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CME

Cardiac Allograft Vasculopathy by Intravascular Ultrasound in Heart Transplant Patients

Substudy From the Everolimus Versus Mycophenolate Mofetil Randomized, Multicenter Trial

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JACC: HEART FAILURE CME

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CME Objective for This Article: After reading this article, the reader should understand: 1) The current understanding of the pathophysiology of cardiac allograft vasculopathy (CAV); 2) Strategies to reduce CAV; and 3) The side effect profiles of immunosuppression agents used to reduce CAV.

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Cardiac Allograft Vasculopathy by Intravascular Ultrasound in Heart Transplant Patients

Substudy From the Everolimus Versus Mycophenolate Mofetil Randomized, Multicenter Trial

Objectives A pre-planned substudy of a larger multicenter randomized trial was undertaken to compare the efficacy of everolimus with reduced-dose cyclosporine in the prevention of cardiac allograft vasculopathy (CAV) after heart transplantation to that of mycophenolate mofetil (MMF) with standard-dose cyclosporine.

Background CAV is a major cause of long-term mortality following heart transplantation. Everolimus has been shown to reduce the severity and incidence of CAV as measured by first year intravascular ultrasound (IVUS). MMF, in combination with cyclosporine, has also been shown to have a beneficial effect in slowing the progression of CAV.

Methods Study patients were a pre-specified subgroup of the 553-patient Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial who underwent heart transplantation and were randomized to everolimus 1.5 mg or MMF 3 g/day. IVUS was performed at baseline and at 12 months. Evaluable IVUS data were available in 189 patients (34.6%).

Results Increase in average maximal intimal thickness (MIT) from baseline to month 12 was significantly smaller in the everolimus 1.5 mg group compared with the MMF group (0.03 mm vs. 0.07 mm, $p < 0.001$). The incidence of CAV, defined as an increase in MIT from baseline to month 12 of greater than 0.5 mm, was 12.5% with everolimus versus 26.7% with MMF ($p = 0.018$). These findings remained irrespective of sex, age, diabetic status, donor disease, and across lipid categories.

Conclusions Everolimus was significantly more efficacious than MMF in preventing CAV as measured by IVUS among heart-transplant recipients after 1 year, a finding, which was maintained in a range of patient subpopulations. CV surgery: transplantation, ventricular assistance, cardiomyopathy (J Am Coll Cardiol HF 2013;1:389–99) © 2013 by the American College of Cardiology Foundation

Cardiac allograft vasculopathy (CAV) is a major cause of long-term mortality following heart transplantation (1). The pathophysiology of CAV is closely linked to immunologic mechanisms, such as the activation of alloreactive T-cells and antibodies, and nonimmunologic factors including history of pre-transplant coronary artery disease, cytomegalovirus (CMV) infection, older age of the donor and recipient, hyperlipidemia, and ischemia/reperfusion injury (2–5). The development of modern immunosuppressive agents to prevent acute allograft rejection and the proliferation of smooth-muscle cells may reduce the frequency and severity of vasculopathy.

Everolimus, a novel proliferation signal inhibitor, also recognized as a mammalian target of rapamycin (mTOR) inhibitor, prevents allograft rejection in rodent and nonhuman primate models of allotransplantation (6–8). It exerts its immunosuppressive effect by inhibiting the proliferation of antigen-activated T-cells, but also restricts the growth factor–stimulated proliferation of hematopoietic and nonhematopoietic cells, for instance vascular smooth muscle cells (9–11). In the first phase 3 clinical trial of everolimus, Study B253, everolimus was more efficacious than azathioprine (AZA) to lower the incidence of CAV as measured by first-year intravascular ultrasound (IVUS) (12). Mycophenolate mofetil (MMF), an inhibitor of the de novo pathway for purine biosynthesis, is commonly employed in heart transplant (13). In a 3-year trial of MMF and AZA, the change in average maximal intimal thickness (MIT) measured by IVUS trended to significantly less for the MMF group than for the AZA group ($p = 0.056$) (8,14). R-analysis of the first year data using site-to-site comparisons revealed significantly less progression of intimal thickening in the MMF group (15).

IVUS is the most sensitive tool for the diagnosis of CAV and is considered the gold standard for investigations of this type. The intravascular catheter provides a sonar image of intimal and media thickness (16). A 5-year IVUS validation

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outcome study suggested that heart transplant patients with progression of MIT ≥0.5 mm in the first year after transplant had a higher incidence of death or graft loss, more nonfatal major adverse cardiac events, and more newly occurring angiographic luminal irregularities through 5 years after heart transplantation (17).

The aim of this pre-planned substudy of Everolimus versus mycophenolate mofetil in heart transplantation: A randomized multicenter trial (A2310 Study) (18) is to investigate whether everolimus provides CAV benefit versus MMF by assessing the change in intimal thickness as measured by first-year IVUS. This study will also compare these IVUS results to subgroups including age, sex, diabetics, and lipid levels at 12 months in heart transplant recipients receiving everolimus-based versus MMF-based immunosuppression.

Abbreviations and Acronyms

AZA = azathioprine
CAV = cardiac allograft vasculopathy
CMV = cytomegalovirus
CsA = cyclosporine
D = donor
HDL = high-density lipoprotein
IVUS = intravascular ultrasound
LDL = low-density lipoprotein
MIT = maximal intimal thickness
MMF = mycophenolate mofetil
mTOR = mammalian target of rapamycin
R = recipient

Methods

Study design. This was a substudy of a phase 3, 24-month, multicenter, open-label, parallel group active controlled study (RAD001 A2310, NCT00300274, by Novartis) in which de novo heart transplant recipients were randomized in a 1:1:1 ratio to receive: 1) everolimus 1.5 mg (target trough concentration 3 to 8 ng/ml; Certican, Novartis Pharma AG, Basel, Switzerland) with reduced-dose cyclosporine (CsA); 2) everolimus 3.0 mg (target trough concentration 6 to 12 ng/ml) with reduced-dose CsA; or 3) MMF 3 g (1.5 mg b.i.d.; Cellcept, Roche Pharma AG, Basel, Switzerland) with full-dose CsA (Fig. 1). Sixty-seven centers (in Europe, North and South America, Australia, New Zealand, and Taiwan) participated in the study, enrolling 721 recipients who were 18 to 70 years old undergoing primary heart transplantation. The study took place during January 2006 to July 2011. Enrollment into the everolimus 3.0 mg/day group was discontinued in March 2008 upon recommendation from the independent Data Monitoring Committee, given an increased rate of death within the first 90 days post-randomization in this treatment group. Thus, of the 721 patients originally enrolled in the trial, 553 remained, who had received either 1.5 mg/day of everolimus or 3 g/day of MMF. Of these, 189 patients had evaluable IVUS at baseline and at 1 year (Fig. 2). The main results of the study have been published elsewhere (18).

Study medication was initiated within 72 h of transplantation. Everolimus and CsA trough levels were assessed at all study visits. Corticosteroids were administered according to local practice. Centers had to choose between 3 induction strategies: 1) basiliximab (Simulect, Novartis Pharma AG, Basel, Switzerland) 20 mg on days 0 and 4 post-transplant; 2) rabbit antithymocyte globulin (Thymoglobulin, Genzyme, Genzyme Corporation, Cambridge Massachusetts) administered as per local practice, starting on day of transplant and ending ≤5 days post-transplant; or 3) no induction. Rejection was treated depending on the histologic grade and the presence or absence of hemodynamic compromise. Severity of rejection was graded according to the International Society of Heart and Lung Transplant guidelines (19,20). Hemodynamic compromise was defined as 1 or more of the following: left ventricular ejection fraction (LVEF) ≤30%, left ventricular ejection fraction ≥25% smaller than baseline, fractional shortening ≤20%, fractional shortening ≥25% lower than baseline, or use of inotropic therapy.

CMV prophylaxis for a minimum of 30 days was mandatory for all cases in which the donor tested positive and the recipient tested negative for CMV. Treatment with gancyclovir, cytomegalovirus hyperimmune globulin, valgancyclovir, or valcyclovir was permitted and was to be administered according to local practice. All cases other than CMV positive donors to CMV negative recipients were to be treated according to local practice. CMV prophylaxis was also recommended following any antibody treatment of acute rejection episodes. In Study A2310, pre-specified definition of CMV events include CMV infection, defined as laboratory evidence for CMV (positive antigenemia, positive polymerase chain reaction); CMV syndrome, defined as fever that lasts 2 days, neutropenia, leukopenia, viral syndrome; and CMV disease, defined as organ involvement.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors were to be administered to all patients even if the patient did not have elevated total or low-density lipoprotein (LDL) cholesterol at baseline. Treatment was to be initiated within the first 2 weeks post-transplant, targeting an LDL cholesterol of <130 mg/dl.

IVUS protocol. An assessment of coronary artery intimal proliferation was performed using IVUS imaging during the first 6 weeks post-transplant (baseline) and at month 12. A minimum of 1 vessel, preferably the left anterior descending coronary artery due to its easy access and the need to standardize, with a maximum of 3 vessels, were interrogated. The tapes were evaluated by the Intravascular Ultrasound Core Laboratory at the Cleveland Clinic (Cleveland, Ohio) by a reviewer blinded to treatment assignment. Hospital stays for IVUS procedures per protocol were not considered serious adverse events. The test parameters, listed subsequently, were reported by the central laboratory to Novartis.

IVUS was performed using an automated, mechanical pullback through the imaged vessel(s) at a rate of 0.5 mm/s. Starting at a distal branch landmark matched between baseline and follow-up, single frames were selected every 1 mm from the digitized pullback sequence and ending at

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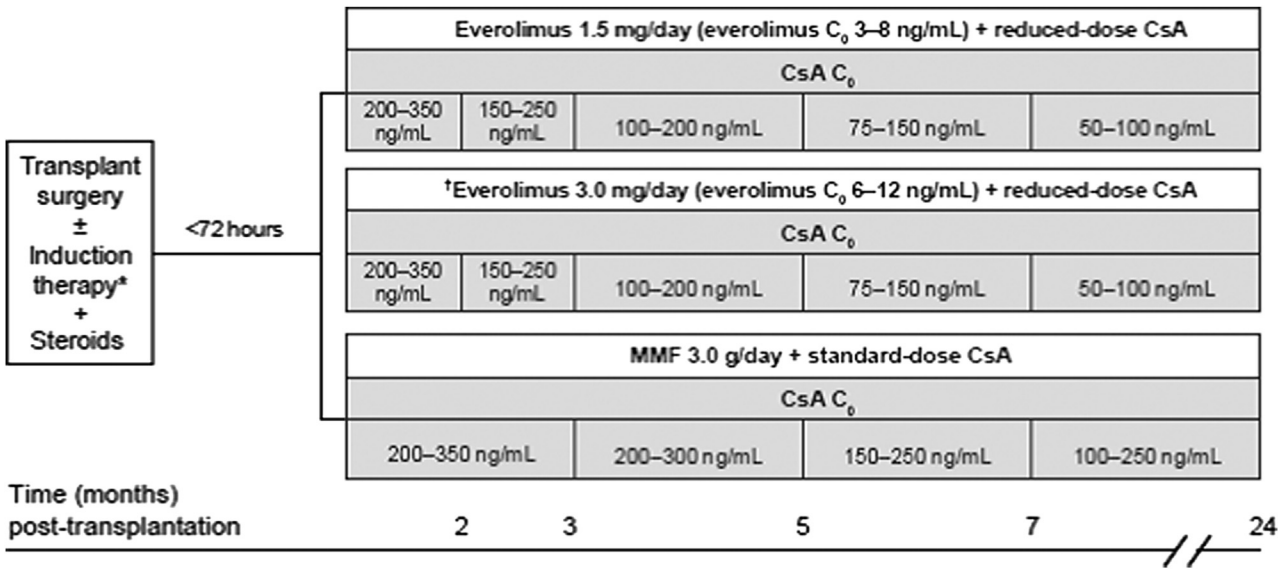

Figure 1 **Study A2310 Protocol**

*Induction therapy was center specific: centers designated as basiliximab, thymoglobulin, or no induction. Steroids to be administered for the duration of the study. †Enrollment into the everolimus 3 mg study arm was closed at recommendation of the study's Data Monitoring Committee. C_0 = trough concentration; CsA = cyclosporine; MMF = mycophenolate mofetil.

a matched proximal branch or aorto-ostial landmark. For each of the selected frames, the lumen and external elastic membrane cross-sectional areas were measured.

The IVUS population was prospectively defined to include patients who had a minimum of 11 matched slices from IVUS images from baseline and from the month 12 visit without missing data at these matched sites. IVUS substudy exclusion criteria included: patients in whom intravenous contrast was contradicted due to renal dysfunction (creatinine >2.0 mg/dl [176 μmol/l] or >1.7 mg/dl

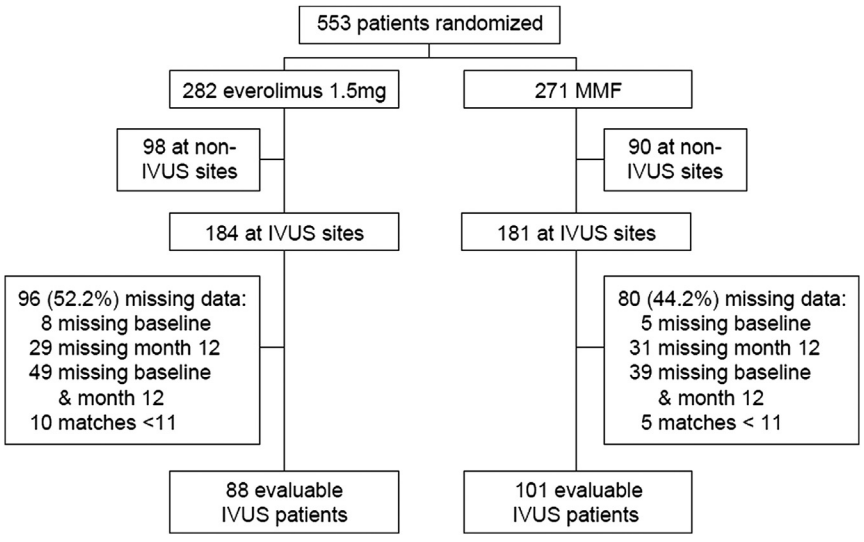

Figure 2 **Patient Enrollment and Disposition**

The figure does not show 168 patients randomized to everolimus 3 mg. MMF = mycophenolate mofetil; IVUS = intravascular ultrasound.

Table 1 Baseline Demographic Characteristics of the Patients

	IVUS Population			IVUS Versus Non-IVUS Population		
	Everolimus 1.5 mg (n = 88)	MMF 3 g (n = 101)	p Value	Total IVUS (n = 189)	Total Non-IVUS (n = 364)	p Value
Age, yrs	51.1 ± 11.8	49.5 ± 13.1	0.564	50.3 ± 12.5	50.9 ± 10.9	0.983
Male	70 (79.5)	88 (87.1)	0.173	158 (83.6)	287 (78.8)	0.213
Caucasian	61 (69.3)	78 (77.2)	0.249	139 (73.5)	312 (85.7)	**<0.001**
Weight, kg	76.5 ± 17.1	77.2 ± 15.1	0.619	76.9 ± 16.0	78.1 ± 16.1	0.437
Height, cm	171.6 ± 9.0	173.3 ± 9.4	0.168	172.5 ± 9.2	173.3 ± 9.6	0.322
BMI, kg/m^2	25.8 ± 4.4	25.6 ± 4.0	0.764	25.7 ± 4.2	25.8 ± 4.6	0.935
Primary reason for heart transplantation						
Idiopathic cardiomyopathy	29 (33.0)	38 (37.6)	0.544	67 (35.4)	161 (44.2)	**0.045**
Coronary artery disease	14 (15.9)	21 (20.8)	0.454	35 (18.5)	78 (21.4)	0.438
Other	45 (51.1)	42 (42.6)	0.241	87 (46.0)	122 (33.5)	**0.005**
Recipient negative for CMV, donor positive	16 (18.2)	23 (22.8)	0.475	39 (20.6)	70 (19.2)	0.735
Induction therapy	65 (73.9)	80 (79.2)	0.394	145 (76.7)	222 (61.0)	**<0.001**
Age of donor, yrs	36.0 ± 13.2	34.1 ± 13.1	0.278	35.0 ± 13.1	34.2 ± 13.1	0.556
Mean duration of cold ischemia, h	3.3 ± 1.0	3.4 ± 1.1	0.957	3.2 ± 1.1	3.1 ± 1.1	0.177
Baseline creatinine, mg/dl	1.32 ± 0.62	1.33 ± 0.49	0.534	1.33 ± 0.55	1.42 ± 0.62	0.129

Values are mean ± SD or n (%). **Bold** values indicate p<0.05.
BMI = body mass index; CMV = cytomegalovirus; IVUS, intravascular ultrasound; IVUS = intravascular ultrasound; MMF = mycophenolate mofetil.

[150 μmol/l] for diabetic patients), patients with advanced CAV in whom IVUS was contraindicated, and patients who did not provide written informed consent.

The primary IVUS efficacy variable was the change in average MIT from baseline to month 12. Secondary IVUS efficacy variables included the incidence of CAV, defined as ≥0.5 mm increase in MIT in at least 1 matched slice from baseline to month 12, change in average intimal area, average intimal index, and total intimal volume in the 10 mm artery subsegment with the greatest disease severity from baseline to month 12, donor disease progression and incidence of de novo disease. Intimal area was defined as external elastic membrane area minus the lumen area. The intimal index was defined as external elastic membrane area minus lumen area/external elastic membrane area. Total intimal volume was defined as mean intimal area per cross section by median number of cross sections for all subjects in study. Donor disease was defined as vessel sites with MIT ≥0.5 mm at baseline. Donor disease progression was defined as vessel sites with MIT ≥0.5 mm at baseline and an increase in MIT of ≥0.3 mm at month 12. De novo disease was defined as MIT <0.5 mm at baseline and an increase of ≥0.5 mm by month 12.

Study conduct. All participants gave written informed consent for Study A2310 and a separate written informed consent for IVUS substudy before randomization. The study was approved by the institutional review board at each center and conducted according to the guidelines of the U.S. Code of Federal Regulations, the European Community Guidance on Good Clinical Practice, and the Declaration of Helsinki.

Statistical analysis. A sample size of 91 evaluable patients per group was required to provide a power of 80% to detect a between-group difference of 0.06 mm (assuming common standard deviation = 0.13 mm) in the primary IVUS efficacy variable (change in average MIT from baseline to month 12) at the significance level of 0.025 (2-sided).

Table 2 Reason for Missing Baseline and Month 12 IVUS

	Baseline		Month 12	
Reason	Everolimus 1.5 mg (n = 38)	MMF (n = 21)	Everolimus 1.5 mg (n = 15)	MMF (n = 15)
Prematurely discontinued from study	NA	NA	2 (13.3)	4 (26.7)
Refused consent	3 (7.9)	3 (14.3)	1 (6.7)	1 (6.7)
Contraindicated due to renal dysfunction	9 (23.7)	4. (19.1)	7 (46.7)	3 (20.0)
Contraindicated due to active infection	1 (2.6)	1 (4.8)	NA	NA
Contraindicated due to hemodynamic unstable patient	1 (2.6)	0 (0.0)	NA	NA
Technical difficulties	1 (2.6)	3 (14.3)	0 (0.0)	2 (13.3)
Missed schedule examination	0 (0.0)	1 (4.8)	1 (6.7)	1 (6.7)
Other	23 (60.5)	9 (42.9)	4 (26.7)	4 (26.7)

Values are n (%).
IVUS = intravascular ultrasound; MMF = mycophenolate mofetil; NA = not applicable.

Table 3 Results of Intravascular Ultrasound

Parameter	Everolimus 1.5 mg (n = 88)	MMF (n = 101)	p Value
Change in average MIT, mm	0.03 ± 0.05	0.07 ± 0.11	**<0.001**
Incidence of CAV	11 (12.5)	27 (26.7)	**0.018**
Change in average intimal area, mm^2	0.14 ± 0.36	0.48 ± 0.81	**0.012**
Change in average intimal index	0.01 ± 0.01	0.03 ± 0.06	**<0.01**
Total intimal volume, mm^3	2.04 ± 7.00	7.74 ± 12.93	**0.005**
Donor disease	42 (47.7)	54 (53.5)	0.468
Donor disease progression	18 (20.5)	26 (25.7)	0.490
Incidence of de novo disease	8 (9.1)	20 (19.8)	**0.042**

Values are mean ± SD or n (%). **Bold** values indicate p<0.05.
CAV = cardiac allograft vasculopathy; MIT = maximal intimal thickness; MMF = mycophenolate mofetil.

To assess the robustness of the main IVUS analysis results, sensitivity analyses were performed with missing values of changes in average MIT imputed. These analyses were performed on the intent-to-treat IVUS population, which consisted of all patients randomized at the IVUS sites.

The primary IVUS efficacy variable was compared between treatment groups by means of a *t* test. For other continuous variables, Wilcoxon rank sum tests were performed. Categorical variables were compared using Fisher's exact test.

Linear regression analyses were performed to investigate the association between the change in lipids (total cholesterol, LDL cholesterol, high-density lipoprotein [HDL] cholesterol, and triglycerides) and the change in average MIT. The regression models include the change in lipid levels from baseline to month 12, treatment (everolimus 1.5 mg or MMF) and the interaction between treatment and the change in lipids as predictors.

A risk factor analysis for CAV was performed using a multivariate logistic regression. The potential risk factors included patient sex, race and age, gender mismatch, body mass index, baseline diabetes, type of induction, use of statins for at least 6 months (180 days) with a maximum of 7 days of temporary interruption, most recent Panel Reactive Antibody (PRA) value (0 vs. >0) and lipid levels at baseline (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides). For this analysis, the backward elimination approach was applied with a criterion of p = 0.10.

Results

Patient characteristics. A total of 189 patients had evaluable IVUS data at baseline and month 12 (88 patients were assigned to the everolimus 1.5 mg group and 101 to the MMF group), and comprised the IVUS population. This

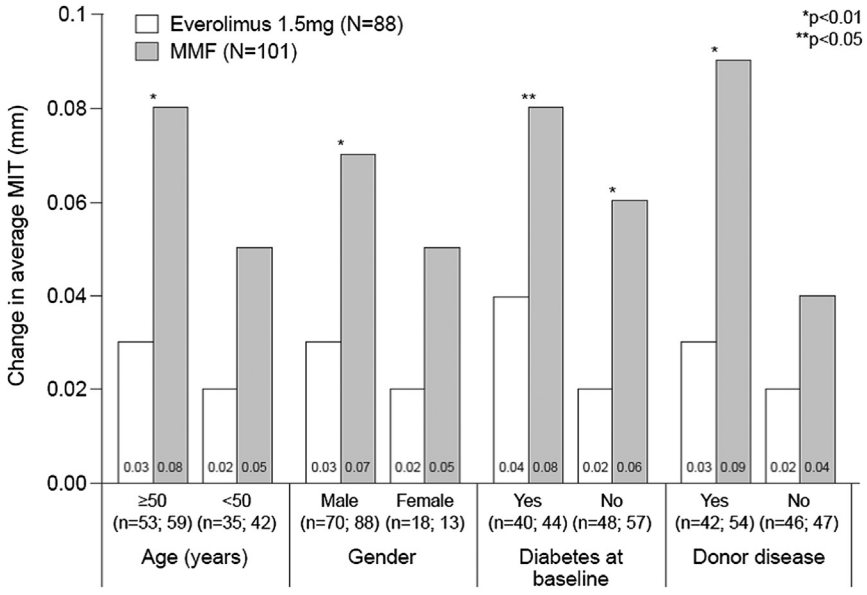

Figure 3 Change in Average MIT From Baseline to Month 12 in Subgroups of Patients

BL = baseline; MIT = maximal intimal thickness; MMF = mycophenolate mofetil.

Table 4 Change in Average MIT From Baseline to Month 12 by Lipid Category According to American Heart Association Criteria Guidelines

			Everolimus 1.5 mg (n = 88)		MMF (n = 101)	
Lipid Parameter	Category (mg/dl)		Change (mm)	Subgroup Number	Change (mm)	Subgroup Number
Total cholesterol	Desirable	<200	0.020	42	0.062	70
	Borderline High	200–239	0.025	24	0.070	24
	High	≥240	0.035	22	0.149	7
LDL cholesterol	Optimal	<100	0.017	35	0.039	42
	Near or above optimal	100–129	0.019	28	0.086	47
	Borderline High	130–159	0.051	16	0.100	8
	High	≥160	0.015	6	0.150	4
Triglycerides	Normal	<150	0.017	22	0.050	48
	Borderline High	150–199	0.028	29	0.076	23
	High	200–499	0.027	37	0.098	30
HDL cholesterol	Low	<40	0.016	6	0.101	16
	Normal	40–59	0.035	33	0.064	64
	Optimal	≥60	0.020	49	0.064	21
Total cholesterol/HDL cholesterol	Normal	≤6.3 (male) ≤5.5 (female)	0.026	80	0.055	86
	High	>5.5 (male) >5.5 (female)	0.017	8	0.159	14
Statin use at Month 12, %			98.9		97.0	

p = 0.222, 0.481, 0.181, and 0.472 for the interactions of change in average maximal intimal thickness (MIT) with total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, respectively. p Value for the interaction with total cholesterol/HDL cholesterol was not calculated because only a few patients had high level of total cholesterol/HDL cholesterol.
HDL = high-density lipoprotein; LDL = low-density lipoprotein; MMF = mycophenolate mofetil.

represented 34.6% of all patients randomized to everolimus 1.5 mg or MMF (31.1% of patients in the everolimus 1.5 mg group [88 of 282] and 37.3% of patients in the MMF group [101 of 271]). Within the IVUS population, the 2 groups had similar baseline demographic characteristics (Table 1). The IVUS group differed, however, from the cohort for which IVUS data was not available. Specifically, there were more Caucasians, more patients with pre-transplant idiopathic cardiomyopathy, and less use of induction therapy immediately post-transplant in the patients without IVUS data (Table 1). The reasons why IVUS examination was not performed showed no significant difference between the everolimus 1.5 mg and MMF groups as summarized in Table 2.

Results of IVUS. The primary IVUS endpoint, mean increase in average MIT from baseline to month 12 was significantly smaller in the everolimus 1.5 mg group compared with the MMF group (0.03 ± 0.05 mm vs. 0.07 ± 0.11 mm, $p < 0.001$). Sensitivity analyses using different imputation methods to account for missing data showed consistently a smaller MIT increase for the everolimus 1.5 mg group compared with the MMF group. The incidence of CAV (first-year change in MIT >0.5 mm) was significantly lower in the everolimus 1.5 mg group compared with the MMF group (12.5% vs. 26.7%, $p = 0.018$). The mean first-year changes in other secondary IVUS variables, including intimal area, intimal index, and intimal volume, were also significantly smaller in the everolimus 1.5 mg group than in the MMF group (Table 3). The percentage of patients with de novo disease was also significantly lower in the everolimus 1.5 mg group compared with the MMF group (9.1% vs.19.8%, $p = 0.042$).

In a risk factor analysis for the development of CAV, donor age and baseline LDL cholesterol were significant independent risk factors for the development of vasculopathy ($p < 0.001$ and $p = 0.014$, respectively). After adjusting for

Table 5 Change in Average MIT From Baseline to Month 12 by Presence or Absence of BPAR ≥2R or Rejection With HDC

	Change in Average MIT (mm)		
Patient subgroup	Everolimus 1.5 mg (n = 88)	MMF (n = 101)	p Value
With BPAR ≥2R/ rejections with HDC	n = 16 0.04 ± 0.06	n = 26 0.06 ± 0.09	0.423
Without BPAR ≥2R/ rejections with HDC	n = 72 0.02 ± 0.05	n = 75 0.071 ± 0.12	**<0.001**

Values are mean ± SD. p = 0.348 for the interaction between change in average MIT and rejection status at Month 12. **Bold** values indicate p<0.05.
BPAR = biopsy proven acute rejection; HDC = hemodynamic compromise; MIT = maximal intimal thickness; MMF = mycophenolate mofetil.

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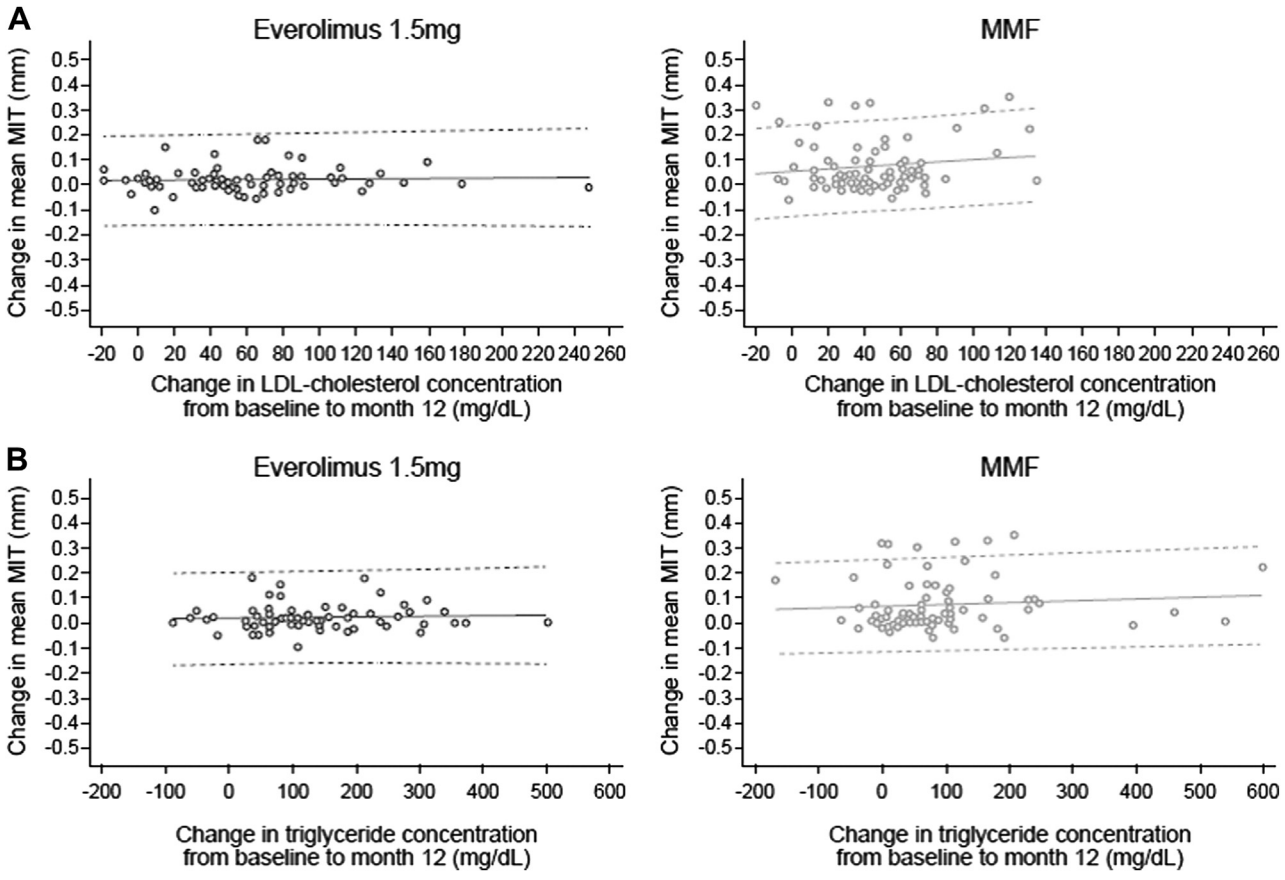

Figure 4 **Slopes of Change According to Treatment Group**

Slopes of change in mean maximal intimal thickness (MIT) according to **(A)** low-density lipoprotein (LDL) cholesterol concentration and **(B)** triglyceride concentration according to treatment group. **Solid lines** = linear regression line. **Dashed lines** = upper and lower limit of 95% confidence intervals. MMF = Mycophenolate mofetil.

these 2 risk factors, patients on everolimus 1.5 mg had a significantly lower risk of CAV than those on MMF treatment (odds ratio = 0.258, p = 0.003).

Pre-planned analysis was performed in specific subpopulations. There was a significantly smaller increase in average MIT between the everolimus 1.5 mg group and the MMF group in the subgroups of patients ≥50 years old, male, with baseline diabetes, and the presence of donor transmitted disease (Fig. 3). There was a trend towards lower average MIT in the everolimus 1.5 mg versus MMF patients who were <50 years old, female, and without donor-transmitted disease.

Higher levels of total cholesterol, LDL cholesterol, and triglycerides at month 12 were present in a larger proportion of patients in the 1.5 mg everolimus group compared with the MMF group, while low levels of HDL cholesterol were less frequent in the 1.5 mg everolimus group (Online Table 1). However, in all lipid categories (including the higher categories), the everolimus 1.5 mg patients showed numerically less of an increase in average MIT after 12 months compared with the MMF patients (Table 4). Changes in patients' lipid values over the 12-month follow-up did not contribute to any change in the MIT. This is shown in Figure 4A, where none of the graphical slopes are significantly different from zero (LDL cholesterol slopes: everolimus 1.5 mg group = 0.0001, p = 0.801; MMF group = 0.0005, p = 0.141). Similarly, higher triglyceride levels were not associated with change in average MIT (Fig. 4B).

In the subgroup of patients with biopsy proven acute rejection greater than or equal to International Society of Heart and Lung Transplant grade 2R or those who experienced rejection with hemodynamic compromise, MIT increase in everolimus 1.5 mg patients was numerically less than in MMF patients. However, patient numbers were small (n = 42) (Table 5).

Incidence of CMV events. In study A2310, 279 and 268 patients received everolimus 1.5 mg/day and MMF 3 g/day treatment, respectively. At month 12, there were significantly fewer CMV infections among everolimus 1.5 mg patients versus MMF patients (8.2% [23 of 279] vs. 20.5% [55 of 268], p < 0.001). The incidence of CMV

Table 6 Incidence of CMV Events by Subgroup

		Everolimus 1.5 mg (n = 279)	MMF (n = 268)	p Value
CMV infection				
	Laboratory evidence of CMV	23 (8.2)	55 (20.5)	**<0.001**
	CMV syndrome*	4 (1.4)	18 (6.7)	**0.018**
	CMV disease†	5 (1.8)	10 (3.7)	0.196
CMV mismatch				
	D+/R+	8/96 (8.3)	20/84 (23.8)	**0.006**
	D+/R-	9/59 (15.3)	18/49 (36.7)	**0.014**
Induction therapy				
	Basiliximab	9/101 (8.9)	17/97 (17.5)	0.092
	Thymoglobulin	10/86 (11.6)	21/83 (25.3)	**0.028**
	No induction	4/91 (4.4)	17/88 (19.3)	**0.002**

Values are n (%) or n/N (%). *Cytomegalovirus (CMV) syndrome was defined as fever lasting 2 days, neutropenia, leucopenia, or viral syndrome. †CMV disease was defined as organ involvement. **Bold** values indicate $p<0.05$.
D = donor; MMF = mycophenolate mofetil; R = recipient.

syndrome was lower in the everolimus 1.5 mg group as compared with the MMF group (1.4% vs. 6.7%, p = 0.018), as was CMV disease (1.8% vs. 3.7%, p = 0.196) (Table 6). Everolimus patients with both CMV Donor+/Recipient+(D+/R+) and D+/R− groups experienced less CMV infection than MMF patients (D+/R+ group: 8.3% vs. 23.8%, p = 0.006; D+/R− group: 15.3% vs. 36.7%, p = 0.014). In subgroups of patients without induction and those who received rabbit antithymocyte globulin therapy, incidence of CMV infections with everolimus 1.5 mg was lower compared with MMF (Table 6).

Discussion

In this pre-planned substudy of a randomized, multicenter trial, the everolimus 1.5 mg group compared to the MMF group demonstrated significant reduction in first-year intimal thickening as measured by IVUS. Of particular note is that change in average MIT from baseline to month 12, and incidence of first-year CAV (first-year change in MIT >0.5 mm), were significantly less in patients treated with everolimus 1.5 mg compared with those treated with MMF. In addition, these results appear robust irrespective of sex, age, diabetic status, and donor disease. The increase in intimal thickening in this first year after transplant most probably represents a heightened immune response and subsequent immune-mediated long-term complications. Therefore, this study's results might suggest that everolimus-treated patients could have long-term outcomes benefit compared to MMF-treated patients as extrapolated from the IVUS validation outcome study (17).

The exact mechanism of everolimus' beneficial effect to reduce the development of CAV is not clear. It may be due to the ability of everolimus to prevent myointimal thickening and inhibit the proliferation of smooth-muscle cells (21,22). Growth factor-stimulated vascular smooth muscle proliferation plays a key role in the pathogenesis of chronic rejection. In vitro, mTOR inhibitor inhibits vascular smooth muscle cell migration (23) and proliferation (24), the rate of which is increased during CAV. mTOR inhibitors also reduce protein and collagen synthesis by 40% to 60% (25) and inhibit endothelial progenitor cells (26).

MMF also has antiproliferative effects (27) that may limit the occurrence of CAV. Such an effect has been described in a previous IVUS study in which patients treated with MMF had significantly less progression of first-year intimal thickening compared to patients treated with AZA (15). A first-year change in MIT of >0.3mm was reported in significantly less patients in the MMF group as compared with the AZA group (p = 0.005) in that study. However, first-year increase in MIT of >0.5 mm, and other IVUS parameters including intimal area and intimal index, were not significantly different between the MMF and AZA groups. In contrast, the current study demonstrated benefit in all IVUS parameters at 1 year post-transplant in patients treated with everolimus compared with MMF.

A number of studies have shown an association between hyperlipidemia and the subsequent development of CAV (28–30). Because lipid levels are increased in the presence of mTOR inhibitors (31), there was concern that this may increase CAV in everolimus-treated patients. In the current analysis, however, everolimus therapy restricted the progression of intimal thickening regardless of lipid levels and higher levels of LDL cholesterol or other lipids did not affect slope of change of MIT in everolimus-treated patients. Therefore, the favorable effect of using everolimus in reducing CAV appears to counteract its hyperlipidemic effect. Interestingly, treatment with an 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor is associated not only with decreased lipid level but also attenuated progression of CAV (32,33). In our study, statin use was balanced between groups, being used in more than 96% of the patients in each group (Table 4).

Episodes of CMV infection appear to be correlated with cardiac rejection and with CAV (2,34). This may due to direct and indirect coronary allograft endothelial cell

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damage, which may be an initiating process in pathogenesis of CAV (2). In the present study, everolimus 1.5 mg with reduced CsA was associated with a lower incidence of CMV infections compared to MMF with standard CsA regardless of induction status and donor-recipient status. This reduction of CMV infection may have contributed to the CAV benefit observed in the everolimus group.

In addition, due to the synergistic effect of everolimus and CsA and the risk of potentiating CsA-related nephrotoxicity, reduced-exposure CsA was administered in the everolimus 1.5 mg arm while MMF-treated patients received standard-exposure CsA. This difference would not be expected to have influenced the IVUS findings because there is no clinical evidence that calcineurin inhibitors contribute to the progression of CAV. Last, the IVUS parameters measured are not clinical endpoints. Extrapolation of data from an IVUS validation outcome study, however, suggest that everolimus-treated patients may experience a long-term clinical benefit compared to patients receiving MMF (17). Follow-up data from the phase 3 randomized trial of everolimus versus azathioprine by Eisen and Yang indicated that the benefit of everolimus in terms of MIT progression and incidence of CAV at 1 year after heart transplantation versus azathioprine translated into a lower rate of major adverse cardiac events at 4 years post-transplant (7.9% vs. 13.6%, $p = 0.033$) (35).

Study limitations. Certain aspects of the study merit consideration. First, evaluable IVUS data were available in approximately 35% of patients in the 2 treatment arms. This is comparable to other cardiac transplant prospective studies that incorporated IVUS as an outcomes measure (12). The demographics of the patients who underwent IVUS procedures were slightly different to those patients who did not undergo IVUS procedures (Table 1), but the differences were unlikely to have resulted in a higher risk for CAV in the non-IVUS population. IVUS procedures were performed in a similar proportion of patients in each treatment group and the high rate of nonevaluable IVUS data reflects the stringent criteria required for inclusion, notably the large number of matched slices that were stipulated. There is an imbalance in the proportion of patients who underwent induction in the population that underwent IVUS and those who did not.

Conclusions

In conclusion, everolimus was significantly more efficacious than MMF in restricting progression of intimal thickening and preventing CAV as measured by IVUS 1 year post-transplant. This finding was robustly observed in various subgroups including lipid categories.

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Key Words: cardiac allograft vasculopathy ■ everolimus ■ heart transplant ■ intravascular ultrasound.

APPENDIX

For the full list of the RAD A2310 IVUS Substudy investigators and a supplementary table, please see the online version of this article.