

ORIGINAL ARTICLE

Everolimus for the Prevention of Allograft Rejection and Vasculopathy in Cardiac-Transplant Recipients

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ABSTRACT

BACKGROUND

Everolimus, a novel proliferation inhibitor and immunosuppressive agent, may suppress cardiac-allograft vasculopathy. We conducted a randomized, double-blind, clinical trial comparing everolimus with azathioprine in recipients of a first heart transplant.

METHODS

A total of 634 patients were randomly assigned to receive 1.5 mg of everolimus per day (209 patients), 3.0 mg of everolimus per day (211 patients), or 1.0 to 3.0 mg of azathioprine per kilogram of body weight per day (214 patients), in combination with cyclosporine, corticosteroids, and statins. The primary efficacy end point was a composite of death, graft loss or retransplantation, loss to follow-up, biopsy-proved acute rejection of grade 3A, or rejection with hemodynamic compromise.

RESULTS

At six months, the percentage of patients who had reached the primary efficacy end point was significantly smaller in the group given 3.0 mg of everolimus (27.0 percent, $P < 0.001$) and the group given 1.5 mg of everolimus (36.4 percent, $P = 0.03$) than in the azathioprine group (46.7 percent). Intravascular ultrasonography showed that the average increase in maximal intimal thickness 12 months after transplantation was significantly smaller in the two everolimus groups than in the azathioprine group. The incidence of vasculopathy was also significantly lower in the 1.5-mg group (35.7 percent, $P = 0.045$) and the 3.0-mg group (30.4 percent, $P = 0.01$) than in the azathioprine group (52.8 percent). The rates of cytomegalovirus infection were significantly lower in the 1.5-mg group (7.7 percent, $P < 0.001$) and the 3.0-mg group (7.6 percent, $P < 0.001$) than in the azathioprine group (21.5 percent). Rates of bacterial infection were significantly higher in the 3.0-mg group than in the azathioprine group. Serum creatinine levels were also significantly higher in the two everolimus groups than in the azathioprine group.

CONCLUSIONS

Everolimus was more efficacious than azathioprine in reducing the severity and incidence of cardiac-allograft vasculopathy, suggesting that everolimus therapy may alleviate this serious problem.

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N Engl J Med 2003;349:847-58.

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AMONG RECIPIENTS OF HEART TRANSPLANTS, vasculopathy of the allograft is the main cause of illness and death after the first year.^{1,2} Early immunologic and nonimmunologic endothelial damage may initiate pathologic remodeling, resulting in progressive luminal narrowing.³⁻⁵ The development of immunosuppressive agents to prevent acute allograft rejection and the proliferation of smooth-muscle cells may reduce the frequency and severity of vasculopathy.

Intravascular ultrasonography is a sensitive approach for the early detection of vasculopathy.⁶⁻⁸ If performed at the same sites at base line and one year after transplantation, ultrasonography can be used to evaluate the progression of intimal proliferation. Intimal thickness one and two years after heart transplantation is predictive of future cardiac events.⁹⁻¹¹

Sirolimus, a macrocyclic immunosuppressive agent and product of *Streptomyces hygroscopicus*, does not inhibit the production of interleukin resulting from antigen-induced T-cell activation but does inhibit cellular proliferation stimulated by growth factor-driven signal transduction in response to alloantigens.¹²⁻¹⁴ It binds to FK506-binding protein 12 (FKBP12), which binds FKBP12–rapamycin-associated protein, arresting the cell cycle in the G₁ phase.^{14,15} This characteristic may allow sirolimus to interact synergistically with cyclosporine. Sirolimus has been shown to reduce the incidence of acute rejection among renal-transplant recipients and to prevent cardiac-allograft vasculopathy in animals.¹⁶⁻¹⁸ Everolimus, a derivative of sirolimus, has similar mechanisms of action.¹⁹⁻²¹

Since the combination of everolimus and cyclosporine reduced the incidence of rejection and cytomegalovirus infection among kidney-allograft recipients,²² we evaluated its effect on rejection and vasculopathy in recipients of a first cardiac allograft. The primary aim of this phase 3 study was to compare the efficacy, safety, tolerability, and incidence of vasculopathy of two doses of everolimus (1.5 mg per day and 3.0 mg per day) with those of azathioprine during the first 12 months after transplantation.

METHODS

STUDY DESIGN

This two-year, prospective, randomized, double-blind trial enrolled 634 recipients of a primary heart transplant at 52 centers (in Europe and North and South America). Recipients who were 18 to 68 years old and who were not pregnant were eligible. Pa-

tients who received a heart from a donor who was older than 60 years of age or had known heart disease, or both, were excluded. All participants gave written informed consent. 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,²³ and the Declaration of Helsinki.²⁴ The study was designed by the authors, who had full access to the data, analyzed the data, and controlled all decisions regarding publication.

Within the first 72 hours after transplantation, patients were randomly assigned to receive one of the following three treatments in a double-blind, double-dummy design: 0.75 mg of everolimus twice daily, 1.5 mg of everolimus twice daily, or azathioprine (1.0 to 3.0 mg per kilogram of body weight per day, according to the patients' weight; maximal dose, 300 mg per day). All patients received oral cyclosporine (Neoral, Novartis) and corticosteroids. Everolimus was administered in tablet form simultaneously with twice-daily cyclosporine.

Cyclosporine was started at the time of transplantation at a dose of up to 12 mg per kilogram per day, and the dose was adjusted to maintain target trough levels of 250 to 400 ng per milliliter during the first four weeks, 200 to 350 ng per milliliter during months 2 through 6, and 100 to 300 ng per milliliter during months 7 through 24. Prednisone was initiated at a dose of 0.5 to 1.0 mg per kilogram per day; the dose was decreased to 0.3 to 0.5 mg per kilogram per day by day 21 and was at least 0.1 mg per kilogram per day by month 6. Induction therapy (≤ 2.5 to 5.0 mg of antithymocyte globulin per kilogram per day or ≤ 5 mg of muromonab-CD3 per day) was used for up to three days in individual centers. Rejection was treated with corticosteroids, with or without antibodies, depending on the histologic grade and the presence or absence of hemodynamic compromise. If cyclosporine was stopped or another immunosuppressant added, the study medication was discontinued.

Lipid-lowering therapy with statins (initial daily dose, 20 mg of pravastatin, 5 mg of simvastatin, or 20 mg of fluvastatin) was mandatory. Cytomegalovirus-negative recipients of hearts from cytomegalovirus-positive donors were treated prophylactically with intravenous ganciclovir for 14 to 28 days, followed by oral ganciclovir or acyclovir for 10 to 12 weeks. Alternatively, a study center could monitor blood samples from such patients weekly for vire-

mia or antigenemia and administer intravenous ganciclovir until antigenemia cleared. Cytomegalovirus infections were identified by each center. Prophylaxis against *Pneumocystis carinii* infection with trimethoprim-sulfamethoxazole was started as soon as oral medications could be tolerated and was continued for one year.

EFFICACY

The primary efficacy end point was the incidence of death, graft loss or a second transplantation, loss to follow-up, or biopsy-proven rejection of at least grade 3A, any episode of rejection associated with hemodynamic compromise in the first six months after transplantation, or both. Endomyocardial biopsy was performed at all study visits and at the time of any suspected episode of rejection, and specimens were graded by each center's pathologist according to the criteria of the International Society for Heart and Lung Transplantation.²⁵ Grades can range from 0 (no rejection) to 4 (severe acute rejection). A grade of 3A indicates the presence of multifocal inflammatory infiltrates and some damage to myocytes. Hemodynamic compromise was defined by the presence of one or more of the following: a left ventricular ejection fraction of 30 percent or less or a value that was at least 25 percent lower than the base-line value on echocardiography, fractional shortening of 20 percent or less or a value that was at least 25 percent lower than the base-line value on echocardiography, or the use of inotropic drugs.

Intravascular ultrasonography was used to assess coronary-artery intimal proliferation²⁶ at base line (performed within six weeks after transplantation) and at month 12 with the use of automated pullback images obtained at a rate of 0.5 mm per second, with a 30-MHz transducer. A minimum of one vessel (the left anterior descending artery) was examined. Frames were selected for measurement at 1-mm intervals. Patients who had fewer than 11 matched slices in the base-line and 12-month studies were excluded from further analysis. Each frame selected was manually traced to determine the following variables: maximal and minimal intimal thickness and luminal, media-adventitial, and intimal areas. The primary efficacy variable measured by intravascular ultrasonography was the mean change in the maximal intimal thickness of a given slice from base line to month 12. Secondary efficacy variables included the incidence of allograft vasculopathy (defined by an increase in the maxi-

mal intimal thickness of at least 0.5 mm from base line in at least one matched slice), the average change in intimal area among the matched slices, the intimal volume (measured in 10 mm of the intimal artery, with the thickest area at the midpoint), and the intimal index, a measure of the area of stenosis. Patients were excluded from intravascular ultrasonography if base-line assessments could not be obtained or the procedure was considered to pose a clinically significant risk.

SAFETY ASSESSMENT

Safety was determined on the basis of the occurrence of infection and other adverse events, findings on physical examination, and laboratory evaluations. Trough levels of cyclosporine and everolimus were determined by liquid chromatography-mass spectrometry in samples of whole blood obtained at every visit.

STATISTICAL ANALYSIS

Efficacy analyses were conducted according to the intention-to-treat principle and included all randomized patients who were assessed at least once after the administration of at least one dose of study medication. Safety and tolerability analyses included all randomized patients who received at least one dose of study medication and underwent at least one safety assessment.

Adjustments for multiple comparisons of the primary efficacy end point (z-test) were made with the use of Hochberg's modified Bonferroni procedure²⁷ to maintain an overall two-sided type I error rate of 0.05. We estimated that we would need 210 patients per treatment group for the study to have a power of 80 percent to detect a 15 percent difference in the primary efficacy end point. All other analyses were unadjusted for multiple comparisons and at a 0.05 level (two-sided). The sample size required for the analyses involving intravascular ultrasonography was one third that of the main study.

Secondary efficacy variables included the proportions of patients who reached the primary efficacy end point and Kaplan-Meier estimates of the probability of reaching the primary efficacy end point at 6 and 12 months, as well as the individual components of this end point at 6 and 12 months, episodes of rejection treated with antibodies by months 6 and 12, and the presence of vasculopathy at 12 months.²⁸ The primary efficacy variable for intravascular ultrasonography was the mean change among matched slices in the maximal intimal thick-

ness from base line to month 12 (assessed with use of the Wilcoxon rank-sum test); secondary end points included the incidence of vasculopathy (assessed with use of Fisher's exact test).

For comparisons between groups, safety data were analyzed with use of the Wilcoxon rank-sum test for continuous variables and Fisher's exact or chi-square tests for categorical data. The Van Elteren test was used to compare the extent of vasculopathy in the three groups after adjustment for the presence of rejection.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 634 patients underwent randomization: 209 were assigned to receive 1.5 mg of everolimus daily, 211 to receive 3.0 mg of everolimus daily, and 214 to receive azathioprine. The patients in the three groups had similar demographic characteristics (Table 1).

IMMUNOSUPPRESSION

The average daily dose of cyclosporine during the 12-month period was significantly lower in the two everolimus groups than in the azathioprine group (3.5 mg per kilogram in the group given 1.5 mg of everolimus, $P < 0.001$; 3.4 mg per kilogram in the group given 3.0 mg of everolimus, $P < 0.001$; 4.2 mg per kilogram in the azathioprine group).

EFFICACY

At six months, significantly more patients in the azathioprine group had reached the primary efficacy end point (46.7 percent) than in the group given 3.0 mg of everolimus (27.0 percent, $P < 0.001$) or the group given 1.5 mg of everolimus (36.4 percent, $P = 0.03$) (Fig. 1). The respective rates at 12 months were 52.8 percent, 32.2 percent ($P < 0.001$), and 41.6 percent ($P = 0.02$). The rates of graft loss and death (Table 2) were similar among the groups. The incidence of multiple episodes of rejection of at least grade 3A was significantly higher in the azathioprine group (14.0 percent) than in the group given 3.0 mg of everolimus (6.6 percent, $P = 0.02$) or in the group given 1.5 mg of everolimus (8.1 percent, $P = 0.06$). The incidence of rejection associated with hemodynamic compromise was lower in the everolimus group than in the azathioprine group, but the difference did not reach statistical significance (8.1 percent in the group given 1.5 mg of everolimus, $P = 0.36$; 6.6 percent in the group given 3.0 mg of everolimus, $P = 0.13$; and 10.7 percent in the azathioprine group). Rejection of at least grade 3A was significantly less frequent in the everolimus groups than in the azathioprine group at month 12 (30.6 percent in the 1.5-mg group, $P < 0.001$; 21.3 percent in the 3.0-mg group, $P < 0.001$; and 45.8 percent in the azathioprine group). There was no significant difference in the incidence of rejection between the group of patients who received induction therapy and the overall study population.

RESULTS OF INTRAVASCULAR ULTRASONOGRAPHY

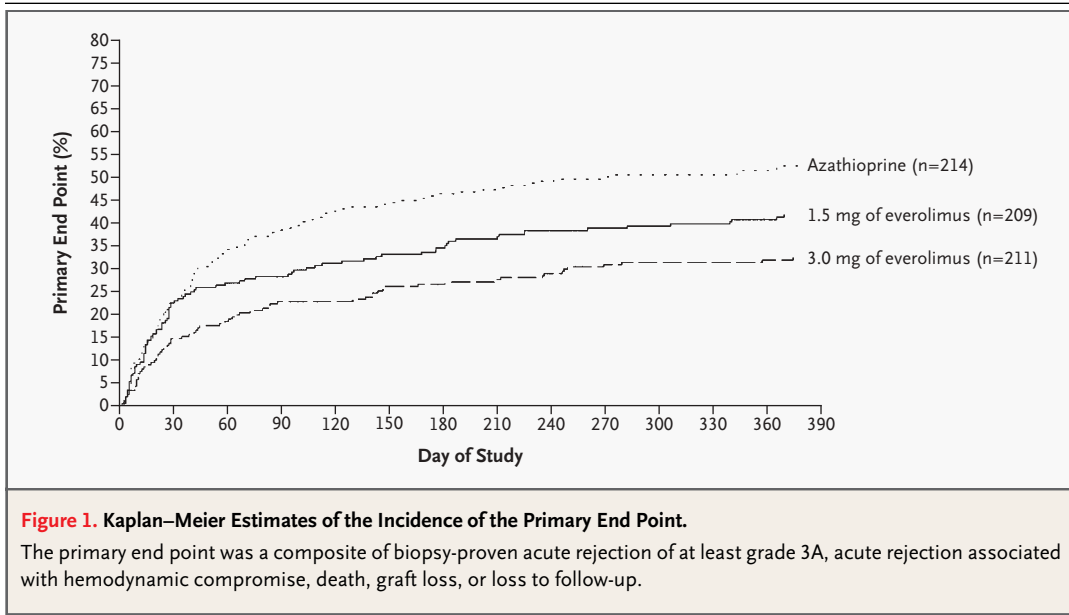
Representative base-line and 12-month intravascular ultrasonograms from a patient with vasculopathy are shown in Figure 2. The average increase in the maximal intimal thickness from base line to 12 months was smaller in the two everolimus groups than in the azathioprine group (0.04 mm in the 1.5-mg group, $P = 0.01$; 0.03 mm in the 3.0-mg group, $P = 0.003$; and 0.10 mm in the azathioprine group) (Fig. 3). The incidence of vasculopathy was also lower in the everolimus groups (35.7 percent

Table 1. Base-Line Demographic Characteristics of the Patients.*

Characteristic	1.5 mg of Everolimus (N=209)	3.0 mg of Everolimus (N=211)	Azathioprine (N=214)
Age — yr	51.2±11.2	52.1±10.8	50.5±11.5
Sex — no. (%)			
Male	166 (79.4)	171 (81.0)	182 (85.0)
Female	43 (20.6)	40 (19.0)	32 (15.0)
Race — no. (%)			
White	181 (86.6)	192 (91.0)	193 (90.2)
Black	21 (10.0)	11 (5.2)	13 (6.1)
Asian	2 (1.0)	3 (1.4)	3 (1.4)
Other	5 (2.4)	5 (2.4)	5 (2.3)
Weight — kg	76.2±15.4	77.6±15.1	77.0±14.9
Height — cm	173.3±10.4	172.5±8.8	172.8±9.3
Primary reason for heart transplantation — no. (%)			
Idiopathic cardiomyopathy	100 (47.8)	98 (46.4)	115 (53.7)
Coronary artery disease	78 (37.3)	84 (39.8)	68 (31.8)
Other	31 (14.8)	29 (13.7)	31 (14.5)
Recipient negative for cytomegalovirus, donor positive — no. (%)	36 (17.2)	48 (22.7)	37 (17.3)
Induction therapy — no. (%)	104 (49.8)	102 (48.3)	109 (50.9)
Age of donor — yr	32.5±12.5	34.1±12.9	33.6±13.2
Duration of cold ischemia — hr	2.9±1.1	3.2±1.1†	3.0±1.1

* Plus-minus values are means ±SD.

† $P = 0.009$ for the comparison with the group given 1.5 mg of everolimus.



in the 1.5-mg group, $P=0.045$; 30.4 percent in the 3.0-mg group, $P=0.01$; and 52.8 percent in the azathioprine group). Vasculopathy was significantly less frequent in the group given 1.5 mg of everolimus ($P=0.02$) and the group given 3.0 mg of everolimus ($P=0.002$) than in the azathioprine group after adjustment for rejection. The mean changes in other intravascular ultrasonographic variables, including the intimal area, intimal volume, and intimal index, were also significantly smaller in the everolimus groups than in the azathioprine group (Fig. 3). The incidence of statin use was similar among the three groups and did not influence the incidence or severity of vasculopathy.

SAFETY

There were no significant differences in death rates among the groups. There were 61 deaths by day 450, the cutoff date for the safety analysis: 19 in the group given 1.5 mg of everolimus, 24 in the group given 3.0 mg of everolimus, and 18 in the azathioprine group. The incidence of specific causes of death varied among the groups, but the number of each type was too low to correlate with study medications.

The overall rate of premature discontinuation of study medication was higher in the everolimus groups than in the azathioprine group (29.7 percent in the 1.5-mg group, $P=0.03$; 39.8 percent in the 3.0-mg group, $P=0.01$; and 28.5 percent in the azathioprine group). Premature discontinuation be-

cause of a lack of efficacy was less frequent in the group given 3.0 mg of everolimus than in either the azathioprine group (0.9 percent vs. 7.0 percent, $P=0.002$) or the 1.5-mg group (6.7 percent).

ADVERSE EVENTS

Almost all patients reported at least one adverse event during the study. The number of patients with adverse events leading to the discontinuation of the study medication did not differ significantly among the groups (15.8 percent in the 1.5-mg group, 21.8 percent in the 3.0-mg group, and 13.1 percent in the azathioprine group) (Table 2). The most frequent reasons for premature discontinuation were renal disorders, infections, leukopenia, gastrointestinal disorders, neurologic disorders, anemia, and thrombocytopenia. The incidence of nonfatal serious adverse events was not significantly greater in the 3.0-mg group than in the 1.5-mg group or the azathioprine group (64.6 percent, 70.6 percent, and 60.3 percent, respectively).

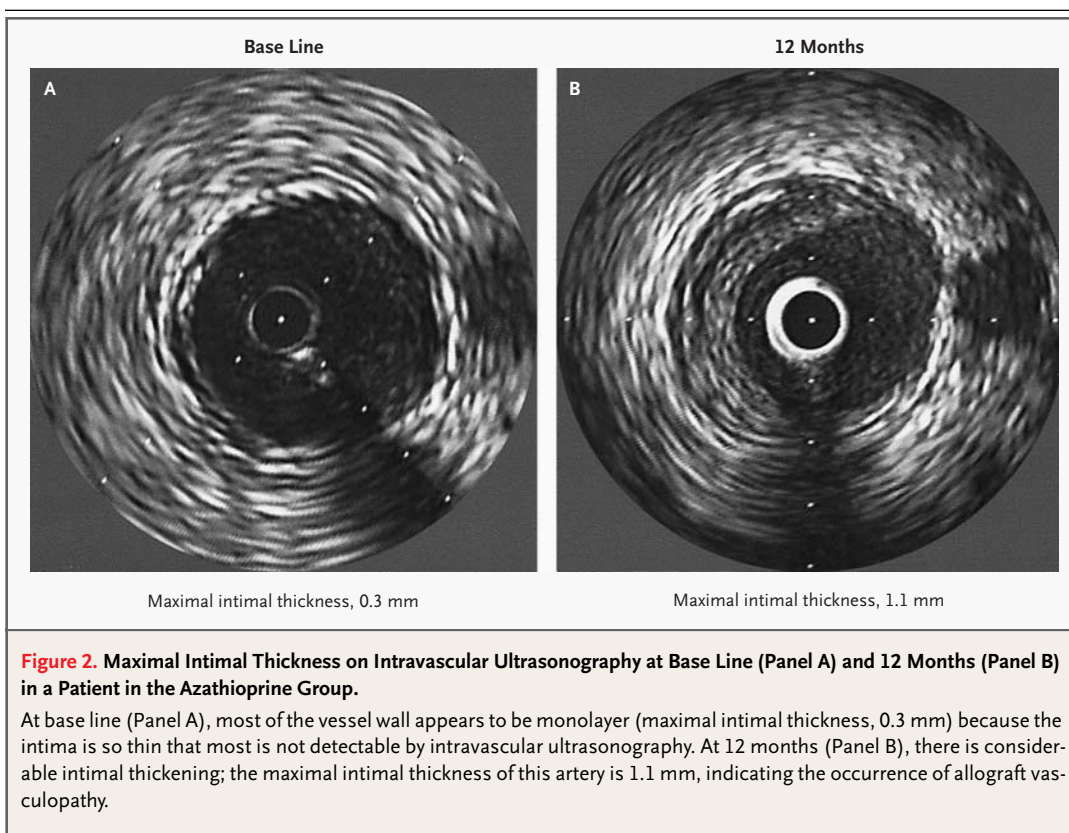
Viral infections, including cytomegalovirus infections, were more frequent during azathioprine therapy (31.3 percent) than during treatment with either 1.5 mg of everolimus (14.8 percent, $P<0.001$) or 3.0 mg of everolimus (17.1 percent, $P<0.001$); the reverse was true with respect to the incidence of bacterial infections (Table 2). Cytomegalovirus infections occurred less frequently with 1.5 mg of everolimus (7.7 percent) or 3.0 mg of everolimus (7.6 percent) than with azathioprine

Table 2. Adverse Events during the 12 Months of the Study.*			
Adverse Event	1.5 mg of Everolimus (N=209)	3.0 mg of Everolimus (N=211)	Azathioprine (N=214)
	<i>number (percent)</i>		
Patients who discontinued study before 12 mo	19 (9.1)	24 (11.4)	21 (9.8)
Death	19 (9.1)	24 (11.4)	18 (8.4)
Withdrawal of consent	0	0	2 (0.9)
Loss to follow-up	0	0	1 (0.5)
Patients who discontinued study treatment before 12 mo†	62 (29.7)	84 (39.8)	61 (28.5)
Adverse events	33 (15.8)	46 (21.8)	28 (13.1)
Anemia	0	5 (2.4)	1 (0.5)
Leukopenia	4 (1.9)	5 (2.4)	6 (2.8)
Thrombocytopenia	0	4 (1.9)	1 (0.5)
Gastrointestinal disorders	2 (1.0)	5 (2.4)	3 (1.4)
Infections	4 (1.9)	10 (4.7)	4 (1.9)
Renal impairment or increased creatinine level	7 (3.3)	8 (3.8)	3 (1.4)
Neurologic disorders	1 (0.5)	5 (2.4)	1 (0.5)
Abnormal laboratory values	4 (1.9)	14 (6.6)	8 (3.7)
Abnormal test result	0	1 (0.5)	0
Unsatisfactory therapeutic effect	14 (6.7)	2 (0.9)	15 (7.0)
Protocol violation	1 (0.5)	4 (1.9)	2 (0.9)
Death	5 (2.4)	8 (3.8)	5 (2.3)
Loss to follow-up	0	0	1 (0.5)
Withdrawal of consent	5 (2.4)	9 (4.3)	2 (0.9)
All patients			
Rejection			
Acute rejection associated with hemodynamic compromise (at mo 12)	17 (8.1)	14 (6.6)	23 (10.7)
Antibody-treated episode of acute rejection of grade $\geq 3A$ or associated with hemodynamic compromise (at mo 12)	15 (7.2)	7 (3.3)	15 (7.0)
Infection	151 (72.2)	162 (76.8)	150 (70.1)
Bacterial	69 (33.0)	80 (37.9)‡	53 (24.8)
Wound	14 (6.7)	11 (5.2)	7 (3.3)
Fungal	16 (7.7)	24 (11.4)	19 (8.9)
Aspergillus	4 (1.9)	5 (2.4)	1 (0.5)
Candidiasis	10 (4.8)	18 (8.5)	16 (7.5)
Viral (cytomegalovirus, herpes simplex, herpes zoster)	31 (14.8)‡	36 (17.1)‡	67 (31.3)

* Patients may have had more than one adverse event.

† Patients are classified according to the event that led to study discontinuation.

‡ P=0.001 for the comparison with azathioprine.



(21.5 percent, $P < 0.001$ for both comparisons) and were even less common among patients who did not receive induction therapy than among those who did (3.8 percent in the 1.5-mg group, $P = 0.01$; 3.7 percent in the 3.0-mg group, $P = 0.01$; and 14.3 percent in the azathioprine group). The percentage of patients who received prophylaxis against cytomegalovirus was similar among the three groups (73.8 percent of the azathioprine group, 79.4 percent of the 1.5-mg group, and 76.8 percent of the 3.0-mg group), as was the percentage of cytomegalovirus-negative recipients who received a transplant from a cytomegalovirus-positive donor (17.3 percent, 17.2 percent, and 22.7 percent, respectively). The incidence of cancer was also similar among the groups (7.7 percent in the 1.5-mg group, 7.1 percent in the 3.0-mg group, and 5.1 percent in the azathioprine group).

Although 90 percent of patients received statins, mean total cholesterol and triglyceride levels were elevated at 12 months in all groups, especially in the two everolimus groups (Table 3). Low-density lipoprotein (LDL) and high-density lipoprotein (HDL)

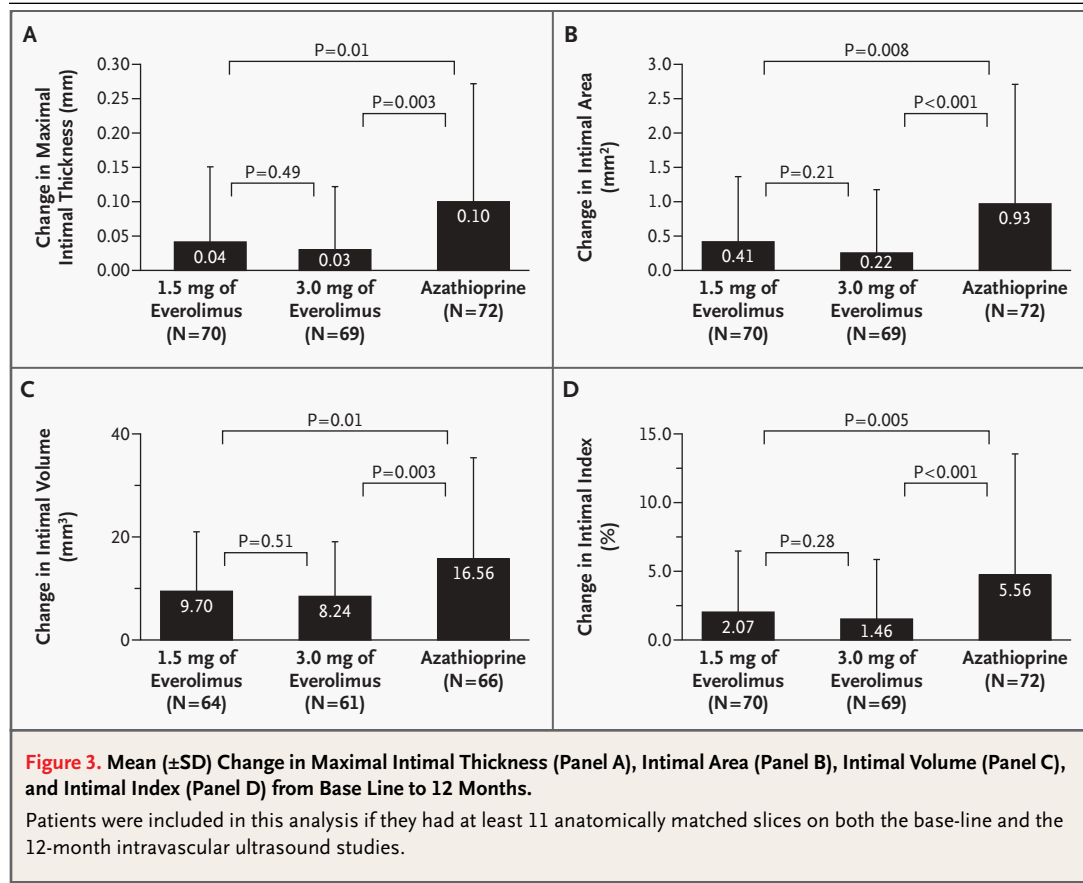
cholesterol levels were also higher at 12 months than at base line, but the values were similar in all three groups. The ratio of LDL to HDL cholesterol was also similar among the groups.

LABORATORY VALUES

Although mean leukocyte counts did not differ significantly among the groups at 12 months, platelet counts were significantly lower in the group given 3.0 mg of everolimus than in the group given 1.5 mg of everolimus ($P = 0.001$) or in the azathioprine group ($P = 0.001$) (Table 3). Everolimus therapy was associated with a significant increase in serum creatinine levels beginning on day 28 ($P = 0.001$ beginning at month 3) (Table 3). There were no significant differences among the groups in blood pressure, pulse rate, or QT interval (data not shown).

DISCUSSION

This 12-month analysis demonstrates that both a 1.5-mg dose and a 3.0-mg dose of everolimus, administered in combination with cyclosporine and



corticosteroids to recipients of a first heart allograft, were superior to azathioprine in preventing the primary efficacy end point of death, graft loss or a second transplantation, or biopsy-proven rejection. The 3.0-mg dose was superior to the 1.5-mg dose. The individual variable most responsible for the benefit was the significant reduction in the frequency of rejection of at least grade 3A. At 12 months, the rates of graft loss and death did not differ significantly among the groups.

Allograft vasculopathy remains the most important cause of late graft deterioration and death. Vascular remodeling develops over a period of months or years and results in decreased caliber of arterial lumens, ischemia, and graft failure. Vasculopathy is associated with immunologic mechanisms, such as the activation of alloreactive T cells and antibodies,⁴ and nonimmunologic factors, including hyperlipidemia, obesity, ischemia or reperfusion injury, older age of the donor, and cytomegalovirus infection.^{1,22,29} Intravascular ultrasonography is considered the most sensitive method of predicting events

and outcomes in patients with vasculopathy.^{5-8,30,31} We used intravascular ultrasonography to demonstrate the ability of both doses of everolimus to limit progressive intimal thickening and decrease the frequency of vasculopathy. The exact mechanism of this beneficial effect is not clear, but it may be due to the ability of everolimus to suppress T cells and inhibit the proliferation of smooth-muscle cells.^{11,13,20}

Viral infections may be associated with the development of vasculopathy.^{1,32} Episodes of rejection and cytomegalovirus infection led to earlier and more frequent episodes of chronic renal allograft nephropathy.³³ The two doses of everolimus resulted in significantly fewer cytomegalovirus infections than did azathioprine therapy and may also have had a role in decreasing the development of vasculopathy.

Interestingly, although it has been suggested that mycophenolate mofetil has antiproliferative effects that might limit the occurrence of vasculopathy more than does azathioprine,^{34,35} such an effect was

Table 3. Relevant Laboratory Abnormalities.*

Variable	1.5 mg of Everolimus (N=209)	3.0 mg of Everolimus (N=211)	Azathioprine (N=214)
Triglycerides			
Mean value at mo 12 (mmol/liter)	3.1†	3.0†	2.1
Triglycerides ≥4.5 mmol/liter (% of patients)	26.6†	32.7†	14.5
Cholesterol			
Mean value at mo 12 (mmol/liter)	5.7†	5.8†	5.2
Cholesterol ≥6.2 mmol/liter (% of patients)	62.8†	66.8†	45.8
LDL cholesterol			
Mean value at mo 12 (mmol/liter)	3.1	3.0	2.9
LDL cholesterol ≥4.1 mmol/liter (% of patients)	35.3	34.6	27.1
HDL cholesterol (mmol/liter)			
Mean value at mo 12	1.3	1.3	1.3
Mean change from base line	0.4	0.4	0.4
Hematology			
Mean leukocyte count at mo 12 (×10 ⁻³ /mm ³)	6.6	5.8	5.8
Mean platelet count at mo 12 (×10 ⁻³ /mm ³)	225	213‡	233
Serum creatinine after transplantation			
Day 28			
No. of patients	193	190	201
Mean (μmol/liter)	145	151	129
Median (μmol/liter)	124†§	142‡§	117
Mo 3			
No. of patients	169	153	167
Mean (μmol/liter)	164	164	135
Median (μmol/liter)	151†§	154†§	128
Mo 6			
No. of patients	156	152	167
Mean (μmol/liter)	178	177	151
Median (μmol/liter)	168†§	169†§	138
Mo 9			
No. of patients	138	119	144
Mean (μmol/liter)	191	181	152
Median (μmol/liter)	169†§	169†§	142
Mo 12			
No. of patients	140	132	151
Mean (μmol/liter)	181	189	147
Median (μmol/liter)	168†§	172†§	141
Last measurement during treatment			
No. of patients	206	211	214
Mean (μmol/liter)	190	190	151
Median (μmol/liter)	169†§	171†§	141

* To convert values for triglycerides to milligrams per deciliter, divide by 0.01129. To convert values for cholesterol to milligrams per deciliter, divide by 0.02586. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein. To convert values for creatinine to milligrams per deciliter, divide by 88.4.

† P=0.01 for the comparison with azathioprine.

‡ P=0.001 for the comparison with 1.5 mg of everolimus.

§ P=0.001 with the use of the Wilcoxon rank-sum test.

not seen in a clinical trial involving cardiac-transplant recipients.³⁶ Statins were reported in single-institution studies to reduce the development of vasculopathy.^{37,38} In our study, statins were used in 90 percent of the patients in each group. Vitamins C and E were reported to reduce the progression of vasculopathy in a small, single-center study.³⁹

Everolimus was generally well tolerated by our patients. There were more adverse events and a higher overall rate of discontinuation with the 3.0-mg dose than the 1.5-mg dose. Preclinical and clinical studies in patients who have undergone renal transplantation suggested that specific adverse events, such as decreased platelet counts and increased levels of cholesterol, triglycerides, and creatinine, might be expected when everolimus was given in combination with full-dose cyclosporine. Therefore, our study protocol was designed with these adverse events in mind. A reduction in the dose or temporary interruption of treatment was allowed for patients with platelet counts of less than 75,000 per cubic millimeter. The use of statins was optimized soon after transplantation. In both everolimus groups, hyperlipidemia was caused mainly by high triglyceride levels, since LDL and HDL cholesterol levels were similar to those in the azathioprine group.

The greater increase in mean serum creatinine levels in the two everolimus groups than in the azathioprine group was thought to be due to the potentiation of the toxic effects of cyclosporine in this setting, since the trough cyclosporine levels were not significantly different among the three groups. This problem was addressed in an amendment to our study, which is ongoing: cyclosporine trough levels were lowered to approximately 100 ng per

milliliter while blood levels of everolimus remained adequate (more than 3 ng per milliliter).

In conclusion, everolimus was significantly more efficacious than azathioprine in preventing vasculopathy among heart-transplant recipients at 12 months, and the 3.0-mg dose was superior to the 1.5-mg dose. Everolimus combined with cyclosporine and corticosteroids during the first 12 months is a safe and effective immunosuppressant regimen for use in recipients of a first heart transplant. Further evaluation and follow-up are required to determine the optimal dose and long-term benefits of everolimus for the prevention of vasculopathy. Our finding that everolimus significantly reduced the frequency and severity of vasculopathy, as measured by intravascular ultrasonography, offers a note of cautious optimism with respect to the control of this disorder.

Supported in part by a grant from Novartis Pharmaceuticals.

Dr. Eisen reports having received consulting fees from Novartis, Roche, AstraZeneca, and GlaxoSmithKline; lecture fees from Roche, AstraZeneca, and GlaxoSmithKline; and grant support from Novartis, Immunex, Roche, Bristol-Myers Squibb, Schering-Plough Research Institute, Menssana Research, and Acorn Cardiovascular. Dr. Dorent reports having received consulting fees from Novartis. Dr. Kobashigawa reports having received grant support from Novartis, Fujisawa, and Roche. Dr. Valentine-von Kaeppler reports having received consulting fees from Novartis and Roche, lecture fees from Novartis, and grant support from Novartis and Roche. Dr. Starling reports having received consulting fees from Acorn Cardiovascular and Novartis, lecture fees from Fujisawa and GlaxoSmithKline, and grant support from Acorn Cardiovascular, Fujisawa, Novartis, Roche, and Guidant. Dr. Sørensen reports having received consulting fees from Novartis. Dr. Hummel reports having received consulting fees from Novartis, lecture fees from Biotest, and grant support from Novartis. Ms. Lind and Drs. Abeywickrama and Bernhardt are employees of Novartis.

We are indebted to Drs. Nathalie Cretin-Buehler, Kenneth Somberg, and Judith Wolf for their thorough and insightful review of the manuscript.

APPENDIX

The following centers and investigators participated in the RAD B253 Study: Novartis Pharma, Basel, Switzerland, and Novartis Pharmaceuticals, Summit, N.J. — P. Bernhardt, K.H. Abeywickrama, J. Lind, N. Cretin, S. Le Breton, J. Kabir, J. Murphy; Data and Safety Monitoring Board — A. Laupacis (Toronto), G.A. Wells (Ottawa, Canada), R. Mills (Cleveland), G. Parry (Newcastle upon Tyne, United Kingdom); Cleveland Clinic Intravascular Ultrasound Core Laboratory: E.M. Tuzcu, S.E. Nissen, P. Schoenhagen, T. Crowe, W. Magyar, J. Coughlin, P. Shalling, C. Werle; **Canada:** Institut de Cardiologie de Montreal, Montreal — M. Carrier, D. Normandin, J. Vézina; University of Ottawa Heart Institute, Ottawa, Ont. — R. Masters, R. Davies; Toronto Hospital, Toronto — H. Ross, C. Cardella, D. Delgado, C. O'Grady; New Halifax Infirmary, Halifax, N.S. — H. Haddad, J. Howlett, G. Hirsch, C. Kells, B.J. O'Neill, K. Giddens; **Argentina:** Fundacion Favaloro, Buenos Aires — S.V. Perrone, L. Favaloro, R. Favaloro, E. Kapplinsky, A. Natello; **Austria:** Allgemeines Krankenhaus Universitaet, Vienna — M. Grimm, G. Laufer, G. Wieselthaler, A. Zuckermann, E. Deviatko; **Belgium:** Universitair Ziekenhuis Gasthuisberg, Leuven — J. Vanhaecke, J. Van Cleemput, W. Drooghe, A. Strijckmans; Cliniques Universitaires St. Luc, Brussels — M. Goenen, T. Timmermans; Onze Lieve Vrouw Ziekenhuis, Aalst — F. Wellens, M. Goethals, W. Tack; **Switzerland:** Universitäts Spital, Zurich — W. Kiowski, E. Oechslin, H. Brunner, R. Schindler; **Germany:** Kliniken der Medizinischen Hochschule, Hannover — A. Haverich, K. Pethig, C. Bara, I. Scheibner; Deutsches Herzzentrum, Berlin — R. Hetzer, M. Hummel, S. Kapell, E. Wenzel; **Denmark:** Skejby Sygehus, Aarhus — K. Soerensen, H. Moelgaard, H. Egeblad, J.E. Nielsen-Kudsk, H. Eiskjær, E.-M. Tram; **Spain:** Hospital Reina Sofia, Cordoba — J.M. Arizon, M. Concha, F. Vallés, A.L. Granados; Hospital Juan Canalejo, La Coruna — M.G. Crespo, M.J. Paniangua-Martín, T. Tabuyo, A. Juffé, J.A. Rodriguez; Clinica Puerta de Hierro, Madrid — L.A. Pulpon, J. Segovia; **France:** Hôpital La Pitié Salpêtrière, Paris — I. Gandjbakhch, R. Dorent, P. Léger, J.-P. Levasseur, E. Vaissier; Hôpital Foch, Suresnes — P. De Lentdecker, G. Dreyfus; Hôpital Cardiologique de Lyon, Lyons — G. Dureau, P. Boissonat, L. Sebbag, A. Roussouliere; **United Kingdom:** Papworth Hospital, Cambridge — J. Parameshwar, P. Schofield, L. Steel, V. Beresford; Wythenshawe Hospital, Manchester — N. Yonan, R. Martyszczuk, J. Reader; **Italy:** Azienda Ospedale Niguarda Ca' Granda, Milan — M. Frigerio, G. Masciooco, M. Grassi, M. Garbellini, F. Oliva; Policlinico San Matteo—Istituto di Ricovero e Cura a Carattere Scientifico, Pavia — M. Viganó, C. Pellegrini, M. Rinaldi, A.M. D'Armini; Policlinico, Università degli Studi, Padua — D. Casarotto, A. Gambino, T. Luca, G. Feltrin,

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