Conversion to Sirolimus as Primary Immunosuppression Attenuates the Progression of Allograft Vasculopathy After Cardiac Transplantation

Eugenia Raichlin, MD; Jang-Ho Bae, MD; Zain Khalpey, MD, MRCS; Brooks S. Edwards, MD; Walter K. Kremers, PhD; Alfredo L. Clavell, MD; Richard J. Rodeheffer, MD; Robert P. Frantz, MD; Charanjit Rihal, MD; Amir Lerman, MD; Sudhir S. Kushwaha, MD

Background—We investigated the potential of conversion to sirolimus (SRL) as a primary immunosuppressant in attenuating cardiac allograft vasculopathy progression.

Methods and Results—Twenty-nine cardiac transplant recipients were converted to SRL 3.8±3.4 years after transplantation with complete calcineurin inhibitor (CNI) withdrawal. Secondary immunosuppressants (azathioprine or mycophenolate) and steroids remained unchanged. Forty patients (controls) 4.8±4.0 years from transplantation were maintained on CNIs. Three-dimensional intravascular ultrasound studies were performed at baseline and 12.1±2.6 months later. Mean plaque (media and intima) volume (PV) and plaque index (PI) (PV/vessel volume percent) increased significantly in the CNI group (1.28±0.86 mm³/mm², P=0.004; and 6±8%, P=0.0001) but not in the SRL group (0.1±0.13 mm³/mm², P=0.63; and 0.1±8%, P=0.94). In patients enrolled within 2 years after transplantation, the increases in PV (0.06±0.106 versus 1.77±1.65 mm³/mm²; P=0.0081) and PI (0±9% versus 10±8%; P=0.0145) were smaller in the SRL group (n=11) than in the CNI (n=12) group. In patients enrolled ≥2 years after transplantation, the increase in PI was less in the SRL group compared with the CNI group (0.1±6.5% versus 5±8%; P=0.033), but changes in PV did not differ significantly. Treatment with azathioprine or mycophenolate did not affect PV or PI in either the SRL group (PV: 0.22±0.66 versus 0.05±1.45 mm³/mm², P=0.46; PI: 1.5±6% versus 1.6±8.5%, P=0.29) or the CNI group (PV: 1.42±1.39 versus 1.06±2.28 mm³/mm², P=0.49; PI: 7.8±8.7% versus 4.8±7.3%, P=0.23).

Conclusions—Substituting CNI with SRL as primary immunosuppression attenuates cardiac allograft vasculopathy progression. (Circulation. 2007;116:2726-2733.)

Key Words: coronary disease ▪ immune system ▪ transplantation

Cardiac allograft vasculopathy (CAV), a complex multifactorial process arising from immune and nonimmune pathogenic mechanisms, is the leading cause of late morbidity and mortality in heart transplant patients. The International Society of Heart and Lung Transplantation (ISHLT) registry in 2006 indicated that 5 years after cardiac transplantation, CAV and late graft failure (likely caused by CAV) together account for 30% of deaths. CAV affects the microcirculation and large epicardial vessels, which results in progressive luminal narrowing and reduced myocardial blood flow.

Editorial p 2666
Clinical Perspective p 2733

The approach to CAV has been modification of underlying traditional risk factors and optimization of immunosuppression. Administration of lipid-lowering medications slows the development of CAVs and improves endothelial dysfunction. Advances in immunosuppressive regimens in the past decade have improved short-term survival after heart transplantation but do not prevent CAV altogether.

Sirolimus (SRL) and derivatives (everolimus) are macrolide antibiotics with antiproliferative and potent antirejection properties. SRL blocks proliferative responses by inhibiting T- and B-cell responses to growth factors, a different pathway than calcineurin inhibitors (CNIs) such as cyclosporin A or tacrolimus (FK506). In cardiac transplant recipients, SRL or its derivative everolimus has generally been used as a secondary immunosuppressive agent in place of azathioprine (AZA) or mycophenolate mofetil (MMF). SRL has been used infrequently as a primary immunosuppressant in cardiac transplant recipients. We have previously reported that SRL is not nephrotoxic and may be used safely as primary immunosuppressant in cardiac allograft recipients to preserve renal function, leaving secondary immunosuppressive agents unchanged. However, no studies have yet demon-
strated a beneficial effect on CAV progression when CNIs are replaced with SRL as primary immunosuppression. We hypothesized that replacement of CNIs with SRL would inhibit CAV progression. The aim of this study was to investigate the efficacy of SRL as primary immunosuppressive agent in preventing CAV progression as assessed by serial 3-dimensional (3D) intravascular ultrasound (IVUS) examination in a cohort of heart transplant patients.

**Methods**

**Study Design**

This was a nonrandomized, single-center study approved by the Meave clinic institutional review board. Since 2004 3D IVUS has been performed on all cardiac transplant recipients, unless otherwise contraindicated, during routine annual coronary angiography as part of the surveillance for CAV. From January 2004 to July 2006, a total of 78 cardiac transplant recipients with impaired renal function secondary to CNI (defined as a corrected glomerular filtration rate [GFR] ≤ 50 mL/min and lack of any other identifiable causes of renal dysfunction) and/or CAV (any stenosis ≥ 50% in any major branch and/or distal pruning of secondary side branches) detected on annual coronary angiography were converted to SRL-based immunosuppression. Twenty-nine of these patients who had undergone 2 consecutive 3D IVUS exams were included (SRL group) in the study. Immunosuppression from CNI was changed to SRL as primary immunosuppression. We investigated the efficacy of SRL as primary immunosuppressive agent in preventing CAV progression when CNIs are replaced with SRL as primary immunosuppression. We hypothesized that replacement of CNIs with SRL would inhibit CAV progression. The aim of this study was to investigate the efficacy of SRL as primary immunosuppressive agent in preventing CAV progression as assessed by serial 3-dimensional (3D) intravascular ultrasound (IVUS) examination in a cohort of heart transplant patients.

**IVUS Examination and Analysis**

IVUS was performed at baseline (before conversion to SRL in the SRL group and at the time of study entry in the CNI group) and at a 12-month follow-up. In 23 patients, baseline IVUS examinations were performed 0.62±0.64 years after transplantation (“early” group included patients who were enrolled in the study within first 2 years after transplantation). Another 46 patients were examined 6.29±3.25 years after transplantation (“late” group included patients enrolled in the study after 2 years after transplantation).

Images were captured at 20 frames per second. A 30 mm IVUS catheter (Volcano Therapeutics, Rancho Cordova, Calif) with a 5 MHz probe was advanced across the aortic valve and across the coronary ostia at the discretion of the investigator. Images were obtained in the mid left anterior descending coronary artery to the left circumflex artery lumen, plaque and vessel volume were analyzed. Each measured volume was normalized to the examined segment length (mm³/mm) to compensate for differences in examined vessel segment length. A plaque index (PI) was calculated as follows: (PV/vessel volume)×100%, where PV is plaque volume. Changes in PV, lumen volume, and vessel volume or PI were defined as follow-up minus baseline volume measures value. The semiautomated contour detection of both the lumen and the media-adventitia interface was performed at intervals of either 16 or 32 frames, depending on the heterogeneity of the image. All other measurements were carried out automatically. Border detection was corrected manually in all frames after automatic border detection. Next, the vessel volume, lumen volume, and PV (mm³) in the exact examined vessel segment length and frames were obtained and calculated as previously described.

**Statistics Analysis**

Data were summarized through the use of mean±SD (range) for numeric variables and percents and counts for categorical variables. Baseline characteristics between the SRL and CNI groups were compared by use of a t test for numerical data and a χ² test for categorical data. Differences from baseline to the 12-month follow-up within groups were compared by use of a paired t test. IVUS values at 12 months between groups were compared by ANCOVA, with the baseline value of the term included in the analysis as a covariate. Multivariable regression was used to assess independent predictors of plaque progression. A value of P<0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Patient Characteristics**

Table 1 provides baseline demographic and clinical characteristics on both groups of patients. The incidence of previous rejections was higher in the SRL group. Other baseline variables were not significantly different between the 2 groups. Laboratory measurements are summarized in Table 2. Higher baseline creatinine, lower baseline GFR, and higher baseline uric acid levels in the SRL group reflect the fact that many patients with renal dysfunction were switched to SRL for preservation of renal function, as previously described. There was no significant difference in baseline lipids and glucose levels between the 2 groups.

Of the 69 patients studied, 97% were on lipid-lowering therapy with pravastatin, simvastatin, or atorvastatin at the start of the study, and changes in lipid values were serially monitored. In the SRL group, additional therapy with ezetimibe (10 mg) in 2 patients, fish oil (2 to 3 g/d) in 7 patients, and gemfibrozil (1200 mg/d) in 2 patients was implemented to control elevations in serum triglycerides.

**Volumetric Changes in the SRL and CNI Groups**

Volumetric data by 3D IVUS at baseline and 12.1±2.6 months are shown in Table 3. At baseline, PV and PI were higher in the SRL group, although not statistically significant. Mean progression in PV was significantly smaller in the SRL group, although not statistically significant. Mean progression in PV was significantly smaller in the SRL group, although not statistically significant. Mean progression in PV was significantly smaller in the SRL group, although not statistically significant. Mean progression in PV was significantly smaller in the SRL group, although not statistically significant. Mean progression in PV was significantly smaller in the SRL group, although not statistically significant.
Table 1. Baseline Demographic and Clinical Characteristics of the Patients

<table>
<thead>
<tr>
<th>SRL (n=29)</th>
<th>CNI (n=40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at heart transplantation, y</td>
<td>50.2±13.2</td>
<td>53.8±14.8</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>20 (69)</td>
<td>31 (77)</td>
</tr>
<tr>
<td>Donor age, y</td>
<td>31.6±14.8</td>
<td>30.4±13.6</td>
</tr>
<tr>
<td>Cold ischemic time, min</td>
<td>180.6±57.0</td>
<td>165.1±45.3</td>
</tr>
<tr>
<td>Time after heart transplant, y</td>
<td>3.81±3.4</td>
<td>4.80±4.0</td>
</tr>
<tr>
<td>Reason for heart transplant, n (%)</td>
<td>Ischemic CMP</td>
<td>8 (28)</td>
</tr>
<tr>
<td></td>
<td>DCMP</td>
<td>15 (51)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>21 (72)</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus, n (%)</td>
<td>10 (34)</td>
</tr>
<tr>
<td></td>
<td>Hypertension, n (%)</td>
<td>19 (66)</td>
</tr>
<tr>
<td></td>
<td>Angiographic CAV present, n (%)</td>
<td>7 (24)</td>
</tr>
<tr>
<td></td>
<td>CMV infection before study entrance, n (%)</td>
<td>5 (17)</td>
</tr>
<tr>
<td></td>
<td>Treated episodes of rejection (ISHLT 2004 grade 2R) before study entry, n (%)</td>
<td>16 (54)</td>
</tr>
<tr>
<td></td>
<td>ACE inhibitor, n (%)</td>
<td>10 (34)</td>
</tr>
<tr>
<td></td>
<td>Statin, n (%)</td>
<td>28 (97)</td>
</tr>
<tr>
<td></td>
<td>Calcium channel blocker, n (%)</td>
<td>8 (28)</td>
</tr>
<tr>
<td></td>
<td>Prednisone, n (%)</td>
<td>13 (45)</td>
</tr>
<tr>
<td></td>
<td>Cyclosporin, n (%)</td>
<td>31 (78)</td>
</tr>
<tr>
<td></td>
<td>Tacrolimus, n (%)</td>
<td>9 (22)</td>
</tr>
<tr>
<td></td>
<td>Sirolimus, n (%)</td>
<td>29 (100)</td>
</tr>
<tr>
<td></td>
<td>AZA, n (%)</td>
<td>14 (48)</td>
</tr>
<tr>
<td></td>
<td>MMF, n (%)</td>
<td>15 (52)</td>
</tr>
<tr>
<td></td>
<td>Aspirin, n (%)</td>
<td>8 (28)</td>
</tr>
</tbody>
</table>

*CMP indicates cardiomyopathy; DCMP, dilated cardiomyopathy; CMV, cytomegalovirus; and ACE, angiotensin-converting enzyme.*

Table 2. Patients’ Laboratory Characteristics

<table>
<thead>
<tr>
<th>SRL (n=29)</th>
<th>CNI (n=40)</th>
<th>P (SRL vs CNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>187.1±39.2</td>
<td>200.6±43.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>206.0±44.9</td>
<td>178.3±40.2</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>96.4±30.5</td>
<td>106.0±34.6</td>
</tr>
<tr>
<td>Follow-up</td>
<td>104.9±44.8</td>
<td>83.9±27.3</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>56.2±17.7</td>
<td>57.2±15.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>61.6±22.9</td>
<td>53.6±16.5</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>Baseline</td>
<td>184.5±88.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>232.2±170.6</td>
<td>180.7±138.5</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>Baseline</td>
<td>103.4±20.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>102.2±28.2</td>
<td>105.3±52.6</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>Baseline</td>
<td>49.6±16.6</td>
</tr>
<tr>
<td>Follow-up</td>
<td>55.9±23.9</td>
<td>56.7±20.1</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>Baseline</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>Follow-up</td>
<td>1.5±0.3</td>
<td>1.6±0.9</td>
</tr>
<tr>
<td>Proteinuria, mg/d</td>
<td>Baseline</td>
<td>299±622</td>
</tr>
<tr>
<td>Follow-up</td>
<td>517±795</td>
<td>369±566</td>
</tr>
<tr>
<td>Albumin, mg/dL</td>
<td>Baseline</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>Follow-up</td>
<td>4.1±0.3</td>
<td>4.0±0.3</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>Baseline</td>
<td>7.4±1.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>5.8±1.6</td>
<td>6.4±1.7</td>
</tr>
</tbody>
</table>
| LDL indicates low-density lipoprotein; HDL, high-density lipoprotein. *ANOVA test; baseline value is the covariate. †Paired t test.*

and whiskers extend from the box to the outermost data point that falls within 1.5 times the interquartile range of the box. Points beyond that are displayed individually. Mean±SD information also is displayed below.) The progression in PI also was significantly lower in the SRL group (−0.1±8% versus 6±8%; P=0.005; Figure 1B). As assessed by PI, 52% of the SRL group and 18% of the CNI group showed no disease progression (P=0.0025). There were no significant changes between the CNI and SRL groups in vessel volume (0.34±2.4 versus 0.92±4.67; P=0.57). There was no association between changes in PV and PI and baseline creatinine (P=0.16 and P=0.43, respectively), GFR (P=0.21 and P=0.61, respectively), and uric acid level (P=0.19 and P=0.59, respectively) in univariate analysis.

Multivariate analysis was performed for all patients in both groups using the following terms: age at first IVUS, indication for transplantation, donor age, ischemic time, presence of hypertension and diabetes, and baseline value of PV. After adjustment for the other factors in the multivariate analysis, the differences in PV (P=0.001) and PI (P=0.009) remained highly significant between the SRL and CNI groups.

Early and Late Plaque Volume Changes After Cardiac Transplantation

In patients studied within the first 2 years (early) after transplantation (mean, 8.5±7.2 months for the SRL group and 6.1±8.3 months for the CNI group), the progression in PV was markedly smaller in the SRL group than in the CNI group (0.06±1.06 versus 1.77±1.65 mm³/mm; P=0.009;
The progression in PI also was significantly smaller in the SRL group compared with the CNI group (0.3% in the SRL group versus 1.0% in the CNI group; P=0.029; Figure 2B). The changes in vessel volume did not differ between groups (1.2 mm³/mm for the CNI group versus 0.3 mm³/mm for the SRL group; P=0.29) during follow-up.

In patients observed beyond the first 2 years (late) after transplantation (5.6±3.1 years for the SRL group and 6.7±3.4 years for the CNI group), PV in the CNI group increased compared with the SRL group, but the difference was not statistically significant (0.13±1.20 versus 0.94±1.92 mm³/mm; P=0.072; Figure 2A). Similarly, PI progression was not statistically significant (0.6% for the SRL group versus 0.9% for the CNI group; P=0.062; Figure 2B). There were no changes between the CNI and SRL groups in vessel volume (0.78±3.70 mm³/mm in the CNI group versus 0.69±4.88 mm³/mm in the SRL group; P=0.98).

Secondary Immunosuppressive Agent Effects on PV and PI

Treatment with AZA or MMF as secondary immunosuppressant had no significant effect on PV in either the SRL group (0.22±0.66 versus −0.05±1.45 mm³/mm; P=0.44) or the CNI group (1.42±1.39 versus 1.06±2.28 mm³/mm; P=0.54; Figure 3A). Patients treated with CNI/MMF, however, demonstrated a nonsignificant increase in PV compared with the SRL/MMF group (1.06±2.28 versus 0.05±1.45 mm³/mm; P=0.085); there was no significant progression in PV compared with the SRL/AZA group (1.06±2.28 versus 0.22±0.66 mm³/mm; P=0.14; Figure 3A).

Treatment with either AZA or MMF as a secondary immunosuppressant did not significantly affect changes in PI in either the SRL group (1.5±6% versus −1.6±8%; P=0.14) or the CNI group (7.8±8.7% versus 4.8±7.3%; P=0.23; Figure 3B). Patients treated with CNI/MMF demonstrated a significantly increased PI compared with patients...
Creatinine decreased significantly (1.9 ± 0.5 mg/dL at baseline versus 1.5 ± 0.3 mg/dL at follow-up; paired \( t \) test, \( P=0.001 \)), and GFR did not change (49.6 ± 16.6 mL/min at baseline versus 55.9 ± 23.9 mL/min at follow-up; paired \( t \) test, \( P=0.12 \)) in the SRL group. There were no significant changes in creatinine (1.5 ± 0.4 versus 1.6 ± 0.9 mg/dL; paired \( t \) test, \( P=0.4 \)) and GFR (59.5 ± 14.8 versus 56.7 ± 20.1 mL/min; paired \( t \) test, \( P=0.36 \)) in the CNI group.

For the SRL group, the degree of proteinuria increased after conversion to SRL from 299 ± 622 to 517 ± 795 mg/dL at 12 months (paired \( t \) test, \( P=0.0002 \)). In the CNI group, proteinuria did not change at 12 months (308 ± 320 versus 369 ± 566 mg/dL; paired \( t \) test, \( P=0.23 \)). The difference between the SRL and CNI groups was significant (\( P=0.046 \)). Blood albumin level did not change in patients treated with SRL (4.1 ± 0.4 versus 4.1 ± 0.3 mg/dL; \( P=0.77 \)), and no patients developed edema or hemorrhatia.

**Uric Acid**

There was a significant reduction in uric acid (7.4 ± 1.9 versus 5.8 ± 1.6 mg/dL; paired \( t \) test, \( P=0.0001 \)) in the SRL group and no change in uric acid levels (6.5 ± 1.8 versus 6.4 ± 1.7 mg/dL; paired \( t \) test, \( P=0.75 \)) in the CNI group.

**Adverse Effects**

Overall, SRL was well tolerated, and no patients were withdrawn from the study. Frequent side effects when SRL was initiated were gastrointestinal disturbance (nausea, diarrhea), greasy skin, and transient mouth ulcers. These symptoms usually resolved after 2 to 3 weeks.

**Discussion**

This is the first comparable serial 3D IVUS study that demonstrates the use of SRL as a primary immunosuppressive agent that mitigates the progression of CAV by reducing intimal hyperplasia. Moreover, our data indicate that a CNI-free regimen is safe and well tolerated and is most effective when initiated within the first 2 years after transplantation.

The diffuse nature of CAV limits the sensitivity of coronary angiography in long-term follow-up of this disease. Therefore, IVUS is now considered the gold standard for the evaluation of CAV. However, 2-dimensional IVUS has limitations in its sensitivity to detect the changes of atherosclerotic burden in CAV, difficulty with spatial registration, and an inability to assess the full extent of vascular disease. We used 3D IVUS, which allows rapid and accurate measurement of volume and plaque dimensions that demonstrate the full extent of atherosclerotic pathology. Because of its superior reproducibility, this technique may be used to assess the progression of coronary artery disease and may allow more accurate evaluation of interventions aimed at preventing or retarding coronary artery disease.

SRL has been shown to prevent vascular remodeling and neointimal proliferation, which are key components of CAV. Although randomized clinical trials have evaluated the role of SRL or its derivative everolimus in combination with cyclosporine for the prevention of CAV, no study has examined the effect of complete CNI withdrawal and replacement with SRL on CAV progression. A recent multicenter, randomized, double-blind everolimus study showed that everolimus initiated 72 hours after transplantation in...
place of AZA was an effective adjunctive agent in reducing the incidence and severity of CAV at a 1-year follow-up.\textsuperscript{10} SRL initiated immediately after transplantation as a secondary immunosuppressive agent in place of AZA also has been demonstrated to be effective in reducing acute allograft rejection and intimal thickening at a 2-year follow-up.\textsuperscript{11} In a single-center, open-label randomized trial including patients with severe CAV, SRL as a secondary immunosuppressive agent in place of AZA also has been shown to lower the risk of subsequent coronary events and to slow the progression of existing CAV.\textsuperscript{9} However, in these studies, questions have been raised regarding the greater increase in serum creatinine in the SRL or everolimus groups.\textsuperscript{10,11} This was most likely due to potentiation of the toxic effects of CNI that occur when SRL is introduced in the face of existing CNI therapy, likely because of increased CNI levels.\textsuperscript{21} Although no wound healing issues were reported in the everolimus study,\textsuperscript{10} in the multicenter SRL study, the trough levels of SRL were broad and high, which led to concerns about nephrotoxicity and wound healing, prompting a reduction in dose.\textsuperscript{11} Similarly, in the study of Mancini et al,\textsuperscript{9} concerns about delayed wound healing and renal dysfunction prompted a change in the therapeutic targets of SRL to lower levels than envisioned at the beginning of the study. In the present study, because all patients were converted to a CNI-free regimen, no exacerbation in renal dysfunction was seen.

In the present study, patients with CAV and significant renal dysfunction were converted to SRL. Therefore, the baseline renal function and plaque burden were worse in the SRL group and improved significantly during their follow-up visit the following year. Furthermore, on instituting the SRL regimen, we preemptively reduced the CNI dose, as previously described, to compensate for the possibility of nephrotoxicity.\textsuperscript{13} Although the degree of proteinuria increased significantly in the SRL group, this increase was not associated with the development of nephrotic syndrome. Severe proteinuria has been seen in renal transplant recipients in whom it can be associated with nephrotic syndrome.\textsuperscript{22}

The antiproliferative effects are the likely mechanism for curtailing the progression of CAV in these patients. SRL attenuates endothelial cell proliferation and arterial smooth muscle cell proliferation and migration induced by platelet-derived growth factor and basic fibroblast growth factor.\textsuperscript{23,24} In contrast, CNIs stimulates fibrogenic growth factor and transforming growth factor-\(\beta\) production, which may contribute to vasculopathy.\textsuperscript{25} Recently, it has been shown that calcineurin pathways (negatively modulated by calsarins) are critical in the remodeling of cardiac tissue when exposed to stress signals.\textsuperscript{26} It is theoretically possible that prolonged use of CNIs may contribute to CAV via this mechanism in genetically predisposed cardiac transplant recipients.

It is possible that the same signals perpetuating intimal proliferation initiate a cascade of pathways that adversely affect the ability of the coronary vessel to positively remodel. Although intimal thickening traditionally has been the focus of research, several observations indicate that impaired positive remodeling also contributes to a net lumen loss.\textsuperscript{27–29} Our study points out no difference in vessel volume between SRL- and CNI-treated patients despite significantly higher plaque burden in the CNI group; this suggests impaired positive remodeling in the CNI group compared with the SRL group.

Coronary endothelial dysfunction is an early characteristic feature of CAV.\textsuperscript{30} CNIs have been shown to impair endothelial function through a number of mechanisms, including inhibition of nitric oxide synthase activity and a decrease in the level of endothelium-derived nitric oxide.\textsuperscript{31,32} In addition, cyclosporin A increases the production of thromboxane \(A_2\) and inhibits local prostacyclin synthesis in vitro.\textsuperscript{34} Cyclosporin A and its metabolites also increase the release of endothelin-1 from endothelial cells\textsuperscript{35} and increase the expression of endothelin-1 receptors\textsuperscript{36} in both human and animal kidney or renal cell cultures. CNIs also may contribute to CAV through other mechanisms, eg, hypertension with cyclosporin A use and diabetes with tacrolimus use.\textsuperscript{37} Taken together, cumulating evidence suggests a therapeutic strategy that involves reducing the use of CNI, which may reduce the incidence of CAV.\textsuperscript{38}

Animal studies\textsuperscript{39,40} and a recent review of major immunosuppressive trials\textsuperscript{41} have suggested that MMF may have a beneficial effect in attenuating the progression of CAV. In our study, the effect of the secondary immunosuppressant (MMF or AZA) was not significant, although patients treated with MMF preferentially had less intimal hyperplasia compared with the AZA treatment group (Figure 3A and 3B).

We recognize that a main limitation of this study is that it is an observational open-label study and is neither blinded nor randomized. Although neither patient nor clinicians were blinded, 3D IVUS operators were unaware of which immunosuppressive agent the patients were taking. Higher incidence of previous rejections and worse baseline renal function in SRL-treated patients may have biased the sample. However, we were able to demonstrate significant mitigation of CAV progression even in this high-risk group of patients.

Another major limitation is that the results cannot be extrapolated to the implementation of SRL as a primary immunosuppressant in the early posttransplant period. We chose stable cardiac transplant recipients with no recent operative intervention because of concerns about SRL and wound healing.\textsuperscript{42,43}

Therefore, further studies are needed to determine whether SRL is safe in the early posttransplant course. This is highlighted by the Heart Spare the Nephron (Heart-STN) trial, which was stopped prematurely and reported to the US Food and Drug Administration (http://www.fda.gov/medwatch/safety/2007/cellcept DHCPletter 02-01-2007.pdf). However, this study proposed an abrupt switch from CNI-based immunosuppression at 3 months after cardiac transplantation without gradual CNI withdrawal, and questions were raised about the adequacy of SRL levels in patients who experienced rejection.

**Conclusions**

SRL not only is effective as a primary immunosuppressant compared with CNIs but also has antiproliferative properties that mitigate CAV progression by reducing intimal hyperplasia, as evidenced by serial 3D IVUS data in heart transplant recipients. Our findings suggest that SRL should be consid-
ered as early as possible for primary immunosuppressive therapy after cardiac transplantation. This strategy has the potential to prevent or retard the development of CAV and to improve long-term survival after cardiac allotransplantation.

Source of Funding
The present study was supported by a CR 20 grant from Mayo Foundation awarded to Dr Kushwaha.

Disclosures
Dr Kushwaha has previously received a research grant from Wyeth Pharmaceuticals. The other authors report no conflicts.

References

CLINICAL PERSPECTIVE
Cardiac allograft vasculopathy (CAV) is the leading cause of late morbidity and mortality after cardiac transplantation. This diffuse coronary disease affects epicardial vessels and the microcirculation and results in luminal narrowing and a decrease in myocardial blood flow. Modification of traditional risk factors may be helpful but does not prevent CAV. Maintenance immunosuppressive regimens after cardiac transplantation typically have been based on calcineurin inhibitors such as cyclosporine A or tacrolimus, with secondary agents such as azathioprine or mycophenolate in addition to steroids. Sirolimus and its derivative everolimus are powerful immunosuppressive agents with antiproliferative properties and previously has typically been used as secondary agents in place of azathioprine or mycophenolate. In the present study, we used 2 consecutive 3-dimensional intravascular ultrasound studies 1 year apart to measure CAV progression in a cohort of cardiac transplant recipients switched to sirolimus as primary immunosuppression compared with recipients maintained on calcineurin inhibitors. We demonstrate that CAV continues to progress in transplant recipients maintained on calcineurin inhibitors but that its progression is significantly decreased in subjects converted to sirolimus. This effect is independent of secondary immunosuppressive agent but appears to be more significant in those converted to sirolimus within 2 years of transplantation. Converting cardiac transplant recipients from calcineurin inhibitors to sirolimus may slow the progression of CAV and significantly improve long-term survival and quality of life. Our findings have significant implications for the long-term management of cardiac transplant recipients in whom CAV greatly limits survival beyond 5 years.
Conversion to Sirolimus as Primary Immunosuppression Attenuates the Progression of Allograft Vasculopathy After Cardiac Transplantation

Circulation. 2007;116:2726-2733; originally published online November 19, 2007; doi: 10.1161/CIRCULATIONAHA.107.692996
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/116/23/2726

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/