Prospective Study of Everolimus With Calcineurin Inhibitor-Free Immunosuppression After Heart Transplantation: Results at Four Years

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Background. Immunosuppression is necessary after transplantation but it is associated with distinct adverse side effects. These negative effects could at least partially be overcome with the mammalian target of Rapamycin (mTOR) inhibitor everolimus. Few studies have examined everolimus therapy with calcineurin inhibitor (CNI) withdrawal in maintenance heart transplant patients (HTx).

Methods. In this prospective, single-arm, single-center study, maintenance patients after HTx were converted from CNI to everolimus. They were followed for 48 months. Primary endpoints were kidney-function and arterial hypertension.

Results. Forty-eight patients were recruited (mean post-transplant time 5.4 ± 3.5 years). Of these, 36 were followed for the entire 4-year period. Median calculated glomerular filtration rate increased from 40.7 (32.4 to 59.1) mL/minute at baseline to 48.9 (29.7 to 67) mL/minute at month 48 (p = not significant). Median systolic and diastolic blood pressure, triglycerides, and high-density lipoprotein and low-density lipoprotein cholesterol, did not change significantly in a comparison of the values at baseline and at 48 months. Early resolution of most non-renal CNI-related adverse events was sustained. Due to adverse events, CNI therapy had to be reintroduced in 6 patients (12.5%). No significant changes in cardiac function parameters were observed.

Conclusions. Calcineurin inhibitor-free immunosuppression with everolimus is an effective and safe option in selected maintenance HTx patients. Most adverse effects under everolimus occurred early after conversion and in most cases resolved without intervention within a few weeks. Refining selection criteria may help both in identifying patients who will profit most from switching and in alleviating the need to reintroduce CNI therapy.


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Immunosuppression is a necessary requirement to prevent rejection after heart transplant (HTx). Calcineurin inhibitors (CNI) were introduced more than 30 years ago [1], resulting in a dramatic improvement in efficacy and thus a marked improvement in survival rates [2]. However, the adverse effects of CNI therapy, particularly renal dysfunction, became increasingly apparent [3]. In addition to their nephrotoxic effect, CNIs induce arterial hypertension, which requires medical therapy, in turn causing further adverse events and reducing therapeutic compliance [3]. Moreover, side effects such as tremor and cosmetic problems are quite common with CNI-based immunosuppression [3].

Patients given everolimus experience superior tolerability and fewer cytomegalovirus infections, compared with other regimens [4–6]. Initially, CNI-free immunosuppression with an mTOR-inhibitor in HTx focused on sirolimus (Rapamune), in combination with mycophenolic acid [7, 8]. After CNI withdrawal, renal function significantly improved in the majority of patients under a mTOR-inhibitor [7, 8]. Several studies show promising results for everolimus with reduced CNI-protocols [9, 10] but data on long-term everolimus-based CNI-free immunosuppressive regimens are still scarce [11].

In this prospective study, we evaluated the effect of switching maintenance HTx patients experiencing CNI-related adverse events or rejection to everolimus with CNI discontinuation. Patients were followed for 4 years after the switch. To the best of our knowledge, this is the first extended long-term follow-up of everolimus-based CNI-free immunosuppression in a large cohort of maintenance HTx recipients.

Patients and Methods

Study Design
This was a prospective, single-arm, single-center study in which maintenance HTx patients were switched from
CNI therapy to everolimus. Patients receiving an immunosuppressive regimen including CNI with a clinical indication for switching immunosuppression (renal impairment, other severe adverse events, or recurring acute rejections) were eligible to take part in the study unless they had severe preterminal chronic kidney insufficiency or were already receiving hemodialysis. Only patients transplanted for at least 1 year were included. A cohort of control patients remaining on a CNI-based regimen, pair-matched for age, sex, and time after transplantation, was used to comparatively assess the course of renal function in patients switched. Target trough levels of cyclosporine A for the control patients were as follows: Year 1 after transplantation, 200 to 250 ng/mL; years 2 to 5 after transplantation, 150 ng/mL; thereafter: 100 ng/mL. The study was conducted in accordance with the Declaration of Helsinki and the US Food and Drug Administration guidelines for good clinical practice, after approval by the local Medical Ethics Committee of our institution.

Immunosuppression
Everolimus was introduced using a standardized protocol described elsewhere [12, 13]. All patients received antihypertensive therapy (beta-blockers, calcium-antagonists, angiotensin converting enzyme inhibitors or angiotensin-I blockers), adjusting the doses as required. In the minority of patients who were not receiving statins at baseline, statins therapy could be initiated as necessary after the introduction of everolimus.

Evaluation
Study visits took place at baseline and every 3 months thereafter up to 48 months post switch. At each visit, a physical examination was performed and blood (electrolytes, kidney and liver function tests, C-reactive protein, interleukin-6, NT-proBNP, lipid status, etc) and urine (creatinine, global protein, albumin, alpha1-microglobulin, alpha2-macroglobulin) biochemistry were analyzed. In addition, the trough concentration of everolimus was measured by liquid chromatography with mass spectrometry [14]. A standard echocardiographic examination and Doppler echocardiography were performed by experienced cardiologists at each study visit. Coronary angiograms and right-heart catheterization, with measurement of cardiac output and pulmonary artery pressure, were undertaken before switching and after 12 months. Catheterization results were analyzed by specialized cardiologists, blinded to the medical treatment, who qualitatively graded (normal, coronary artery disease, or allograft vasculopathy) each vessel. Right ventricular biopsy was performed yearly, when rejection was clinically suspected, and before and after larger operations with deep surgical wounds, because these operations required patients to temporarily revert to CNI-based protocols. Myocardial single photon emission computed tomography was performed before conversion to everolimus and 12 months after switching.

Study Endpoints
In previous studies [13, 15], renal function and blood pressure after 24 months served as the primary endpoints. Accordingly, in this follow-up study we continued to observe these primary endpoints up to 4 years from the time of conversion. Secondary endpoints for this study were changes in CNI-related adverse events (tremor, hirsutism, gingival hyperplasia, and peripheral edema), changes in lipid status, and the occurrence of adverse events, hospital readmissions, and rejection episodes after the switch to everolimus.

Statistical Analysis
Statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY). Due to the small sample size and the partial lack of a normal distribution of our data, nonparametric methods were applied. A Friedman test with post hoc analyses for multiple comparisons was used to determine differences between the time intervals of assessment, both for continuous and for ordinal data. The Mann-Whitney U test was performed to compare the 2 groups, regarding the effect of changes at 1-year intervals, beginning at baseline and ending 48-months later. The McNemar $\chi^2$ test was used to compare the dichotomous proportions of the presence and absence of different periods at baseline and after 48 months. Changes in parameters over all 5 time intervals were investigated by means of the Cochran Q, followed by post-hoc analyses in case of overall significance. A $p$ value less than 0.05 was considered statistically significant.

Results
The results of the first 6 and 24 months after switching to everolimus, including adverse events, have already been published before [13, 15, 16]. In the following, the results from month 25 to month 48 and the overall course of a 4-year follow-up are presented.

Patient Population
A total of 48 patients were recruited, of whom all could be followed for 12, 44 for 24, 38 for 36, and 36 for the full 48 months. Six patients (12.5%) discontinued everolimus due to adverse events. Five patients died during the study period. There was no indication of an acute or chronic rejection as the reason for these deaths. One patient was lost to follow-up.

The study population consisted of 43 males and 5 females, with a mean age of 54.4 years (range: 22 to 74 years) at inclusion. At the time of conversion, the mean posttransplant time was $5.4 \pm 3.5$ years. In all but 1 case, the indication for switching was CNI-related adverse events, comprising mostly renal impairment and allograft vasculopathy. One patient was switched due to recurrent rejection episodes that developed despite several immunosuppressive regimens.

A control group of 34 patients remaining on a CNI-based regimen was used to comparatively assess renal
function in the cohort of patients switched to everolimus. There was no statistical difference between the intervention and control group in terms of age, sex, posttransplant time, and the duration of renal function follow-up (mean age at inclusion 54.1 ± 12.2 years, mean time posttransplant 4.7 ± 2.7 years).

Immunosuppression
At the beginning, all patients were on a CNI-based regimen, with 46 patients concomitantly receiving mycophenolic acid and two patients receiving different immunosuppressive regimens. After switching to everolimus, all patients achieved the target everolimus trough-concentration range (4 to 8 ng/mL). The mean everolimus trough level was 6.3 (4.75 to 7.75) ng/mL at 12, 5.6 (5.03 to 6.7) ng/mL at 24, 5.1 (4.2 to 6.8) at 36, and 5.7 (4.05 to 6.9) ng/mL at 48 months. The mean dose was 1.5 mg per day during the first 3 years and 1.75 mg per day during the fourth year. All patients discontinued CNI, but in 7 cases CNI therapy had to be reintroduced due to adverse events (n = 6) or withdrawal of consent (n = 1).

Renal Function
Median serum creatinine at baseline was 1.77 mg/dL (1.47–2.16), decreasing to 1.4 mg/dL (1.12 to 1.89) at month 12 (p < 0.001 versus baseline), and stabilizing at 1.39 mg/dL (1.19 to 1.87) at month 24 and 36 months (p < 0.01 versus baseline, respectively). After 48 months, median serum creatinine was 1.53 (1.1 to 2.31) but compared with baseline this reduction did not reach statistical significance (Table 1). Creatinine clearance (Modification of Diet in Renal Disease formula) improved significantly from baseline to month 12, a difference that was sustained at months 24 and 36. After 48 months, creatinine clearance had increased from 40.7 (32.4–59.1) to 48.9 (29.7–67) mL/min/1.73 m², but this difference also did not reach statistical significance (Table 1). The decrease in serum urea nitrogen concentration compared with baseline was significant at months 12 and 24 but not at months 36 and 48. Serum urea nitrogen was within the normal range over the entire study period.

When patients were divided into groups according to their baseline creatinine concentration in steps of 0.5 mg/dL, the calculated absolute decline in creatinine concentration was only relevant in those with a baseline creatinine concentration of 1.6 mg/dL and higher. The collection of data on proteinuria was started only after the first 2 years. As such, there was neither a long-term course over the 4 years nor a sufficient amount of data to perform reasonable statistical testing.

In the control group, renal parameters did not significantly change at month 48 compared with baseline. Absolute changes from baseline to the end of the study were calculated and did not significantly differ for all 3 renal parameters between the everolimus and control group (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Values Over the Time</th>
<th>Baseline</th>
<th>12 Months</th>
<th>24 Months</th>
<th>36 Months</th>
<th>48 Months</th>
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<td>Range</td>
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<td>Creatinine (mg/dL)</td>
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<td>46</td>
<td>1.4-1.97</td>
<td>46</td>
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<td>Urea (mg/dL)</td>
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<td>44</td>
<td>2.3-4.5</td>
<td>44</td>
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<td>GFR (ml/min/1.73 m²)</td>
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<td>78-120</td>
<td>48</td>
<td>75-120</td>
<td>44</td>
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<td>Syst. Bp (mm Hg)</td>
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<td>48</td>
<td>120-130</td>
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<td>EF (ejection fraction)</td>
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<td>LVEDD (mm)</td>
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<td>Triglyceride (mg/dL)</td>
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<td>NT-proBNP (pg/mL)</td>
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<td>37</td>
<td>50-83</td>
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*p all = significance over all time points; *bl-48 = significance baseline versus 48 months; *np = not performed; Syst. Bp = systolic blood pressure; LVESD = left ventricular end-systolic diameter; LVEDD = left ventricular end-diastolic diameter; EF = ejection fraction; LVEDD = left ventricular end-diastolic diameter; Syst. Bp = systemic blood pressure.
Blood Pressure, Lipid Profile, and Serum Chemistry

Systolic and diastolic blood pressures did not significantly change over the 4-year study period (Table 1). Serum concentrations of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides did not significantly differ at 48 months versus baseline (Table 1). At baseline, 37 patients (77.1%) were receiving statins therapy compared with 33 patients (91.7%) at month 48, with simvastatin and pravastatin the most commonly used statins.

Bilirubin levels decreased from 0.6 (0.5 to 0.9) at baseline to 0.5 (0.4 to 0.6) at month 48, with significant differences between the baseline and months 12, 24, and 36, and a trend versus month 48 ($p = 0.07$). All other laboratory parameters did not change significantly (Table 1).

Over the 48 months, there was also no significant change in interleukin-6 as a marker of inflammation.

Cardiac Function

There were no significant changes in any echocardiographic parameter from baseline to month 48. All values were within the normal range throughout the study period. There were also no significant differences in cardiac output or in the incidence of pulmonary hypertension, as indicated by right-heart catheterization (data not shown). Data on transplant vasculopathy could not be statistically assessed due to the small sample numbers. Concerning the prevalence of coronary artery disease, there was no significant change compared with the control group (data not shown). There were also no significant differences in perfusion defects or myocardial scars from baseline (n = 39) to month 48 (n = 20) (data not shown), as determined by single photon emission computed tomography.

The NT-proBNP, as a marker of heart insufficiency, did not significantly change over the entire study period.

Median body mass index decreased from 25.5 kg/m² (23.5 to 28.1 kg/m²) at baseline to 23.9 kg/m² (21.9 to 26.9 kg/m²) at the end of the study. The difference was statistically significant when baseline was compared with the measurements at 48 months.

CNI-Related Adverse Events

As previously reported [13, 15, 16], the incidence of CNI-related tremor, hirsutism, gingival hyperplasia, and peripheral edema, decreased within the first few weeks after the switch to everolimus. In our patients, this effect was sustained to month 48, with no de novo cases of CNI-related side effects and no deterioration of those adverse events that had not completely resolved after switching.

Safety and Tolerability

The majority of everolimus-related adverse events developed shortly after the switch [13, 15, 16]. Adverse events reported for the first time after month 24 included influenza infections (n = 13), peripheral edema (n = 9) that could not be explained by deteriorating cardiac or renal function, diarrhea (n = 3), unintended loss of
bodyweight (n = 3), or infectious pneumonia (n = 2). No further adverse events involving the skin were reported after month 24. Two patients developed interstitial pneumonia during the first 6 months after switching, both requiring reversion to a CNI regimen. Both patients were greater than 65 years of age and 1 was a patient who had been re-transplanted. Only 4 episodes of biopsy-proven acute rejection (≥ grade 2B) occurred after month 24, including two grade 3 rejections in the patient who had been switched to everolimus due to previous recurrent rejections under different several immunosuppressive regimens.

During the study, 9 patients had to be transiently re-converted to CNI as they underwent surgery with deep wounds. In the course of the study, 50 patients had unscheduled re-admittance to the hospital, including admissions for coronary angiography with percutaneous coronary intervention.

Comment

In this large cohort of maintenance HTx patients, renal function initially improved after the first 3 years of everolimus therapy and remained stable after patients were converted from CNI- to everolimus-based immunosuppression. Renal function did not significantly differ between the everolimus group and the control group kept on a CNI-based immunosuppressive regimen. Most other CNI-related adverse events resolved after switching to everolimus, including tremor, peripheral edema, hirsutism, and gingival hyperplasia. Severe adverse events in patients receiving everolimus were infrequent, although discontinuation was necessary in some cases.

Our finding that renal function initially improved after patients were switched from CNI to everolimus is consistent with the results of studies in kidney transplantation [17] and other data from heart transplantation [11, 18], although an extended delay post transplantation [19] or high baseline creatinine levels [20] before conversion can obviate any benefit. To the best of our knowledge, there are no data obtained from a large cohort of patients on completely CNI-free regimens using everolimus and followed for 48 months. Actually, patients have been switched to everolimus due to relevant renal dysfunction, i.e. reduced glomerular filtration rate or progradient rise of renal retention values. Thus, there is a bias to the disadvantage of patients switched to everolimus although not statistically significant at baseline. Against this background, statistical equality concerning renal function can already be rated as a success.

In our experience, it is not the duration of exposure to CNIs but the extent of renal impairment that is the key determinant of whether CNI elimination following the introduction of everolimus will be advantageous. Pathophysiologically, it seems logical that the switch should be made at an early stage of renal dysfunction, before irreversible structural changes have occurred [21]. Even if renal function does not improve significantly under everolimus, a reduction of CNI-related toxicity can slow the progress of impairment and thus postpone the need for dialysis. We were able to show, especially in patients with a baseline creatinine >1.5 mg/dL, that the conversion to everolimus was beneficial. This finding supports the conclusions of Arora and colleagues [22], who also showed that patients benefit most when switched to everolimus within the first 5 years after transplantation.

Over the course of this study, blood pressure did not change significantly after conversion from CNI to everolimus. Indeed, a reduction of blood pressure after the cessation of CNI was expected. A possible explanation for the missing decrease is a progression of the preexisting damage by CNI. Alternatively, it may be due to the fact that, for historical reasons, patients transplanted at our institution are routinely maintained on low-dose prednisolone (5 mg/day).

There were no clinically relevant changes in lipid status; however, significantly more patients received statins therapy, although the doses were not higher than in other patients. No patient had to revert to CNI due to hypertriglyceridemia or hypercholesterolemia. Other trials have shown mixed results in terms of lipid parameters after the conversion from CNI to everolimus therapy [20, 23, 24]. Rosing and colleagues [25] could show that everolimus reduces Lp-PLA2-levels, thus probably reducing the risk for the development of coronary artery disease and transplant vasculopathy. The frequency of all CNI-related side effects in our patients improved early and significantly after the switch to everolimus, suggesting lower neurologic toxicity and less induction of cellular hyperplasia. Moreover, once resolved, the adverse events did not recur.

The rate of adverse events during everolimus therapy was within the normal range reported with the use of other immunosuppressive drugs. Most side effects occurred early post conversion and were mild and temporary, usually resolving spontaneously within a few weeks or after mild reduction of the everolimus dosage within the target trough range of 4 to 8 ng/mL [13]. By the final evaluation, 6 patients (~12.5%) who had been switched to everolimus were again on a CNI-based regimen, due to adverse events over the course of the 48-month follow-up. This experience is consistent with reconversion rates in other studies [21, 26, 27]. The rate of CNI reintroduction may be reduced by refining patient selection for everolimus therapy, particularly by excluding patients undergoing re-transplantation and those older than 65 years.

Several studies show that everolimus exerts an inhibitory effect on vascular smooth muscle cell growth and on the progression of allograft vasculopathy [5, 28]. In our study, the prevalence of allograft vasculopathy was too minimal for statistical analysis and an increase was by no means apparent. Thus, given the usual continuous exacerbation of transplant vasculopathy, this stabilization could be regarded as an improvement. In addition, cardiac function was stable, without any signs of deterioration. These findings are comparable with data reported in the literature [9, 16].

In conclusion, our results suggest that CNI-free immunosuppression using everolimus is an effective and safe option in maintenance HTx patients with stable function in whom the elimination of CNI is desirable due
to renal impairment or other complications. In our experience, immunosuppression should be switched early, but not too early, after transplantation because switching within the first months post-transplantation may increase the likelihood of rejection [29]. Adverse effects under everolimus often resolved without intervention, within a few weeks. An improvement or stabilization in renal function and a resolution of CNI-related adverse effects therefore justify a conversion to everolimus in selected patients.

There are some limitations in this single-center study. The control group was recruited retrospectively and was smaller than the everolimus group. Also, an adequate pair match for every patient switched to everolimus was not possible. Proteinuria data were collected beginning 2 years after the study was initiated, considerably reducing the data available for evaluation.

Part of this work will appear in the doctoral thesis of Mortimer Phil Klarner.

References