclusion	Time since transplantation	Follow-up, month	Kidney function	Graft survival	Acute rejection
Afzali *et al.* [21]	89	CsA/Tac + AZA + S → Red CsA/Tac + MMF + S	NRCT	CsA C0: 25–50 ng/ml Tac C0: 5 ng/ml	CCl 37.2 ± 16.3 ml/min	Median 43 months	12	Improved 1sCr	Not available	1.2%
Pascual *et al.* [22]	64	CsA + MMF + S → *CsA + MMF + S (n = 32) * Red CsA + MMF + S (n = 32)	RCT	Reduction of CsA by 50%	sCr <170 µmol/l.	>1 year. Mean 22 months	6	Improved CCl in the reduced CsA arm	100%	0%
Etienne *et al.* [23]	208	CsA + MMF → *CsA + MMF (n = 102) * Red CsA + MMF (n = 106)	RCT	$AUC_{0-12\ h}$ Usual exposure: 4.3 (3.5–4.8) mg h/l Low exposure: 2 (2–2.6) mg h/l	eGFR (MDRD): 52 ± 12 ml/min	1.4 ± 0.3 years	24	Improved eGFR	98% vs. 100% P = NS	2.9% vs. 5.7% P = NS
Kamar *et al.* [24]	94	Tac + MPA ± S → * Tac + EC-MPS (720 mg/day) ± S (n = 48) * Red Tac + EC-MPS (1440 mg/day) ± S (n = 46)	RCT	Tac: 5.5–10 ng/ml Red Tac: 2–4.5 ng/ml	eGFR (MDRD) ≥30 and <60 ml/min/1.73 m^2 Mean 45 ± 10 ml/min/1.73 m^2	>1 year	6	Improved change in eGFR between day 0 and month 6	100%	0%

CsA, cyclosporine A; Tac, tacrolimus; MMF, mycophenolate mofetil; EC-MPS, enteric-coated mycophenolate sodium; S, steroids; RCT, randomized controlled trial; NR, nonrandomized controlled trial; sCr, serum creatinine; GFR, glomerular filtration rate; CCl, creatinine clearance.

MPA. Kidney transplant patients with stable kidney function and receiving tacrolimus and MPA, with or without steroids, were randomized to receive either low tacrolimus exposure (target trough level between 2 and 4.5 ng/ml) plus enteric-coated mycophenolate sodium at a daily dose of 1440 mg, or a standard tacrolimus dose (target trough level between 5.5 and 10 ng/ml) plus enteric-coated mycophenolate sodium at a dose of 720 mg/day [24]. Steroid doses were left unchanged. The change in eGFR between day 0 and month 6 improved in the low-dose tacrolimus group (2.48 ± 0.95 vs. -0.48 ± 0.93 ml/min/1.73 m^2, $P = 0.03$). In this multicenter study, 67 patients agreed to participate in the extension follow-up study: 32 were in the reduced tacrolimus arm and 35 in the standard-exposure arm. At 24 months after initial randomization, the improvement in eGFR since day 0 in the low-dose tacrolimus group was maintained (4.5 ± 10.1 vs. 1.9 ± 11.9 ml/min/1.73 m^2, $P = 0.02$) [24]. One acute rejection was observed in each arm.

In summary, MPA-based CNI withdrawal strategies in maintenance patients who have been recipients of a graft for <30 months and have stable kidney function show an unacceptable rate of acute rejection and severe allo-immune responses. However, the reduction of CNIs showed a beneficial impact on kidney function without a significant impact on graft survival. No longer term data are available.

CNI-sparing strategies in *de novo* kidney transplant patients

CNI avoidance regimens

Several studies have evaluated CNI-free regimens in low-risk immunological patients and/or in elderly patients [26–31]. In all studies, immunosuppression was based on an induction therapy, MMF, plus steroids. The induction therapy consisted of an anti-IL2 receptor blocker [26,27,30] or anti-thymocyte globulins [28,29], or both [31]. Disappointing results were obtained from all these studies because the acute rejection rate ranged from 24 to 70%, and after a follow-up period that varied from 6 months to 5 years, the proportion of patients that required CNIs to be introduced ranged from 26 to 62%. In addition, for patients given a polyclonal antibody induction therapy, there was an increased risk of opportunistic infections and malignancies [28] (Table 4).

Based on these data, it seems that the combination of mycophenolic acid, steroids, and anti-IL2 receptor blockers or a polyclonal antibody induction therapy is not potent enough to avoid needing CNIs. Consequently, CNI avoidance protocols based on MPA without the use of mTOR inhibitors and/or belatacept have been abandoned.

Table 4. Avoidance of calcineurin inhibitors in *de novo* kidney transplant patients.

Study	No.	Inclusion criteria	Study design	Study type	Follow-up	Kidney function at last follow-up	Graft survival at last follow-up	Acute rejection rate	CNIs at last follow-up, %
Tran *et al.* [26]	45	PRA <20%	Daclizumab/MMF/S	NRCT	6 months	124 ± 133 µmol/l	95%	38%	51
Vincenti *et al.* [27]	98	PRA ≤20% Negative TCXM	Daclizumab/MMF/S	NRCT	1 year	139 (125–152) µmol/l	96%	53%	62
Grinyo *et al.* [28]	30	PRA <50% Donors >50 years	ATG/MMF/S	NRCT	5 years	218 ± 155 µmol/l	65%	24%	53
Arbogast *et al.* [29]	89	Donors >50 years Recipients >50 years	ATG/MMF/S	NRCT	5 years	136 ± 45 µmol/l	69.8%	23.6%	26
Asberg *et al.* [30]	54	PRA <20% Zero DR MM	Daclizumab/MMF/S (n = 27) vs. CsA/MMF/S (n = 27)	RCT	1 year	52 ± 20 ml/min vs. 69 ± 29 ml/min	88.9% in both arms	70.4% vs. 29.6% P = 0.006	13
Guba *et al.* [31]	56	PRA <30% Recipient >50 years	ATG/Basiliximab/MMF/S	NRCT	1 year	44.5 ± 21.8 ml/min	85.4%	53.6%	57

PRA, panel-reactive antibodies; TCXM, T-cell cross-match; MM, mismatches; ATG, antithymocyte globulins; MMF, mycophenolate mofetil; S, steroids; RCT, randomized controlled trial; NR, nonrandomized controlled trial.

Early withdrawal of CNIs in *de novo* kidney transplant patients

In a prospective study, Hazzan *et al.* assessed the effect of early withdrawal of CsA in *de novo* kidney transplant patients. Low- to moderate-risk immunological patients (panel-reactive antibodies <30%) were given polyclonal antibodies induction therapy, CsA, MMF, plus steroids until month 3 post-transplantation. At that time, patients with stable kidney function and without a recent history of acute rejection were randomized to be converted from CsA, MMF, plus steroids to receive either MMF plus steroids (n = 54) or CsA plus steroids (n = 54) [32]. At 1 year after the intervention, kidney function was significantly better in the MMF arm (67.7 ± 18.7 ml/min in the MMF group vs. 56.5 ± 18 ml/min in the CsA group, P = 0.03). However, the rate of acute rejection was higher in the MMF arm (18.5% vs. 5.6%, P = 0.045). Graft survival was 100% [32]. They also found that borderline changes in kidney biopsies with lower MPA exposure, when assessed at 12 h in area-under-the-curve analysis and before randomization, were independent predictive factors for acute rejection after CsA withdrawal [32]. At 2 years, kidney function remained significantly better in the MMF arm (49.1 ± 17.8 ml/min in the MMF group vs. 40.1 ± 11.1 ml/min in the CsA group, $P < 0.05$) [33]. The rate of acute rejection was higher in the MMF arm (22.2% vs. 5.6%, P = 0.04). There was a higher incidence of C4d deposits in the MMF group at 1 year, which suggested an ongoing humoral allo-immune response [33]. Graft survival was 98% in the MMF arm and 93% in the CsA arm [33].

The CEASAR study was a prospective multicenter study that aimed to evaluate different CsA-sparing strategies and to replace it with MMF in *de novo* kidney transplant patients [34]. Low- to moderate-risk immunological patients who had received a first allograft were randomized to receive one of the following three regimens: (i) CsA was withdrawn (n = 179): Initially, patients received daclizumab, MMF, steroids, plus CsA (target trough levels 50–100 ng/ml) until month 4, and then, CsA was progressively reduced until it was stopped at month 6; (ii) a low-dose CsA group (n = 183) that received daclizumab, MMF, steroids, plus CsA (target trough level 50–100 ng/ml); and (iii) those that received a standard CsA dose (n = 173): that is, daclizumab, MMF, steroids, plus CsA (target trough level 150–300 ng/ml until month 4, and then 100–200 ng/ml). At 12 months, the patient and graft survival rates, as well as measured GFR, were similar in all three groups. Within the first 6 months, that is, before CsA was withdrawn in the first arm, acute rejection rates were similar in all three arms. However, by month 12, the biopsy-proven acute rejection rate was significantly higher in the CsA withdrawal group (38%) compared to both the low-dose

CsA group (25.4%, $P = 0.03$) and the standard-dose CsA group (27.5%, $P = 0.04$) [34].

Hence, early CsA withdrawal does not seem to be an optimal option to treat *de novo* kidney transplant patients, whereas reduced CsA dose with MMF was efficient at preventing acute rejection and preserving kidney function.

CNI minimization in *de novo* kidney transplant patients

The Symphony study is the largest prospective study to have studied reduced CNI exposure in *de novo* kidney transplant patients [35]. Low-risk immunological patients were randomized to receive one of four treatments: (i) standard CsA therapy (target trough level 150–300 ng/ml until month 3 and then 100–200 ng/ml), plus MMF and steroids ($n = 390$); (ii) low-dose CsA (target trough level 50–100 ng/ml), plus MMF, steroids, and daclizumab ($n = 399$); (iii) low-dose tacrolimus (target trough level 3–7 ng/ml), plus MMF, steroids, and daclizumab ($n = 401$); or (iv) low-dose sirolimus (target trough level 4–8 ng/ml), plus MMF, steroids, and daclizumab ($n = 399$). After 1 year, the acute rejection rate was significantly lower in the tacrolimus arm (12.3%) compared to the standard-dose CsA (25.8%), low-dose CsA (24%), and low-dose sirolimus (37.2%) groups. Mean GFR was also significantly higher in the tacrolimus arm (65.4 ml/min) than in the other three arms (57.1 $\pm$ 25.1 ml/min in the standard-dose CsA, 59.4 $\pm$ 25.1 ml/min in the low-dose CsA, and 56.7 $\pm$ 26.9 in the low sirolimus dose) [35]. Finally, death-censored graft survival was also significantly higher in the tacrolimus arm (96.4%) compared to the standard-dose CsA arm (91.9%), the low-dose CsA arm (94.3%), and the low-dose sirolimus arm (91.7%) [35]. A pharmacokinetic substudy showed that MPA exposure was higher in the tacrolimus arm, which may explain, at least in part, the better results observed in this latter group [36].

Interestingly, in the Symphony study, the best kidney function was observed in the tacrolimus arm regardless of whether the patients had experienced an acute rejection episode or not [37]. At 3 years post-transplantation, kidney function became comparable between all four arms [38]. Creatinine clearance values were comparable between the tacrolimus arm (68.6 $\pm$ 23.8 ml/min), the standard-dose CsA arm (65.9 $\pm$ 26.2 ml/min), and the sirolimus arm (65.3 $\pm$ 26.2 ml/min). Conversely, creatinine clearance value in the low-dose CsA arm (64 $\pm$ 23 ml/min, $P = 0.04$) was significantly lower than in the tacrolimus arm [38]. The lack of difference between the three different arms can be related to the conversion from CsA or sirolimus to tacrolimus during the follow-up period. In addition, overall patient survival rates were similar across all four arms. Death-censored graft survival was significantly higher in the tacrolimus arm (93%) compared to the sirolimus arm (89%; $P = 0.02$) and was similar to the CsA arms (91% for the standard-dose arm, $P = 0.051$; and 95% for the low-dose arm; $P = 0.2$). Biopsy-proven acute rejection rate was significantly lower in the tacrolimus arm (14%) compared to the standard-dose CsA arm (27%, $P < 0.0001$), to the low-dose CsA arm (27%, $P < 0.0001$), and to the sirolimus arm (27%, $P < 0.0001$) [38].

Hence, this study shows that an induction therapy of anti-interleukin 2 receptor blockers followed by low-dose tacrolimus, MPA, plus steroids was highly efficient at preventing acute rejection and did not have any negative effects on kidney function.

Conclusion

The use of mycophenolic acid has prompted transplant physicians to establish CNI-sparing protocols to decrease the nephrotoxicity and cardiovascular-induced side effects it can cause. Protocols on CNI withdrawal when based on MPA without mTORi or belatacept, in *de novo* and maintenance kidney transplant patients, are associated with an increased risk of acute rejection. Consequently, these strategies have been abandoned and are not recommended.

Protocols on CNI minimization show a beneficial impact of kidney function and acceptable acute rejection rates mainly in patients who have been recipients of a graft for more than 3–5 years. However, no significant improvement in graft survival has been observed. These results have been confirmed in a meta-analysis on CNI-sparing regimens where MPA was given to stable kidney transplant patients [39]. It was found that CNI-sparing significantly improved kidney function but did not significantly improve graft survival. However, the acute rejection rate was significantly increased in CNI avoidance strategies [39]. The lack of a beneficial effect on graft survival could be related to an increased risk of an allo-immune response, namely the occurrence of *de novo* DSAs, which have a negative impact on graft survival [40].

Transplant physicians need to keep in mind that there are several limitations to previously published studies. Indeed, most have been underpowered and have included <200 patients, the follow-up periods have been relatively short (ranging from 1 to 2 years, except a couple of studies that have reported 5-year follow-ups), no protocol biopsies have been performed, and the occurrence of *de novo* DSAs was not assessed.

Indeed, in light of our recent improved knowledge regarding the incidence and harmful impacts of DSAs on graft survival, immunological risk can now be assessed using very sensitive immunological tools before using any CNI-sparing strategies. Thus, the incidence of DSAs in patients undergoing CNI-sparing strategies should be prospectively addressed before recommending any of these strategies. In

addition, the target levels of immunosuppressants that can be safely used during a CNI minimization protocol are still unknown. Hence, prospective, well-designed, and long-term studies are required before recommending a CNI-sparing regimen based on mycophenolic acid. The target levels that can be used to avoid acute cellular and antibody-mediated rejection still need to be determined.

Funding

The authors have declared no funding.

References

1. Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715.
2. Vincenti F, Friman S, Scheuermann E, *et al.* Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007; **7**: 1506.
3. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; **346**: 580.
4. El-Zoghby ZM, Stegall MD, Lager DJ, *et al.* Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; **9**: 527.
5. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326.
6. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931.
7. Klintmalm GB, Feng S, Lake JR, *et al.* Belatacept-based immunosuppression in de novo liver transplant recipients: 1-year experience from a phase II randomized study. *Am J Transplant* 2014; **14**: 1817.
8. Diekmann F. Immunosuppressive minimization with MTOR inhibitors and belatacept. *Transpl Int* 2015; **28**: 921.
9. McGrath JS, Shehata M. Chronic allograft nephropathy: Prospective randomised trial of cyclosporin withdrawal and mycophenolate mofetil or tacrolimus substitution. *Transplant Proc* 2001; **33**: 2193.
10. Weir MR, Ward MT, Blahut SA, *et al.* Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy. *Kidney Int* 2001; **59**: 1567.
11. Weir MR, Blahut S, Drachenburg C, *et al.* Late calcineurin inhibitor withdrawal as a strategy to prevent graft loss in patients with suboptimal kidney transplant function. *Am J Nephrol* 2004; **24**: 379.
12. Suwelack B, Gerhardt U, Hohage H. Withdrawal of cyclosporine or tacrolimus after addition of mycophenolate mofetil in patients with chronic allograft nephropathy. *Am J Transplant* 2004; **4**: 655.
13. Dudley C, Pohanka E, Riad H, *et al.* Mycophenolate mofetil substitution for cyclosporine a in renal transplant recipients with chronic progressive allograft dysfunction: the "creeping creatinine" study. *Transplantation* 2005; **79**: 466.
14. Stoves J, Newstead CG, Baczkowski AJ, Owens G, Paraoan M, Hammad AQ. A randomized controlled trial of immunosuppression conversion for the treatment of chronic allograft nephropathy. *Nephrol Dial Transplant* 2004; **19**: 2113.
15. Frimat L, Cassuto-Viguier E, Charpentier B, *et al.* Impact of cyclosporine reduction with MMF: a randomized trial in chronic allograft dysfunction. The 'reference' study. *Am J Transplant* 2006; **6**: 2725.
16. Frimat L, Cassuto-Viguier E, Provot F, *et al.* Long-term impact of cyclosporin reduction with MMF treatment in chronic allograft dysfunction: REFERENCE Study 3-year follow up. *J Transplant* 2010; **2010**: pii: 402750. doi: 10.1155/2010/402750.
17. Smak Gregoor PJ, van Gelder T, van Besouw NM, van der Mast BJ, Ijzermans JN, Weimar W. Randomized study on the conversion of treatment with cyclosporine to azathioprine or mycophenolate mofetil followed by dose reduction. *Transplantation* 2000; **70**: 143.
18. Keunecke C, Rothenpieler U, Zanker B, *et al.* Mycophenolate mofetil monotherapy: an example of a safe nephrotoxicity/atherogenicity-free immunosuppressive maintenance regimen in a selected group of kidney-transplanted patients. *Transplant Proc* 2000; **32**: 6S.
19. Abramowicz D, Manas D, Lao M, *et al.* Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen in stable kidney transplant recipients: a randomized, controlled study. *Transplantation* 2002; **74**: 1725.
20. Abramowicz D, Del Carmen Rial M, Vitko S, *et al.* Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J Am Soc Nephrol* 2005; **16**: 2234.
21. Afzali B, Shah S, Chowdhury P, O'Sullivan H, Taylor J, Goldsmith D. Low-dose mycophenolate mofetil is an effective and safe treatment to permit phased reduction in calcineurin inhibitors in chronic allograft nephropathy. *Transplantation* 2005; **79**: 304.
22. Pascual M, Curtis J, Delmonico FL, *et al.* A prospective, randomized clinical trial of cyclosporine reduction in stable patients greater than 12 months after renal transplantation. *Transplantation* 2003; **75**: 1501.
23. Etienne I, Toupance O, Benichou J, *et al.* A 50% reduction in cyclosporine exposure in stable renal transplant recipients: renal function benefits. *Nephrol Dial Transplant* 2010; **25**: 3096.
24. Kamar N, Rostaing L, Cassuto E, *et al.* A multicenter, randomized trial of increased mycophenolic acid dose using enteric-coated mycophenolate sodium with reduced tacrolimus exposure in maintenance kidney transplant recipients. *Clin Nephrol* 2012; **77**: 126.
25. Cortinovis M, Gotti E, Pradini S, Gaspari F, Perico N. Renal graft function and low-dose cyclosporine affect

mycophenolic acid pharmacokinetics in kidney transplantation. *Transplantation* 2011; **92**: 550.
26. Tran HT, Acharya MK, McKay DB, *et al.* Avoidance of cyclosporine in renal transplantation: effects of daclizumab, mycophenolate mofetil, and steroids. *J Am Soc Nephrol* 2000; **11**: 1903.
27. Vincenti F, Ramos E, Brattstrom C, *et al.* Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. *Transplantation* 2001; **71**: 1282.
28. Grinyo JM, Gil-Vernet S, Cruzado JM, *et al.* Calcineurin inhibitor-free immunosuppression based on antithymocyte globulin and mycophenolate mofetil in cadaveric kidney transplantation: results after 5 years. *Transpl Int* 2003; **16**: 820.
29. Arbogast H, Huckelheim H, Schneeberger H, *et al.* A calcineurin antagonist-free induction/maintenance strategy for immunosuppression in elderly recipients of renal allografts from elderly cadaver donors: long-term results from a prospective single centre trial. *Clin Transplant* 2005; **19**: 309.
30. Asberg A, Midtvedt K, Line PD, *et al.* Calcineurin inhibitor avoidance with daclizumab, mycophenolate mofetil, and prednisolone in DR-matched de novo kidney transplant recipients. *Transplantation* 2006; **82**: 62.
31. Guba M, Rentsch M, Wimmer CD, *et al.* Calcineurin-inhibitor avoidance in elderly renal allograft recipients using ATG and basiliximab combined with mycophenolate mofetil. *Transpl Int* 2008; **21**: 637.
32. Hazzan M, Labalette M, Copin MC, *et al.* Predictive factors of acute rejection after early cyclosporine withdrawal in renal transplant recipients who receive mycophenolate mofetil: results from a prospective, randomized trial. *J Am Soc Nephrol* 2005; **16**: 2509.
33. Hazzan M, Buob D, Labalette M, *et al.* Assessment of the risk of chronic allograft dysfunction after renal transplantation in a randomized cyclosporine withdrawal trial. *Transplantation* 2006; **82**: 657.
34. Ekberg H, Grinyo J, Nashan B, *et al.* Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. *Am J Transplant* 2007; **7**: 560.
35. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
36. Grinyo JM, Ekberg H, Mamelok RD, *et al.* The pharmacokinetics of mycophenolate mofetil in renal transplant recipients receiving standard-dose or low-dose cyclosporine, low-dose tacrolimus or low-dose sirolimus: the Symphony pharmacokinetic substudy. *Nephrol Dial Transplant* 2009; **24**: 2269.
37. Frei U, Daloze P, Vitko S, *et al.* Acute rejection in low-toxicity regimens: clinical impact and risk factors in the Symphony study. *Clin Transplant* 2010; **24**: 500.
38. Ekberg H, Bernasconi C, Tedesco-Silva H, *et al.* Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am J Transplant* 2009; **9**: 1876.
39. Moore J, Middleton L, Cockwell P, *et al.* Calcineurin inhibitor sparing with mycophenolate in kidney transplantation: a systematic review and meta-analysis. *Transplantation* 2009; **87**: 591.
40. Lefaucheur C, Loupy A, Hill GS, *et al.* Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1398.
41. Schnuelle P, van der Heide JH, Tegzess A, *et al.* Open randomized trial comparing early withdrawal of either cyclosporine or mycophenolate mofetil in stable renal transplant recipients initially treated with a triple drug regimen. *J Am Soc Nephrol* 2002; **13**: 536.