Transplant International

Transplant International ISSN 0934-0874

REVIEW

Calcineurin-inhibitor minimization protocols in heart transplantation

Andreas Oliver Zuckermann and Arezu Z. Aliabadi

Department of Cardiothoracic Surgery, Medical University of Vienna, Vienna, Austria

Keywords
cardiac transplantation, immunosuppression, minimization, mycophenolate mofetil, rapamycin, renal dysfunction.

Correspondence
Andreas Oliver Zuckermann MD, Department of Cardiothoracic Surgery, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Tel.: +43 140400 5642; fax: +43 140400 5643; e-mail: andreas.zuckermann@meduniwien.ac.at

Received: 12 May 2008
Revision requested: 7 June 2008
Accepted: 8 September 2008

doi:10.1111/j.1432-2277.2008.00771.x

Summary

Cardiac transplantation has become an established method for end-stage heart disease. A calcineurin-inhibitor (CNI)-based regimen is the cornerstone of immunosuppressive therapy after cardiac transplantation. CNIs have reduced acute rejection and infection and markedly increased survival of cardiac transplantation patients. However, the dose- and time-dependent nephrotoxic effects of CNIs can limit long-term survival, and chronic renal failure is a major cause of morbidity and mortality in long-term cardiac transplant patients. Early experience on withdrawal of CNIs (and maintenance of patients on azathioprine and steroids) in patients, who developed chronic renal dysfunction, resulted in rejection episodes with, sometimes, fatal outcome. The introduction of newer immunosuppressive drugs, like thymoglobulin, anti CD-25 monoclonal antibodies, mycophenolate mofetil, everolimus or sirolimus into clinical practice, has given transplant physicians new tools to adapt immunosuppression to patients' needs. Changes of immunosuppressive protocols by using new drugs early and late after transplantation and simultaneous reduction or weaning of CNIs have become attractive options. The aim of this article is to review strategies to delay, reduce or prevent CNIs after cardiac transplantation as means to improve short- and long-term outcome mainly by protecting renal function.

Introduction

A calcineurin-inhibitor (CNI)-based regimen is the cornerstone of immunosuppressive therapy after cardiac transplantation. CNIs have reduced acute rejection and infection and markedly increased survival of cardiac transplantation patients [1,2]. However, the dose- and time-dependent nephrotoxic effects of CNIs can limit long-term survival [3–5], and chronic renal failure is a major cause of morbidity and mortality in long-term cardiac transplant patients. In recent analyses of cardiac transplant patients, the rate of end-stage renal failure (ESRF) 5 years after cardiac transplantation was between 3.9% and 10.9% [4]. In addition, cardiac transplant patients on chronic hemodialysis had a much higher risk of death (relative risk 4.55) [4–6]. Independent risk factors for developing ESRF were pretransplant renal function, postoperative renal failure, diabetes and age [4].

Early experience in patients, on withdrawal of CNIs (and maintenance on azathioprine and steroids), who developed chronic renal dysfunction (RD), resulted in rejection episodes (some fatal) in several patients [7].

The introduction of newer immunosuppressive drugs, like thymoglobulin, anti CD-25 monoclonal antibodies, mycophenolate mofetil (MMF), everolimus (Evl) or sirolimus (Srl) into clinical practice, has given transplant physicians options that were not available in the past [8–11]. Changes of immunosuppressive protocols by using new drugs early and late after transplantation and simultaneous reduction or weaning of CNIs have become attractive options.

The aim of this article was to review strategies to delay, reduce or avoid CNIs after cardiac transplantation as

means to improve short- and long-term outcome mainly by protecting renal function.

CNI delay

One technique to prevent early renal insufficiency is to delay having to use CNIs by starting with the use of antibody induction therapy. In a prospective trial, Rosenberg *et al.* [12] examined if basiliximab induction therapy with delayed use of CNIs can prevent RD early after transplantation. Twenty-five patients who were considered to be at high risk for development of early RD [preoperative serum creatinine (sCrea) ≥ 221 μM/l or creatinine clearance (creaCl) 35–50 ml/min], received 20 mg basiliximab preoperatively and on day 4 after transplant. Cyclosporine A (CsA) was started on day 4 at a dose of 1–2 mg/kg and then gradually increased to 2–6 mg/kg until targeted serum levels (300–350 ng/ml) were achieved. These patients were compared with 33 patients with normal renal function, who received standard regimen with pretransplant start of CsA and without antibody induction. Moreover, both groups were compared with a historical control group with pretransplant RD, which also received CsA at the time of transplantation. The increase in sCrea after transplantation was less in the group receiving basiliximab induction (−0.1 ± 0.7) than in the historical high-risk group (0.5 ± 1.0; $P < 0.02$) and comparable to the low- risk group (0.03 ± 0.6). The basiliximab protocol was not associated with an increased risk for acute rejection.

In another study, Cantarovich *et al.* [13] evaluated the efficacy of anti-thymocyte globulin (thymoglobulin) induction and delayed initiation of CsA in 15 patients with postoperative RD (≥150 μM/l sCrea). These patients were compared with 17 patients with normal renal function. ATG was given every 2–5 days (1.5 mg/kg depending on total lymphocyte count <200/mm^3) in RD patients and for 5 days in control patients. In the RD group, CsA was delayed until sCrea decreased to less than 150 μM. Both groups received similar ATG doses (7.0 ± 1.0 vs. 6.1 ± 1.8 mg/kg). However, CsA was started significantly later in the RD group (12 ± 9 vs. 2 ± 1 days; $P < 0.0001$). SCrea improved in RD patients and did not differ from controls after the first month. One year survival and rejection rates were 87% and 27% in RD patients and 88% and 59% in control patients.

Delgado *et al.* [14] investigated a direct comparison of basiliximab and thymoglobulin with delayed CsA therapy in patients with RD. Patients in the basiliximab group received two doses of 20 mg on day 0 and day 4 respectively and CsA was started on an average of 7.3 days after transplant. In contrast, the patients in the ATG group received a 10-day course of ATG (dosing depending on lymphocyte and thrombocyte counts) and CsA was started on an average of 3.2 days after transplant. Pretransplant creatinine levels were similar in both groups (>243 μM/l). Both groups resulted in equivalent benefit in renal function at 1 week, 1 month and 6 months (179 ± 45 vs. 154 ± 30 μM/l). However, there were significantly less acute rejections in the ATG group.

These results show that delay of CNIs by using antibody induction therapy may have beneficial effects on early renal function in selected patients without increasing the risk of acute rejection.

CNI avoidance

Calcineurin-inhibitor avoidance has extensively been tested in renal transplantation with mixed results [15–17]. In contrast, there exist only two publications on CNI avoidance in cardiac transplantation. In a pilot trial, Meiser *et al.* [18] examined CNI avoidance immunosuppression in 8 *de novo* patients after cardiac transplantation. Immunosppression consisted of Srl (target levels: 10–15 ng/ml), MMF (2 g/day; target levels: 2.5–4 μg/ml) and steroids (1–0.1 mg/kg/day taper until week 4 after transplant and complete weaning after 6 months). Moreover, all patients received r-ATG antibody induction for 4 days immediately after transplantation. With a follow-up of 3–12 months, patient survival was 100% and freedom from rejection was 75%. Mean creatinine levels initially decreased and remained stable thereafter. Most frequent adverse events were pericardial (25%)-, pleural effusions (13%), peripheral edema (50%), and wound-healing complications (50%). One patient needed operative sternal re-fixation and three surgical repair of sub-xiphoidal hernia. Moreover, moderate myelosuppressive effects were seen as well as intermittently elevated blood lipids.

Vazquez de Prada *et al.* [19] describe case reports of two patients with severe RD (chronic dialysis) prior to transplantation, who were treated with CNI avoidance protocols after transplantation to avoid further renal damage. Although intermittent hemofiltration or dialysis was needed in the early postoperative course, renal function was regained. One patient received daclizumab antibody induction, whereas the other did not receive any induction therapy. Srl was started on day 1 (target levels: 8–12 ng/ml) in combination with standard MMF and steroid doses. Both patients had complicated post-transplant courses, yet recovered completely. There were no signs of acute rejection during the whole follow-up and both patients survived the first post-transplant year.

Both studies suggest that further evaluation of CNI avoidance protocols is warranted.

CNI holiday

Anti-CD25 monoclonal antibody induction has been used to reduce the risk of acute rejection in solid organ transplantation [20,21]. Cantarovich *et al.* has examined the potential of basiliximab and daclizumab to prevent acute rejection during a temporary interruption (holiday) of CNI therapy because of either acute or chronic RD [22–24] in 11 transplant patients, including seven cardiac- and two heart-kidney transplant patients, who experienced 15 events of acute RD after initial post-transplant hospitalization [22]. Acute RD was defined as ≥25% sCrea increase from baseline. The CNI dose was temporarily withheld until sCrea had decreased to baseline. Basiliximab or daclizumab temporarily replaced CNI therapy. Basiliximab was given in days1, 4 and then every 20 days and daclizumab was given every 7 days during CNI holiday. Patients received an average of 3.1 ± 1.9 doses of antibody during a mean duration of 21 ± 51 days of CNI holiday. SCrea decreased significantly from 301 ± 92 to 143 ± 55 μM/l ($P < 0.0001$). Anti CD25 mAb therapy was well tolerated without evidence of side-effects and no episodes of acute rejection were recorded. In a second publication, the same group describe a similar successful case with long-term CNI holiday lasting 171 days [23].

However, there is a need to confirm these preliminary results in prospective randomized trials.

CNI minimization

Minimization with MMF

Mycophenolate mofetil is a non-nephrotoxic immunosuppressive drug. In comparison with azathioprine, superior safety and efficacy have been demonstrated in cardiac transplant patients with improved 1-year survival and lower rejection incidence [8]. Based on these observations, different investigators examined, if CsA could be reduced after switch from azathioprine to MMF in long-term cardiac transplant patients with chronic RD (Table 1). A total of eight studies with similar design demonstrated safety and efficacy of CNI reduction after switch to MMF [25–32]. All Patients were >6 months post-transplant, with the majority of patients >48 months after transplantation. CsA was lowered in all patients from baseline levels 129–170 to 82–129 ng/ml after 6 months and 57–110 ng/ml at end of follow-up (12–54 months postswitch). SCrea decreased in almost all patients from 152–248 μM/l at baseline to 140–195 μM/l at 6 months and to 135–206 μM/l at the end of follow-up. There were only few patients with further deteriorating renal function who were in need of dialysis at the end of follow-up. A total of 27 rejection-episodes ISHLT grade ≥1b were detected in 314 patients (8.7%) included in the eight studies [25–32]. In studies with control groups, study patients showed less rejection episodes. Moreover, rejections had a lower incidence after the switch when compared with preswitch periods. Some of the studies reported a decrease in blood lipids after CsA decrease [25,28].

In 2004, IMPROVED, a prospective comparative multicenter trial confirmed these results in 161 patients [33]. In the intervention arm ($n = 109$, recruited from nine centers), MMF was introduced additionally or instead of azathioprine, followed by CsA reduction (target through levels 2–4 and 50 ng/ml, respectively). In controls ($n = 52$, recruited from one center), immunosuppression remained unchanged. In the MMF group, CsA levels were 57 ± 24 vs. 116 ± 36 ng/ml in controls. In the MMF arm, SCrea decreased by 23 ± 50.7 μM/l ($P < 0.0001$). In controls SCrea increased insignificantly (+7.3 ± 46.9 μM/l; $P = 0.992$). At the end of follow-up, SCrea was significantly lower in the MMF group (186.8 ± 86 vs. 203.1 ± 72.1 μM/l, $P = 0.0001$). Switch to MMF was immunologically safe. The incidence of acute rejection was 2.7% during follow-up.

In 2007, Hamour *et al.* [34] published a sequential study, comprising 240 cardiac transplant patients who were treated either with MMF ($n = 119$) or azathioprine ($n = 121$), both in combination with CsA, steroids and ATG induction therapy. By protocol, lower CsA levels were targeted in the MMF group during the first year (6 months: MMF: 203 ± 52 ng/ml vs. Aza: 236 ± 59 ng/ml, $P = 0.0006$; 24 months: MMF: 147 ± 44 ng/ml vs. Aza: 171 ± 46 ng/ml, $P = 0.001$). Patient survival at 1 year (82% MMF vs. 79% AZA, $P = 0.55$) and at 3 years was similar in both groups. The cumulative probability of receiving anti-rejection treatment within 1 year was lower in the MMF group, as was biopsy-proven acute rejection with ISHLT grade ≥3A (24% vs. 35%, $P = 0.03$). In the MMF group, more patients had steroids withdrawn by 1 year (66% vs. 32%, $P < 0.001$). Renal function was better in the MMF group with lower creatinine levels at 1 year (133 ± 45 vs. 155 ± 46 μM/l, $P = 0.0004$). Calculated creaCl (Cockcroft–Gault) at 1 year was also better (MMF 74 ± 32 ml/min vs. AZA 62 ± 24 ml/min, $P = 0.004$). Although this trial did not achieve minimization of CsA, it showed potential feasibility to lower CsA even during the first 6 months. Overall, it appears that CNI minimization shows a benefit to improve renal function in heart transplantation.

Minimization with sirolimus or everolimus

Sirolimus as well as its derivate Evl have been tested with full dose CsA and steroids to be more effective as azathioprine in prevention of acute rejection [9,10] (Table 1).

Table 1. Calcineurin inhibitor minimization after cardiac transplantation.

Author	Year	*N*	Time post-TX (months)	MMF dose (g/day) PSI level (ng/ml)	Preswitch CNI level (ng/ml)	Postswitch CNI level (ng/ml)	*P*-value	Follow-up (months)	Preswitch renal function (Screa/CrCl)	Last renal function (Screa/CrCl)	*P*-value
MMF											
Aleksic	2000	12	4–60	2	169 ± 58	101 ± 28	0.002	6	221 ± 71	158 ± 63	0.02
Tedoriya	2002	30	90 ± 9	2.3 ± 0.1	124 ± 11	81 ± 8	0.001	12	248 ± 15	206 ± 19	<0.05
Baryalei	2003	14	24 ± 18	2	173 ± 56	110 ± 33	0.02	6 ± 2	239 ± 71	168 ± 44	0.005
Zuckermann	2001	98	52 ± 35	2	148 ± 78	75 ± 26	0.001	36	173 ± 44	158 ± 44	0.03
Sanchez	2004	25	42 ± 26	1.6 ± 0.5	159 ± 70	86 ± 38	0.01	30 ± 13	210 ± 44	141 ± 35	0.001
Boyer	2005	14	97	600*	144.2/6.5	59.4/3.5	0.02	24	47 ± 10	78 ± 16	0.019
Manito	2005	45	40 ± 27	1.97 ± 0.2	151 ± 40	83 ± 34	0.004	12	172 ± 59	153 ± 57	0.001
Al-Aly	2006	5	84 ± 36	1.9 ± 0.2	?	?	NA	39 ± 14	230 ± 62	343 ± 149	NS
Angermann	2004	109	8	1.3 ± 0.6	129 ± 42	57 ± 24	?	8	210 ± 65	187 ± 86	<0.001
Hamour†	2007	119	*De novo*	2.0 ± 1.0	231 ± 53	127 ± 26	NA	36	114 ± 54	142 ± 61	NA
Srl/Evl											
Meiser†	2007	33	*De novo*	6.9 ± 1.7 (Srl)	8.0 ± 2.3	6.4 ± 1.5	NA	24	122 ± 35	121 ± 35	NA
Lehmkuhl†	2007	38	*De novo*	5.81 ± 1.75 (Evl)	240 ± 57	101 ± 26	NA	12	148 ± 52	135 ± 50	NA
Trösch	2004	12	48 ± 31	8–12 (Srl)	180 ± 40	132 ± 46	0.002	6	274 ± 53	212 ± 35	0.032
Schweiger	2006	17	69 ± 11	6.69 ± 0.5 (Evl)	109	68.5	0.001	8	148	165	NS
Balfour	2006	15	74 ± 11	8–10 (Srl)	150–200/6–10	60–100/6–8	NA	1	88	70	0.019
Ross	2008	36	>12	6.4 ± 2.0 (Evl)	124 ± 64	87 ± 43	0.001	3	133 ± 30	146 ± 39	NS

IS, immunosuppressive; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; Srl, sirolimus; Evl, everolimus; ?, not reported; NA, not applicable; NS, nonsignificant; Screa, serum creatinine in μm/l; CrCl, creatinine clearance in ml/min.

*****MMF dose: mg/m^2.

†*De novo* study; CsA and creatinine are listed at 3 months post-transplant and at end of follow-up.

Both drugs, although not nephrotoxic, have been associated with aggravation of CNI-induced nephrotoxicity [9,10]. However, both drugs have complementary mechanisms of action, resulting in a synergistic effect that may enable to reduce the dose of CNIs without loss of efficacy.

Two studies were carried in *de novo* cardiac transplant patients to examine efficacy and safety with two different CNI-minimization protocols in combination with either Srl or Evl [35,36]. Meiser *et al.* [35], report a prospective pilot study in 33 low-risk patients (creatinine < 177 μM/l) using low-dose tacrolimus (Tac: 6–8 ng/ml) and Srl (6–8 ng/ml) with low-dose steroids. Survival was 100% and only one rejection episode occurred during the 24-month follow-up. Steroids were weaned completely after 6 months. Baseline sCrea was 122 ± 35 μM/l. At 12 months, sCrea increased to 144 ± 44 μM/l and decreased again to 121 ± 35 μM/l until month 24. Only two patients developed renal insufficiency and had to be switched from Tac to MMF. In another observational trial Lehmkhul *et al.* [36], compared the combination of reduced-dose CsA (initial target levels: 200–240 ng/ml) in combination with Evl (3–8 ng/ml) in 38 patients with 14 patients receiving CsA (initial target levels: 200–250 ng/ml) in combination with MMF (1.5–2.5 g/day). Starting with week 2, CsA was minimized to 128 ± 38 ng/ml at 6 months and 101 ± 26 ng/ml at 12 months. In contrast, patients in the MMF group underwent a slow reduction protocol (201 ± 48 ng/ml at 6 months and 160 ± 41 ng/ml at 12 months). Mean pretransplant sCrea levels of 148 ± 52 μM/l decreased to 135 ± 50 μM/l under Evl and increased from 108 ± 32 to 176 ± 66 μM/l in the MMF group by Month 12 post-transplant. Efficacy was high in both groups with a rejection rate of 23.6% (Evl) vs. 28.5% (MMF) by Month 12.

Four studies have been published that report about CNI minimization in patients with RD late after cardiac transplantation. Troesch *et al.* [37], reported about 12 patients with severe RD (sCrea > 221 μM/l) with an average time of 4 years after transplantation. CNIs were reduced by 50%, Srl added (target levels 8–12 ng/ml) and azathioprine stopped. SCrea dropped from 274 ± 53 to 212 ± 35 μM/l ($P = 0.032$). Thereafter, no statistically significant changes were noted up to 6 months post transplant ($P = 0.41$). Serum CsA levels dropped from 180 ± 40 to 132 ± 46 ng/ml ($P = 0.002$). Side-effects occurred in four patients and were all related to a Srl level exceeding 12 ng/ml. Schweiger *et al.* [38], describe 20 long-term patients switched to Evl (target levels 3–8 ng/ml) in combination with CsA minimization. Twenty patients with standard therapy (CsA, MMF, steroids) were used as control group. Whereas patients in the control group had stable CsA levels (60–160 ng/ml) during follow-up (8 months), CsA dose was reduced by >25% in study patients. CsA levels decreased significantly from median of 109–68.5 ng/ml ($P < 0.001$) at the end of follow-up. Renal function remained stable in both groups (148–165 μM/l). In pediatric patients, Balfour *et al.* [39] described CNI minimization in 15 long-term patients after switch to Srl (8–10 ng/ml). CNI was reduced by 20–50% and CNIs were weaned completely in 33% of patients. SCrea decreased from 88 to 70 μM/l after 30 days. Rejection occurred in two patients 9 and 17 months after Srl was started. Main adverse events were mouth ulcers in about 30% of patients. The latest study on CNI minimization in combination with Evl initiation has been published by Ross *et al.* [40]. In a multicenter pilot study, MMF or azathioprine was switched to Evl with simultaneous reduction of CsA by 25% in 36 long-term cardiac transplant patients. Further CNI minimization was performed according to renal function. By 12 weeks, 75% of patients were reduced by 26.9 ± 2.9% CsA dose. These patients did not show any reduction of renal function (creaCl: 69.5 ± 14.4 and 66.6 ± 8.6 ml/min; $P = 0.132$). One case of acute rejection of Grade ≥ 3A occurred (2.7%). There was no graft loss or death. Hemoglobin and hematocrit levels decreased significantly, whereas cholesterol and triglyceride levels increased (all $P < 0.0001$).

Based on early experience with CNI minimization, an international expert panel as well as a German-Austrian Consensus group has established similar guidelines for CNI reduction after initiation of Evl. Stepwise CNI-reduction to levels 50–80 ng/ml is thought to be sufficient and effective [41,42]. Moreover, patients should have GFR > 40 ml/min/1.73 m^2 to benefit substantially from CNI minimization after switch to Evl [43]. Nevertheless, more controlled studies are needed to get more information on the beneficial effect of CNI minimization in long-term cardiac transplant patients.

Minimization with C2 measurement

Monitoring of CsA using 2-h postdose (C2) levels is stated to have better correlation with AUC_{0-4} and therefore might have a better association with clinical outcome [44,45]. *De novo* studies comparing C0–C2 monitoring showed contrasting reports about CsA dosing with C2. Whereas Kittleson as well as Cantarovich described lower CsA dosing with C2 monitoring, Barnard did not report any differences [46–48]. Two new trials have examined the ability to lower CsA levels in *de novo* patients. Cantarovich reported about 87 patients receiving high, intermediate and low CsA ranges, depending on pretransplant renal function. Target C2 levels were between 1300 ng/ml (low) and 1700 ng/ml (high) in the early phase (month 1) and between 800 ng/ml (low) and 1200 ng/ml (high)

in the late phase (month 6–12) [49]. Patients with RD, who were randomized to intermediate and low CsA levels, had 28–43% lower CsA levels after 12 months compared with the high groups. Interestingly, rejection rates during the first year were similar in all groups [49]. In another prospective randomized trial, regular and low dose CsA C2 levels were compared in *de novo* patients who received CsA in combination with Evl. Although CsA C2 blood levels were above target levels in the 'reduced' group (target levels: 300–500 ng/ml, actual levels: 600 ng/ml), they were 20% lower than in the standard dose group. As C0 levels were also measured the difference was even larger (153 ± 68 vs. 119 ± 63 ng/ml). Rejection rate was similar between the groups [50].

In long-term patients, two groups report about CsA reduction if C2 monitoring was used instead of C0 monitoring. CsA dose could be reduced by 14–26% with C2 monitoring [51,52].

CNI elimination

Early elimination

A prospective randomized Trial investigated whether renal function benefit could be achieved with the withdrawal of CNI therapy followed by the introduction of Srl at 12 weeks post heart transplantation [53] (Table 2). Of the seven patients randomized to the CNI-weaning arm, four (one with hemodynamic compromise) experienced a ISHLT grade 3A rejection within 5 weeks of discontinuing the CNI. There were no similar episodes of rejection in patients in the CNI-based control arm. Although there are not enough data to allow firm conclusions, the study was terminated because of safety concerns. Possibly SRL and MPA concentrations were not adequate to maintain satisfactory immunosuppression after abrupt CNI withdrawal early after Transplantation. An optimum protocol for CNI withdrawal early after cardiac transplantation remains to be defined.

Late elimination

Sell *et al.* [54] published the first report about CNI-free immunosuppression in thoracic transplantation, in 2002. Twenty-five thoracic transplant patients (including five cardiac transplant patients) were switched to Srl and CNIs were minimized (reduction by 64%). Renal function increased and four patients could be weaned from dialysis. In 48% of the patients, CNI therapy was stopped within 2 months after Srl was started. However, 25% of patients had no benefit from the switch and their renal function decreased. In 2003, Grötzner reported about CNI-free immunosuppression in long-term cardiac transplant patients with RD [55]. He reported on 30 patients (47 ± 50 months after transplant) with CNI-based immunosuppression and creatinine levels > 168 μM/l. Conversion was started with 6 mg Srl, continued with 2 mg, and the dose was adjusted to achieve target trough levels between 8 and 14 ng/ml. MMF was continued with trough level adjusted (1.5–4 μg/ml). Subsequently, the CNIs were tapered down and stopped. Survival was 90% after a mean follow-up of 13 ± 95 months. No acute rejection episode was detected during the study period. Renal function improved significantly after conversion: creatinine preconversion versus post conversion: 279 ± 67 vs. 195 ± 73 μM/l, $P = 0.001$. In three patients, dialysis therapy was stopped completely after conversion.

In contrast, Zakliczynsky, reported about accelerated renal failure in five patients with severe renal damage, where CsA was replaced by Srl [56].

Between 2004 and 2007, a total of seven small single-center pilot-studies were published that entailed CNI conversion to Srl, late after cardiac transplantation [57–63]. Chronic RD was the indication for switch in all the studies. Between eight and 19 patients were included in each study with 24–98 months between transplantation and conversion. Centers used abrupt CNI stop or weaning protocols (weaning period 1–6 weeks) for conversion. Srl target levels were 5–15 ng/ml. Preswitch sCrea was between 186 and 345 μM/l. In all studies, creatinine decreased during follow-up (115–250 μM/l between 6 and 12 months of follow-up). In 50% of the studies, acute rejection episodes were detected during follow-up (time to rejection: 3.4 ± 1.8 months postswitch). Overall Incidence of acute rejection was 20% (Min: 0%; Max: 65%). Most could be treated easily and only a few patients had to be re-converted to CNI-therapy. However, experience with larger patient numbers might be associated with lower incidence of rejection. Studies with higher patient numbers ($n = 23$–80), showed less rejection incidence (4.3% vs. 20%). About 33% of patients were re-converted to CNIs because of side-effects. Main side-effects were mouth ulcers, gastrointestinal problems, acne, edema and infection. In 2005, Kushwaha *et al.* [64] concluded that substitution of CNIs with Srl in cardiac transplant recipients leads to an improvement in renal function, without compromise in cardiac function and rejection. He described 34 stable cardiac transplant recipients with CNI-induced nephrotoxicity (iothalamate clearance 25–50 ml/min) or allograft vasculopathy. Twelve patients (Group A) were prospectively enrolled for RD. The remaining patients ($n = 22$, Group B) were converted to Srl on clinical grounds because of poor renal function or the presence of allograft vasculopathy. A further 24 patients (Group C) were retrospective controls, stable (range 2–10 years post-transplant), and maintained on a standard CNI-based immunosuppressant regimen. CNI was withdrawn gradually over 12 weeks, adjunct immu-

Table 2. Calcineurin inhibitor elimination after cardiac transplantation.

					IS protocol							
Author	Year	*N*	Time post-TX (months)	Indication	CNI wean	Srl, Evl target levels (ng/ml)	PI therapy	Preswitch CNI level (ng/ml)	Preswitch renal function (Screa/CrCl)	Last renal function (Screa/CrCl)	Follow-up (months)	*P*-value
Early												
Hunt	2007	7	3		Stop	5–10	MMF	Center	NA	NA	Stop	NA
Late												
Snell	2002	25	36	Ren	Slow	5–10	MMF/Aza	?	290 ± 210	210	10	0.03
Grötzner	2003	30	47 ± 50	Ren	Slow	8–14	MMF	80–250/7–10	279 30	195 41	12	0.001
Zaklysinski	2003	5	60	Ren	Stop	12–20	?	?	289	448	2	?
Lyster	2004	14	48 ± 36	Ren	Stop	8–20	MMF	181 ± 21	321 ± 107 28	180 42	12	?
Cabezon	2005	8	74 ± 60	Ren	Slow	5–10	MMF	81 ± 49	212 ± 44	150 ± 18	3	<0.05
Fernandez	2005	19	89 ± 44	Ren	Slow	10–12	MMF/Aza	?	243 ± 52	250 ± 124	9	NS
DeMeester	2005	9	96	Ren	Stop	5–15	MMF/Aza	?	186 ± 40	182 ± 55	6	NS
Kushwaha	2005	34	50	Ren	Slow	10–15	MMF	100–150/8–12	36.1	48.7	12	0.001
Hunt	2005	80	57	Ren	Stop	6–12	MMF	?	181	145	2	0.001
Bestetti	2006	10	24	Ren	Stop	6–14	MMF	249 ± 11/13 ± 3	345 ± 159	115 ± 35	6	0.001
Aranda-Dios	2006	17	78 ± 43	Ren	Slow	8–12	MMF	186 ± 40	186 ± 40	135 ± 35	17	0.019
Gleissner	2006	16	98 ± 52	Ren	Fast	7–12	MMF	62 ± 26	186 ± 40 48 ± 21	142 ± 45 62 ± 27	6	0.001
Moro	2007	23	66 ± 54	Ren, Tu	?	?*	?	?	168 ± 45	177 ± 124	11	NS
Rothenburger	2007	60	65 ± 38	Ren, SE	Slow	4–8*	MMF	167	185 40	115 61	6	0.001
Gustafsson	2007	18	82	Ren	Slow	5–15	MMF/Aza	259|5	22.9	29	21	NS
Raichlin	2007	78	56 ± 57	Ren, Tu, Cav	Slow	10–15	MMF/Aza	100–150/6–8	179 ± 18 48 ± 18	140 ± 42 61 ± 25	24	0.001
Aliabadi	2008	61	97 ± 54	Ren, Tu, Cav	Fast	5–10	MMF	83 ± 38	45	50	24	NS

IS, immunosuppressive; CNI, calcineurin inhibitor; Cav, graft vaculopathy; Ren, renal dysfunction; Tu, tumor; SE, side-effects; slow, weaning duration > 2 weeks; fast, weaning duration 1–2 weeks; Stop, CNI stop, Srl or Evl start; Srl, sirolimus; Evl, everolimus; Aza, azathioprine; MMF, mycophenolate mofetil; Center, according to center practice; study stop, trial stopped because of high incidence of rejection; ?, not reported; NA, not applicable; NS, nonsignificant; Screa, serum creatinine in μm/l; CrCl, creatinine clearance in ml/min.
*Everolimus.

nosuppression was left unchanged, and Srl was started at 1 mg/day with titration over two weeks to achieve target levels of 10–15 ng/ml. Iothalamate clearance (improved significantly (Group A baseline: 36.08 ± 2.4 to 48.67 ± 4.1 ml/min, $P = 0.004$; Group B baseline: 48.14 ± 3.2 to 55.77 ± 4.2 ml/min, $P < 0.001$) without exacerbating rejection or compromising cardiac function. By contrast, in controls, Group C, the baseline renal clearance declined from 40.04 ± 1.86 to 34.63 ± 1.6 ml/min over the course of 1 year ($P < 0.01$). Similarly, another study reported about 80 patients with RD in whom CNIs were abruptly discontinued and Srl added at 5 mg twice a day for 2 days and then 2 mg daily [65]. The treatment goal was a trough level of 6–12 ng/ml. All patients were also managed with MMF at 1000 mg twice daily. At a mean of 304 days postconversion, the mean sCrea decreased from 181 ± 55 μM/l preconversion to 145 ± 42 μM/l ($P < 0.001$). Four patients with sCrea ≥ 221 μM/l became dialysis-dependent during follow-up despite conversion.

Gleissner *et al.* [66] reported the first randomized trial between CNI minimization and CNI-free protocols. Thirty-nine patients with renal failure on low-dose CsA (64.0 ± 19.9 ng/ml) were studied. All patients had been treated with low-dose CsA >6 months, renal function was stable or slowly decreasing (creatinine 150–310 μM/l). Nineteen patients were randomized to discontinuation of CsA and overlapping Srl therapy initiation, 20 patients continued low-dose CsA (control). Three patients (16%) discontinued Srl medication because of side-effects (diarrhea, skin rash). No rejections were seen during follow-up. After 6 months, renal function in the control group was unchanged. In the Srl group, renal function markedly improved [creatinine: 186 ± 40 to 142 ± 45 μM/l, cGFR: 48.5 ± 21.4 to 61.7 ± 21.4 ml/min ($P < 0.001$ within and between groups)].

Recently new information about switch to Srl or Evl has been published. Rothenburger *et al.* [67] reported about the first experience with Evl in a CNI-free protocol. Sixty heart transplant recipients underwent standardized switching protocols and completed 6 months of follow-up. Evl was started at a fixed dose of 0.75 mg twice a day, whereas other immunosuppressive drugs were not changed (CsA, MMF, steroids). After 1 week, CsA dose was reduced by 30% and stopped one week later. Evl target levels were 4–8 ng/ml. After switching to Evl, most patients recovered from the side-effects associated with CNIs. Renal function improved significantly after 6 months (creatinine, 185 ± 53 vs. 115 ± 80 μM/l, $P = 0.001$; creaCl, 42.2 ± 21.6 vs. 61.8 ± 23.4 ml/min, $P = 0.018$). Arterial hypertension improved after 3 months and remained decreased during the observation period. Tremor, peripheral edema, hirsutism, and gingival hyperplasia markedly improved. Adverse events occurred in eight patients (13.3%), including interstitial pneumonia ($n = 2$), skin disorders ($n = 2$), reactivated hepatitis B ($n = 1$), and fever of unknown origin ($n = 3$). These preliminary data suggest that CNI-free immunosuppression using Evl is safe, with acceptable efficacy in maintenance heart transplant recipients.

Gustafsson *et al.* [68] examined predictors of improvement in renal function after conversion to Srl in 38 long-term patients. Median creaCl at conversion was 22.9 ml/min (19.1–30.6 ml/min), which increased after 1, 3, and 6 months to 25.9 (18.6–37.1; $P = 0.015$), 25.6 (17.9–34.5; $P = 0.11$), and 28.8 (18.7–38.7; $P = 0.28$) ml/min, respectively. Age, gender, creaCl at baseline, CNI reduction versus discontinuation, and presence or absence of diabetes or hypertension did not predict improvement in creaCl after conversion. Only time from transplantation to conversion, and creaCl 3 months before conversion was correlated to the improvement in renal function after conversion to Srl ($P < 0.05$ and $P < 0.01$ for correlation after 1 month, respectively). Five patients (13%) experienced a grade 3A rejection episode while being treated with Srl.

Raichlin *et al.* [69] reported the longest follow-up with CNI-free immunosuppression after cardiac transplantation. The aim of this study was to assess over 2 years the safety and effect on renal function of switch from CNI to Srl in stable recipients, 56 ± 57 months post-transplant. CNI was substituted with Srl in 78 cardiac transplant recipients (Srl group) of whom 58 (Group A) had CNI-induced renal impairment (GFR < 50 ml/min) and 20 (Group B) had preserved renal function (GFR > 50 ml/min). Fifty-one patients (CNI group) with renal impairment (GFR ≤ 50 ml/min) maintained on CNI served as controls. Secondary immunosuppressants were unchanged. In the Srl group, GFR increased from 47.0 ± 18.0 to 61.2 ± 22.2 ml/min ($P = 0.0001$) 24 months after Srl initiation. In Group A, GFR increased from 40.5 ± 12.7 to 53.9 ± 19.8 ml/min ($P < 0.0001$). In Group B, GFR increased marginally from 67.2 ± 15.8 to 83.5 ± 27.8 ml/min ($P = 0.10$). In the CNI group, GFR declined from 40.5 ± 14.0 to 36.4 ± 12.5 ml/min ($P = 0.23$) after 24 months of follow-up. There was no significant difference in cardiac rejection or cardiac allograft function. In Srl group, proteinuria increased from 299 ± 622 to 517 ± 795 mg/day ($P = 0.0002$) 12 months after Srl initiation and then stabilized; it did not differ from CNI group at 24 months (637 ± 806 vs. 514 ± 744 mg/day, $P = 0.39$).

Aliabadi *et al.* [70] was the first to show the influence of proteinuria development on renal function after switch to Srl. In 61 long-term cardiac transplant patients after switch from CNI to Srl, proteinuria increased significantly

from a median of 0.13 g/day (range 0–5.7) preswitch to 0.23 g/day (0–9.88) at 24 months postswitch ($P = 0.0024$). Before the switch, 11.5% of patients had high-grade proteinuria (>1.0 g/day); this increased to 22.9% postswitch ($P = 0.006$). ACE inhibitor (ACEi) and angiotensin-releasing blocker (ARB) therapy reduced proteinuria development. Patients without proteinuria had increased renal function (median 42.5 vs. 64.1, $P = 0.25$), whereas patients who developed high-grade proteinuria showed decreased renal function at the end of follow-up (median 39.6 vs. 29.2, $P = 0.125$). Thus, proteinuria may develop in cardiac transplant patients after switch to Srl, which may have an adverse effect on renal function in these patients. The cause of proteinuria is still unclear. Experimental as well as clinical studies in renal transplant patients have shown either tubular or glomerular damage. At this stage, it is not established whether hemodynamic effect, direct toxic effects of Srl or both could be implicated as causative agent for the development of proteinuria. Patients with high-grade proteinuria should not be switched to Srl. Srl should be used in combination with ACEi/ARB therapy and patients should be monitored for proteinuria and increased RD.

Conclusion and perspective

Calcineurin-inhibitors are associated with significant side-effects, including nephrotoxicity and chronic RD. Strategies to limit these effects include CNI delay, CNI avoidance, CNI holiday, CNI minimization, and CNI elimination. CNI delay is a novel strategy to prevent early RD in selected patients. CNI avoidance has been used in only a few patients and needs further investigation before any clear statement is allowed. Only one study group has described CNI holiday until now. Nevertheless, it might be an attractive option if more groups can confirm results achieved in the past. The most reliable data has been published on CNI minimization. Many groups investigated MMF introduction in combination with CNI minimization. There exists data from two large trials (one prospective randomized) that proved safety and efficacy of early as well as late minimization. Less data has been published on proliferation signal inhibitors (PSIs) and CNI minimization as well as use of CsA C2 monitoring. Early CNI minimization seems to be attractive as it might reduce the risk of chronic RD. However, it is not clear which CNI target levels are safe. There is a strong need for prospective randomized trials that evaluate the lowest CNI target levels that are still safe. CNI minimization in long-term patients might be safer that CNI elimination, however results concerning renal function seem to be worse.

Calcineurin-inhibitor elimination appears to be an appealing alternative in patients with CNI toxicities (especially renal toxicity). However, early elimination seems to be not safe enough to be used in a general way. Late CNI elimination seems to be possible, though; most studies had low patient numbers and short follow-up. In our center, we have experience with >100 patients after CNI elimination. We strongly feel that it is safe concerning the risk of rejection. Based on our experience in patients with RD, we recommend switching early enough. There might be a threshold of renal damage where CNI elimination does not improve kidney function. Nevertheless, PSIs have special abilities that might have paramount impact on long-term complications after cardiac transplantation. The anti-proliferative effects have beneficial effects on development of graft vasculopathy (CAV). *De novo* therapy with PSIs showed significantly reduced incidence of CAV. Moreover, Mancini *et al.* [71] demonstrated that late switch to PSIs has a beneficial impact on progression of CAV. New data by Raichlin *et al.* [72], revealed that CAV can be attenuated by Srl after CNI elimination.

In conclusion, CNI-delay, -minimization, and -elimination seem to be possible treatment options after cardiac transplantation, yet there is still a lack of prospective randomized trials confirming promising results of small single center experience. Nevertheless, these strategies bear huge potential to counteract the increasing problems and complications of long-term immunosuppression.

Disclosures

Andreas Zuckermann has the following disclosures: Speakers bureau: Genzyme, Novartis, Wyeth; Scientific funding Roche; Advisory board: Astellas. Novartis Investigator: Novartis, Astellas, Wyeth.

References

1. John R, Rajasinghe HA, Chen JM, Weinberg AD, *et al.* Long-term outcomes after cardiac transplantation: an experience based on different eras of immunosuppressive therapy. *Ann Thorac Surg* 2001; **72**: 440.
2. Taylor DO, Edwards LB, Boucek MM, *et al.* International Society for Heart and Lung Transplantation. Registry of the International Society for Heart and Lung Transplantation: twenty-third official adult heart transplantation report-2006. *J Heart Lung Transplant* 2006; **25**: 869.
3. Bloom RD, Doyle AM. Kidney disease after heart and lung transplantation. *Am J Transplant* 2006; **6**: 671.
4. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931.
5. Alam A, Badovinac K, Ivis F, Trpeski L, Cantarovich M. The outcome of heart transplant recipients following the

development of end-stage renal disease: analysis of the Canadian Organ Replacement Register (CORR). *Am J Transplant* 2007; **7**: 461.
6. Senechal M, Dorent R, du Montcel ST, *et al.* End-stage renal failure and cardiac mortality after heart transplantation. *Clin Transplant* 2004; **18**: 1.
7. Hunt S, Stinson E, Oyer P, *et al.* Results of immunoconversion from cyclosporine to azathioprine in heart transplant recipients with progressive nephrotoxicity. *Transplant Proc* 1987; **19**: 2522.
8. Kobashigawa J, Miller L, Renlund D, *et al.* A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Mycophenolate Mofetil Investigators. *Transplantation* 1998; **66**: 507.
9. Keogh A, Richardson M, Ruygrok P, *et al.* Sirolimus in de novo heart transplant recipients reduces acute rejection and prevents coronary artery disease at 2 years: a randomized clinical trial. *Circulation* 2004; **110**: 2694.
10. Eisen HJ, Tuzcu EM, Dorent R, *et al.* Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. *N Engl J Med* 2003; **349**: 847.
11. Kobashigawa JA, Miller LW, Russell SD, *et al.* Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant* 2006; **6**: 1377.
12. Rosenberg PB, Vriesendorp AE, Drazner MH, *et al.* Induction therapy with basiliximab allows delayed initiation of cyclosporine and preserves renal function after cardiac transplantation. *J Heart Lung Transplant* 2005; **24**: 1327.
13. Cantarovich M, Giannetti N, Barkun J, Cecere R. Antithymocyte globulin induction allows a prolonged delay in the initiation of cyclosporine in heart transplant patients with postoperative renal dysfunction. *Transplantation* 2004; **78**: 779.
14. Delgado DH, Miriuka SG, Cusimano RJ, Feindel C, Rao V, Ross HJ. Use of basiliximab and cyclosporine in heart transplant patients with pre-operative renal dysfunction. *J Heart Lung Transplant* 2005; **24**: 166.
15. Larson TS, Dean PG, Stegall MD, *et al.* Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. *Am J Transplant* 2006; **6**: 514.
16. Webster AC, Lee VWS, Chapman JR, Craig JC. Target of rapamycin inhibitors (sirolimus, and everolimus) for primary immunosuppression of kidney transplant recipients: a systematic review and meta-analysis of randomized trials. *Transplantation* 2006; **81**: 1234.
17. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
18. Meiser B, Reichart B, Adamidis I, Uberfuhr P, Kaczmarek I. First experience with de novo calcineurin-inhibitor-free immunosuppression following cardiac transplantation. *Am J Transplant* 2005; **1**: 827.
19. Vazquez de Prada JA, Vilchez FG, Cobo M, *et al.* Sirolimus in de novo heart transplant recipients with severe renal impairment. *Transpl Int* 2006; **19**: 245.
20. Hershberger RE, Starling RC, Eisen HJ, *et al.* Daclizumab to prevent rejection after cardiac transplantation. *N Engl J Med* 2005; **352**: 2705.
21. Mehra MR, Zucker MJ, Wagoner L, *et al.* A multicenter, prospective, randomized, double-blind trial of basiliximab in heart transplantation. *J Heart Lung Transplant* 2005; **24**: 1297.
22. Cantarovich M, Metrakos P, Giannetti N, Cecere R, Barkun J, Tchervenkov J. Anti-CD25 monoclonal antibody coverage allows for calcineurin inhibitor ''holiday'' in solid organ transplant patients with acute renal dysfunction. *Transplantation* 2002; **73**: 1169.
23. Potter B, Giannetti N, Cecere R, Cantarovich M. Long-term calcineurin inhibitor ''holiday'' using daclizumab in a heart transplant patient with acute renal dysfunction. *J Heart Lung Transplant* 2005; **24**: 1126.
24. Cantarovich M. Renal protective strategies in heart transplant patients. *Curr Opin Cardiol* 2007; **22**: 133.
25. Aleksic I, Baryalei M, Busch T, *et al.* Improvement of impaired renal function in heart transplant recipients treated with mycophenolate mofetil and low-dose cyclosporine. *Transplantation* 2000; **69**: 1586.
26. Tedoriya T, Keogh AM, Kusano K, *et al.* Reversal of chronic cyclosporine nephrotoxicity after heart transplantation-potential role of mycophenolate mofetil. *J Heart Lung Transplant* 2002; **21**: 976.
27. Baryalei M, Zenker D, Pieske B, Tondo K, Dalichau H, Aleksic I. Renal function and safety of heart transplant recipients switched to mycophenolate mofetil and low-dose cyclosporine. *Transplant Proc* 2003; **35**: 1539.
28. Zuckermann A, Ploner M, Keziban U, *et al.* Benefit of mycophenolate-mofetil (MMF) in patients with cyclosporine (CYA) induced nephropathy after cardiac transplantation. *J Heart Lung Transplant* 2001; **20**: 163.
29. Sanchez V, Delgado JF, Morales JM, *et al.* Chronic cyclosporine-induced nephrotoxiciy in heart transplant patients: long-term benefits of treatment with mycophenolate mofetil and low-dose cyclosporine. *Transplant Proc* 2004; **36**: 2823.
30. Boyer O, Le Bidois J, Dechaux M, Gubler MC, Niaudet P. Improvement of renal function in pediatric heart transplant recipients treated with low-dose calcineurin inhibitor and mycophenolate mofetil. *Transplantation* 2005; **79**: 1405.
31. Manito N, Kaplinsky EJ, Roca J, *et al.* Heart transplant recipient clinical profile improvement following mycophenolate mofetil late incorporation into the treatment schedule. *Clin Transplant* 2005; **19**: 304.
32. Al-Aly Z, Sachdeva A, Philoctete Ashley JM, Bastani B. Preliminary experience with mycophenolate mofetil for preservation of renal function in cardiac transplant

patients with documented cyclosporine nephrotoxicity. *Nephrology* 2006; **11**: 151.
33. Angermann CE, Störk S, Costard-Jäckle A, *et al.* Reduction of cyclosporine after introduction of mycophenolate mofetil improves chronic renal dysfunction in heart transplant recipients–the IMPROVED multi-centre study. *Eur Heart J* 2004; **25**: 1626.
34. Hamour IM, Lyster HS, Burke MM, Rose ML, Banner NR. Mycophenolate mofetil may allow cyclosporine and steroid sparing in de novo heart transplant patients. *Transplantation* 2007; **83**: 570.
35. Meiser B, Kaczmarek I, Mueller M, *et al.* Low-dose tacrolimus/sirolimus and steroid withdrawal in heart recipients is highly efficacious. *J Heart Lung Transplant* 2007; **26**: 598.
36. Lehmkuhl HB, Mai D, Dandel M, *et al.* Observational study with everolimus (Certican) in combination with low-dose cyclosporine in de novo heart transplant recipients. *J Heart Lung Transplant* 2007; **26**: 700.
37. Trösch F, Rothenburger M, Schneider M, *et al.* First experience with rapamycin-based immunosuppression to improve kidney function after heart transplantation. *Thorac Cardiovasc Surg* 2004; **52**: 163.
38. Schweiger M, Wasler A, Prenner G, *et al.* Everolimus and reduced cyclosporine trough levels in maintenance heart transplant recipients. *Transpl Immunol* 2006; **16**: 46.
39. Balfour IC, Srun SW, Wood EG, Belsha CW, Marshall DL, Ferdman BR. Early renal benefit of rapamycin combined with reduced calcineurin inhibitor dose in pediatric heart transplantation patients. *J Heart Lung Transplant* 2006; **25**: 518.
40. Ross H, Pflugfelder P, Haddad H, *et al.* Cyclosporine reduction in the presence of everolimus: 3-month data from a Canadian pilot study of maintenance cardiac allograft recipients. *J Heart Lung Transplant* 2008; **27**: 197.
41. Lehmkuhl H, Ross H, Eisen H, Valantine H. Everolimus (certican) in heart transplantation: optimizing renal function through minimizing cyclosporine exposure. *Transplant Proc* 2005; **37**: 4145.
42. Zuckermann A, Manito N, Epailly E, *et al.* Multidisciplinary insights on clinical guidance for the use of proliferation signal inhibitors in heart transplantation. *J Heart Lung Transplant* 2008; **27**: 141.
43. Rothenburger M, Zuckermann A, Bara C, *et al.* Recommendations for the use of everolimus (Certican) in heart transplantation: results from the second German-Austrian Certican Consensus Conference. *J Heart Lung Transplant* 2007; **26**: 305.
44. Cantarovich M, Elstein E, de Varennes B, Barkun JS. Clinical benefit of neoral dose monitoring with cyclosporine 2-hr post-dose levels compared with trough levels in stable heart transplant patients. *Transplantation* 1999; **68**: 1839.
45. Cantarovich M, Besner JG, Barkun JS, Elstein E, Loertscher R. Two-hour cyclosporine level determination is the appropriate tool to monitor Neoral therapy. *Clin Transplant* 1998; **12**: 243.
46. Kittleson M, Skye A, Felker G, *et al.* De novo C2 monitoring immediately after cardiac transplantation is not associated with improved renal function. *J Heart Lung Transplant* 2006; **25**: S127.
47. Cantarovich M, Giannetti N, Cecere R. Impact of cyclosporine 2-h level and mycophenolate mofetil dose on clinical outcomes in de novo heart transplant patients receiving anti-thymocyte globulin induction. *Clin Transplant* 2003; **17**: 144.
48. Barnard JB, Thekkudan J, Richardson S, *et al.* Cyclosporine profiling with C2 and C0 monitoring improves outcomes after heart transplantation. *J Heart Lung Transplant* 2006; **25**: 564.
49. Cantarovich M, Ross H, Arizón JM, *et al.* Benefit of neoral C2 monitoring in de novo cardiac transplant recipients receiving basiliximab induction. *Transplantation* 2008; **85**: 992.
50. Keogh A, Wang SS, Zuckermann A, *et al.* Everolimus in de novo heart transplant recipients six month data on cyclosporine reduction and impact on renal function. *J Heart Lung Transplant* 2007; **26**: S202.
51. Cantarovich M, Elstein E, de Varennes B, Barkun JS. Clinical benefit of neoral dose monitoring with cyclosporine 2-hr post-dose levels compared with trough levels in stable heart transplant patients. *Transplantation* 1999; **68**: 1839.
52. Mathias HC, Ozalp F, Will MB, *et al.* A randomized, controlled trial of C0- Vs C2-guided therapeutic drug monitoring of cyclosporine in stable heart transplant patients. *J Heart Lung Transplant* 2005; **24**: 2137.
53. Hunt J, Bedánová H, Starling RC, *et al.* Premature Termination of a Prospective, Open Label, Randomized, Multicenter Study of Srl to Replace CNIs (CNI) in a Standard Care Regimen of CNI, MMF and Corticosteroids Early after Heart Transplantation. *J Heart Lung Transplant* 2007; **26**(2S): S203.
54. Snell GI, Levvey BJ, Chin W, *et al.* Sirolimus allows renal recovery in lung and heart transplant recipients with chronic renal impairment. *J Heart Lung Transplant* 2002; **21**: 540.
55. Groetzner J, Meiser B, Landwehr P, *et al.* Mycophenolate mofetil and sirolimus as calcineurin inhibitor-free immunosuppression for late cardiac-transplant recipients with chronic renal failure. *Transplantation* 2004; **77**: 568.
56. Zakliczynski M, Nozynski J, Zakliczynska H, Rozentryt P, Zembala M. Deterioration of renal function after replacement of cyclosporine with sirolimus in five patients with severe renal impairment late after heart transplantation. *Transplant Proc* 2003; **35**: 2331.
57. Lyster H, Panicker G, Leaver N, Banner NR. Initial experience with sirolimus and mycophenolate mofetil for renal rescue from cyclosporine nephrotoxicity after heart transplantation. *Transplant Proc* 2004; **36**: 3167.
58. Bestetti RB, Theodoropoulos TA, Burdmann EA, Santos MA, Abbud-Filho M. Switch from cyclosporine to sirolimus as a treatment of acute renal failure complicating

cardiogenic shock in a heart transplant recipient. *Int J Cardiol* 2006; **112**: 83.
59. Fernandez-Valls M, Gonzalez-Vilchez F, de Prada JA, Ruano J, Ruisanchez C, Martin-Duran R. Sirolimus as an alternative to anticalcineurin therapy in heart transplantation: experience of a single center. *Transplant Proc* 2005; **37**: 4021.
60. De Meester JM, Van Vlem B, Walravens M, *et al.* Preservation of renal function after heart transplantation: initial single-center experience with sirolimus. *Transplant Proc* 2005; **37**: 1835.
61. Aranda-Dios A, Lage E, Sobrino JM, *et al.* Sirolimus experience in heart transplantation. *Transplant Proc* 2006; **38**: 2547.
62. Cabezón S, Lage E, Hinojosa R, Ordóñez A, Campos A. Sirolimus improves renal function in cardiac transplantation. *Transplant Proc* 2005; **37**: 1546.
63. Moro J, Almenar L, Martinez-Dolz L, *et al.* mTor Inhibitors and their secondary effects in cardiac transplant recipients: a descriptive study. *Transplant Proc* 2007; **39**: 2365.
64. Kushwaha SS, Khalpey Z, Frantz RP, *et al.* Sirolimus in cardiac transplantation: use as a primary immunosuppressant in calcineurin inhibitor-induced nephrotoxicity. *J Heart Lung Transplant* 2005; **24**: 2129.
65. Hunt J, Lerman M, Magee MJ, Dewey TM, Herbert M, Mack MJ. Improvement of renal dysfunction by conversion from calcineurin inhibitors to sirolimus after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 1863.
66. Gleissner CA, Doesch A, Ehlermann P, *et al.* Cyclosporine withdrawal improves renal function in heart transplant patients on reduced-dose cyclosporine therapy. *Am J Transplant* 2006; **6**: 2750.
67. Rothenburger M, Teerling E, Bruch C, *et al.* Calcineurin inhibitor-free immunosuppression using everolimus (Certican) in maintenance heart transplant recipients: 6 months' follow-up. *J Heart Lung Transplant* 2007; **26**: 250.
68. Gustafsson F, Ross HJ, Delgado MS, Bernabeo G, Delgado DH. Sirolimus-based immunosuppression after cardiac transplantation: predictors of recovery from calcineurin inhibitor-induced renal dysfunction. *J Heart Lung Transplant* 2007; **26**: 998.
69. Raichlin E, Khalpey Z, Kremers W, *et al.* Replacement of calcineurin-inhibitors with sirolimus as primary immunosuppression in stable cardiac transplant recipients. *Transplantation* 2007; **84**: 467.
70. Aliabadi AZ, Pohanka E, Seebacher G, *et al.* Development of proteinuria after switch to sirolimus-based immunosuppression in long-term cardiac transplant patients. *Am J Transplant* 2008; **8**: 854.
71. Mancini D, Pinney S, Burkhoff D, *et al.* Use of rapamycin slows progression of cardiac transplantation vasculopathy. *Circulation* 2003; **108**: 48.
72. Raichlin E, Bae JH, Khalpey Z, *et al.* Conversion to sirolimus as primary immunosuppression attenuates the progression of allograft vasculopathy after cardiac transplantation. *Circulation* 2007; **116**: 2726.