

# Safety, Tolerability, and Efficacy of Everolimus in De Novo Liver Transplant Recipients: 12- and 36-Month Results

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Everolimus is a macrolide immunosuppressive agent with known consistent absorption. In this double-blind study, we examined the safety and tolerability of everolimus vs. placebo in de novo liver transplant recipients. One hundred and nineteen liver allograft recipients were randomized to 1 of 4 groups: everolimus 0.5 mg bid, everolimus 1.0 mg bid, everolimus 2 mg bid, or placebo. Patients received oral cyclosporine to achieve a target trough level of 150-400 ng/mL in combination with prednisone. Primary and secondary endpoints of safety, tolerability, and efficacy were determined at 12 months, and patients were followed through 36 months. There was a trend toward fewer treated acute rejections in the everolimus group than in the placebo group: everolimus 0.5 mg: 39.3%; everolimus 1.0 mg: 30.0%; everolimus 2 mg: 29.0%; placebo: 40.0% ( $P =$  not significant). Adverse events were higher in everolimus-treated patients especially at the 4-mg/day dose, but there was no difference in the incidence of thrombocytopenia or leukopenia between all groups and renal function as determined by serum creatinine, and creatinine clearance remained stable to 36 months in everolimus-treated patients. Mean cholesterol and triglycerides increased from baseline in all treatment groups, and maximum levels were seen at 6 months. In conclusion, this study demonstrates that everolimus in combination with oral cyclosporine had an acceptable safety and tolerability profile, paving the way for additional studies in this transplant indication. *Liver Transpl* 12:1640-1648, 2006. © 2006 AASLD.

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Transplantation is the treatment of choice for patients with acute and chronic end-stage liver disease for whom there are no medical alternatives.<sup>1</sup> Despite high rates of 1- to 3-year survival, major concerns remain over long-term adverse effects of immunosuppressant

agents, such as diabetes, dyslipidemia, renal insufficiency, hypertension, and osteoporosis, all of which compromise quality of life and long-term patient survival.<sup>1-6</sup> The current clinical challenge is to develop regimens that maintain high rates of transplantation success while also minimizing unwanted and harmful metabolic and other effects.

It appears increasingly likely that efficacy and toxicity

**Abbreviations:** HAT, hepatic arterial thrombosis; BPAR, biopsy-proven and treated acute rejection; CMV, cytomegalovirus. Presented at the American Transplant Congress in Boston, MA, May 15-19, 2004 (abstracts 250461 and 251150).

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may be balanced by tailoring immunosuppressive therapy to individual patients by the use of proliferation signal inhibitors. These agents, which include the macrolide compound sirolimus (rapamycin) and its more recently introduced semisynthetic derivative, everolimus, appear to be well tolerated especially when used at lower dosages.<sup>5,7,8</sup> Use of sirolimus has been associated with disturbances in hematologic function including anemia, leukopenia and thrombocytopenia, hypertriglyceridemia and hypercholesterolemia, mouth ulceration, edema, and joint pain.<sup>9,10</sup> There have also been a number of reports suggesting that sirolimus is associated with an increased incidence of wound infection and dehiscence.<sup>11,12</sup> Furthermore, in 2 clinical trials involving de novo liver transplant recipients, there was an increase in the incidence of hepatic arterial thrombosis (HAT) resulting in stoppage of the trials and issuance of a black-box warning by the U.S. Food and Drug Administration.<sup>10</sup> More recently however, reports from 2 centers have suggested that with reduced dosage, the incidence and severity of sirolimus related side effects are manageable.<sup>10,13</sup>

Everolimus (Certican, Novartis, Basel, Switzerland) is a signal inhibitor that targets T-cell proliferation and complements the inhibitory effect of cyclosporine on T-cell-dependent growth factors.<sup>14-16</sup> Unlike cyclosporine, however, everolimus does not inhibit interleukin production arising from antigen-induced T-cell activation.<sup>15</sup> In vitro and preclinical studies have demonstrated that everolimus enhances immunosuppression in cyclosporine-based regimens<sup>17-19</sup> and has equivalent clinical efficacy to mycophenolate mofetil when used in combination with cyclosporine in renal transplant recipients.<sup>20,21</sup> Although everolimus is generally well tolerated in clinical studies in cardiac<sup>22</sup> and renal<sup>23</sup> transplant recipients, there has been a reported increase in average creatinine levels due to an interaction between everolimus and cyclosporine. There are few reports of the use of everolimus in liver transplantation. In a phase I study in orthotopic liver transplant recipients, there were no clinically significant changes in laboratory parameters or increases in rates of infection related to the use of the drug when used in combination with cyclosporine and corticosteroids.<sup>24</sup> The present randomized and placebo-controlled phase II study was performed to determine the long-term tolerability and safety of everolimus at 3 dose levels (1 mg/day, 2 mg/day and 4 mg/day) over 1 year. After completion of the 12-month double-blind period, patients on active treatment were followed in an open-label treatment protocol for an additional 2 years. The secondary objective was to compare everolimus with placebo in terms of a composite end point of biopsy-proven and treated acute rejection (BPAR), graft loss, death, or loss to follow-up. Results are reported for 12 and 36 months.

## PATIENTS AND METHODS

### Ethics, Study Design, and Patients

This multicenter de novo liver transplant study was initiated in February 1999 and patient follow-up com-

pleted in December 2003. The trial was carried out in accordance with the principles of good clinical practice and the Declaration of Helsinki (amended), and patients were provided with written informed consent before inclusion. The core phase was a 12-month multicenter, randomized, double-blind comparison of 3 different doses of everolimus with placebo in patients also taking cyclosporine (Neoral, Novartis, Basel, Switzerland) and corticosteroids. This was followed by an open-label extension phase in which patients originally randomized to everolimus therapy continued treatment for up to 36 months (placebo patients were discontinued at 12 months).

De novo whole-organ or split-liver transplant recipients who were over 16 years of age, who had a maximum cold ischemia time of 16 hours, and who were to receive cyclosporine posttransplant were randomized into 4 treatment groups on a 1:1:1:1 basis. Female patients of childbearing potential must have had a negative pregnancy test within the 48 hours immediately prior to randomization, and they were required to practice an approved method of birth control while receiving study medication and for 3 months following its discontinuation.

Exclusion criteria included prior organ transplant (patients scheduled to receive multiple organ transplants were also excluded); receipt of ABO incompatible transplants; receipt of living related or unrelated transplants; pregnant or lactating females; use of any investigational drug within 4 weeks prior to baseline; receipt of induction antibody therapy prior to randomization; receipt of any drugs known to interact with any study medication; thrombocytopenia ( $\leq 40,000/\text{mm}^3$ ), leukopenia ( $\leq 3,000/\text{mm}^3$ ), and/or an absolute neutrophil count of  $\leq 1000/\text{mm}^3$  as measured within 48 hours prior to transplant.

Baseline assessments were performed within 24 hours of transplant prior to first administration of the study drug and included vital signs, physical examination, electrocardiogram, safety laboratory tests (including hematology, biochemistry, and urinalysis) and endocrinology.

### Study Drugs and Concomitant Immunosuppressive Medication

Patients were randomized to oral everolimus 1 mg/day, 2 mg/day, or 4 mg/day in divided doses twice daily, or to matching placebo. All patients also took oral cyclosporine at doses necessary to maintain trough levels in the following target ranges: weeks 1 to 4, 150 to 450 ng/mL; months 2 to 6, 100 to 300 ng/mL; and months 7 to 12, 75 to 300 ng/mL. If required, everolimus/placebo was started via nasogastric tube until tablets could be tolerated.

Intravenous methylprednisolone was administered before, during, or immediately after transplantation. Oral prednisone (or its methylprednisolone equivalent) was tapered to  $\geq 5.0$  mg/day by month 3, after which it was given at maintenance doses, was tapered, or was discontinued.

Dose reduction or interruption of study medication was permitted for those patients with reduced platelet count, a decreased white blood cell count, elevated lipids, or any other moderate or severe adverse event suspected to be related to the study medication.

### Pharmacokinetics and Pharmacodynamics

Pharmacokinetic and pharmacodynamic analyses were done to relate everolimus and cyclosporine exposure to efficacy (BPAR) in the time-window of days 1-225 post-transplantation. Everolimus and cyclosporine exposure was calculated as the geometric mean of the trough levels till event or censoring.

### Safety and Efficacy End Points and Assessments

The primary objective was to evaluate the safety and tolerability of everolimus over the 12-month double blind phase, and in the long-term during the 24-month open-label extension. Secondary objectives were to investigate the pharmacokinetics of twice-daily multiple oral dosing of everolimus during steady-state administration of cyclosporine, and to evaluate the efficacy relative to placebo of everolimus 1 mg, 2 mg, and 4 mg per day. Efficacy was expressed in terms of a composite end point, including graft failure requiring retransplant, BPAR, graft loss, death, or loss to follow-up, and in terms of individual components of the composite end point, at 12 months. These end points were also assessed during the 24-month extension phase. Clinically suspected acute rejection was confirmed by biopsy unless contraindicated because of coagulation abnormalities or ascites. Standard core needle biopsy was specified either before or no later than 24 hours after the start of antirejection therapy.

Everolimus and cyclosporine peak and trough levels, the area under the curve of everolimus level vs. time and clearance values were determined on day 7 and at months 2 and 3. Adverse events, including infections, were monitored throughout the study period. Cyclosporine whole-blood trough levels were assessed on days 7, 14, 21, and 28, and at months 2, 3, 6, 9, and 12. Laboratory tests, including hematology, biochemistry, and urinalysis, were performed on days 1, 7, 14, 21, and 28 and at months 2, 3, 6, 9, and 12. Particular attention was made to renal function, including repeated measurements of serum creatinine ( $\mu\text{mol/L}$ ), creatinine clearance as calculated from the Cockcroft-Gault ( $\text{mL/min}$ ), and serum lipids (triglycerides and cholesterol [ $\text{mmol/L}$ ]). Electrocardiograms were performed on days 1 and 7 and at months 2 and 12. Vital signs were assessed on days 1, 7, 14, and 28 and at months 2, 3, 6, 9, and 12. Endocrinology was assessed at months 6 and 12. All concomitant medications and relevant nondrug therapies were recorded.

### Statistical Analyses

Safety and tolerability analyses were performed on the safety population, which included all randomized pa-

tients who received at least 1 dose of study medication and who had at least 1 post-baseline safety evaluation. Efficacy assessments were performed on the intent-to-treat population of all randomized patients at 12 months.

Primary efficacy was compared using the Pearson chi-square test for equal proportions for calculation of overall *P* values. A 2-sided Fisher exact test was used for pair-wise comparisons. Planned enrollment was 120 patients (30 per treatment group). No formal sample-size calculation was performed.

## RESULTS

### Demographics and Patient Disposition

A total of 119 patients were enrolled (placebo, *n* = 30; everolimus, 1 mg, *n* = 28; everolimus, 2 mg, *n* = 30; everolimus, 4 mg, *n* = 31). There were no significant differences overall in demographic variables between treatment groups, although more females were enrolled into the everolimus 1 mg and 4 mg groups. The most frequent reason for transplantation was active hepatitis or cirrhosis (54-65% in all groups), and the proportion of hepatitis C-positive recipients was comparable between treatment groups (Table 1). The proportion of cytomegalovirus (CMV)-negative recipients who received an organ from a CMV-positive donor was highest in the everolimus 4-mg/day group (36%, compared with 27%, 18%, and 20% in the placebo, everolimus 1-mg/day, and 2-mg/day groups, respectively).

The most common pre-existing medical conditions were ascites (46.4-54.8%), cardiovascular disorders (33.3-53.3%), and central and peripheral nervous system disorders (30.0-51.6%, mostly encephalopathy). Patients randomized to placebo in the core phase were discontinued after 12 months in the double-blind phase or within 1 month of the start of the extension phase, as unblinding could not take place until all patients had received a full 12 months of therapy. A total of 56 patients completed the core phase, and 28 continued in the extension phase (11, 11, and 6 in the everolimus 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively).

The disposition of patients discontinuing treatment at 12 and 36 months is shown in Table 2. A higher proportion of patients discontinued treatment before month 12 in the everolimus treatment groups than with placebo, but discontinuation was not dose-related (43.3%, 57.1%, 46.7%, and 64.5% in placebo and everolimus 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively) (Table 2).

### Drug Exposure

At least 20% of patients in each treatment group discontinued medication within 2 weeks of randomization, mainly because of technical or surgical complications with the allograft. Discontinuation rates were 20.0%, 32.1%, 26.7%, and 22.6% in the placebo and everolimus 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively. Mean daily everolimus doses from random-

TABLE 1. Patient Demographics and Baseline Characteristics

	Placebo (n = 30)	Everolimus 1 mg/day (n = 28)	Everolimus 2 mg/day (n = 30)	Everolimus 4 mg/day (n = 31)
Age <50 years	12 (40.0%)	18 (64.3%)	16 (53.3%)	15 (48.4%)
Age ≥50 years	18 (60.0%)	10 (35.7%)	14 (46.7%)	16 (51.6%)
Mean age ± SD (years)	51.6 ± 9.0	46.3 ± 11.2	48.2 ± 12.0	48.8 ± 10.2
Gender (% male)	50.0	64.3	43.3	71.0
Ethnicity				
Caucasian	27 (90.0%)	27 (96.4%)	27 (90.0%)	31 (100%)
Black	2 (6.7%)	1 (3.6%)	1 (3.3%)	0
Other	1 (3.3%)	0	2 (6.7%)	0
Mean weight ± SD (kg)	80.7 ± 18.5	81.2 ± 25.2	77.7 ± 19.6	82.8 ± 20.7
Mean height ± SD (cm)	171 ± 10.6	172 ± 8.6	168 ± 10.8	174 ± 9.7
Primary cause of end-stage liver disease				
Chronic active hepatitis/cirrhosis	17 (56.7%)	15 (53.6%)	17 (56.7%)	20 (64.5%)
Primary biliary cirrhosis	6 (20.0%)	6 (21.4%)	5 (16.7%)	2 (6.5%)
Sclerosing cholangitis	3 (10.0%)	3 (10.7%)	6 (20.0%)	5 (16.1%)
Other	4 (13.3%)	4 (14.3%)	2 (6.7%)	4 (12.9%)
Hepatitis C positive	6 (20.0%)	9 (32.1%)	7 (23.3%)	8 (25.8%)
Patients with diabetes	4 (13.3%)	7 (25.0%)	5 (16.7%)	8 (25.8%)
Split-liver transplant	1 (3.3%)	4 (14.3%)	1 (3.3%)	1 (3.2%)
Mean cold ischemia time ± SD (hours)	9.1 ± 3.2	10.0 ± 2.9	9.4 ± 2.6	8.3 ± 2.9

TABLE 2. Patient Disposition, Months 12 and 36

	Placebo n = 30	Everolimus 1 mg/day n = 28	Everolimus 2 mg/day n = 30	Everolimus 4 mg/day n = 31
Month 12				
Completed 12-month visit on treatment	17 (56.7%)	12 (42.9%)	16 (53.3%)	11 (35.5%)
Discontinued treatment before 12-month visit	13 (43.3%)	16 (57.1%)	14 (46.7%)	20 (64.5%)
Reasons for discontinuation				
Adverse events	4 (13.3%)	9 (32.1%)	10 (33.3%)	10 (32.3%)
Laboratory abnormality	0	2 (7.1%)	0	0
Lack of efficacy	7 (23.3%)	2 (7.1%)	1 (3.3%)	5 (16.1%)
Protocol violation	0	0	1 (3.3%)	0
Consent withdrawn	2 (6.7%)	3 (10.7%)	2 (6.7%)	3 (9.7%)
Death*	5 (16.7%)	5 (17.9%)	1 (3.3%)	4 (12.9%)
Month 36	n = 0	n = 11	n = 11	n = 6
Completed 36-month visit on treatment	–	10 (90.9%)	8 (72.7%)	2 (33.3%)
Discontinued treatment before 36-month visit	–	1 (9.1%)	3 (27.3%)	4 (66.7%)
Reasons for discontinuation				
Adverse events	–	1 (9.1%)	2 (18.2%)	3 (50.0%)
Administrative problems	–	0	1 (9.1%)	1 (16.7%)

\*Numbers for patients who died are also included in adverse events or lack of efficacy discontinuations.

ization to month 12 were 0.9, 1.7, and 3.4 mg/day in the 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively. Variation in the daily everolimus dose throughout the study was small. Although two hour post-dose measurement monitoring has now been proposed for patients receiving cyclosporine, at the time of initiation of this study trough monitoring was the standard of practice and thus all patients were monitored by cyclosporin A trough level measurement. The mean daily cyclosporine dose was comparable between all treatment groups at all visits, and was reduced to a

similar extent in all groups as the study progressed (from 7.05-8.07 mg/kg/day on day 1 to 2.57-3.48 mg/kg/day at month 12).

### Tolerability

Most patients experienced at least 1 adverse event. Rates of adverse events were higher in everolimus-treated patients, especially those receiving 4 mg/day. Although differences were not statistically different, the incidence of anemia, tachycardia, constipation, edema,



TABLE 3. Clinically Significant Adverse Events (Safety Population; 36 Months)

	Placebo (n = 30)	Everolimus 1 mg/day (n = 28)	Everolimus 2 mg/day (n = 30)	Everolimus 4 mg/day (n = 31)
Hematologic events				
Thrombocytopenia*	3 (10.0%)	4 (14.3%)	6 (20.0%)	6 (19.4%)
Leukopenia <sup>†</sup>	0	4 (14.3%)	2 (6.7%)	2 (6.5%)
Hypercholesterolemia <sup>‡</sup>	1 (3.3%)	2 (7.1%)	3 (10.0%)	3 (9.7%)
Neoplasms	2 (6.7%)	1 (3.6%)	4 (13.3%)	2 (6.5%)
Renal dysfunction	2 (6.7%)	6 (21.4%)	3 (10.0%)	5 (16.1%)
HAT	1 (3.3%)	0	1 (3.3%)	1 (3.2%)
Death <sup>§</sup>	5 (16.7%) <sup>b</sup>	5 (17.9%)	1 (3.3%)	4 (12.9%)
Nonfatal serious adverse event	13 (43.3%)	16 (57.1%)	20 (66.7%)	21 (67.7%)
Discontinuation due to adverse event	1 (3.3%)	3 (10.7%)	2 (6.7%)	1 (3.2%)

\* $\leq 25 \times 10^9/L$ .<sup>†</sup> $\leq 3.0 \times 10^9/L$ .<sup>‡</sup> $\geq 9.1$  mmol/L.<sup>§</sup>Deaths are reported to 12 months in the placebo group. Between 12 and 36 months there was one additional death, for a total of 6 deaths.

elevated blood creatinine, and agitation were increased with everolimus treatment relative to placebo. Adverse effects suspected to be related to study drug treatment were generally more frequent with increasing everolimus dose. Infections, gastrointestinal events, laboratory findings such as decreased platelets, and metabolic disorders such as hypercholesterolemia and hypertriglyceridemia that were suspected to be drug-related were most frequently observed with the 2 highest dosages of everolimus.

The overall incidence of infection was comparable between groups (61-77%). Of interest, only a small number of patients developed CMV disease (7 of 119, or 6%), and although not statistically significant, the incidence of CMV disease was higher in the everolimus groups (placebo, 1; everolimus 1 mg/day, 1; 2 mg/day, 2; and 4 mg/day, 3). This may be explained by the fact that the everolimus 2-mg/day and 4-mg/day groups received more organs from CMV-positive donors (60% and 65%, respectively) than the everolimus 1-mg/day or placebo groups (37% and 39%, respectively). Potentially clinically significant events (regardless of frequency) are shown in Table 3. Hematologic adverse events (thrombocytopenia and leukopenia) were associated with higher everolimus dosages (Table 3). Overall, the rate of malignancy was low, with neoplasms being reported in 9 patients (2, 1, 4, and 2 patients in the placebo and everolimus 1- mg/day, 2-mg/day, and 4-mg/day groups, respectively) (Table 3).

There were 3 cases of HAT, 1 in each of the everolimus 2-mg/day and 4-mg/day groups (retransplanted and recovered, respectively) and 1 in the placebo group (died). Neither event in the active treatment groups was considered to be related to drug treatment. As shown in Table 3, there were few deaths in any group (16.7%, 17.9%, 3.3%, and 12.9% in the placebo and everolimus 1 mg/day, 2 mg/day, and 4 mg/day groups, respectively); none were thought to be drug-related.

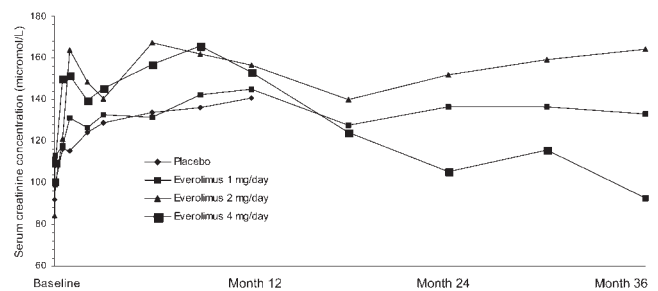


Figure 1. Mean serum creatinine concentrations in de novo liver transplant recipients treated with placebo (baseline through month 12) or everolimus 1 mg/day, 2 mg/day or 4 mg/day (baseline through month 36).

## Safety

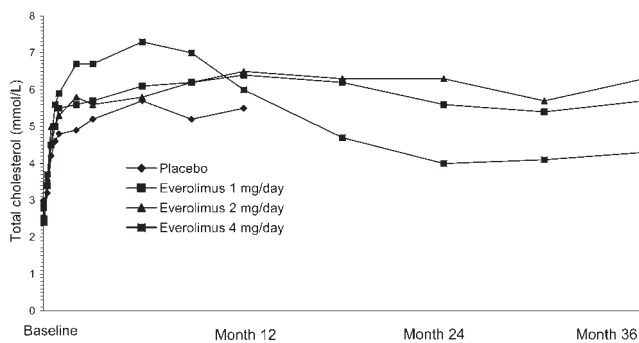
There were no dose-related differences from baseline among groups for hematology parameters (hemoglobin, leukocytes, neutrophils, or platelets). Mean platelet counts increased as expected immediately following transplantation, but they returned to normal values and stabilized from month 3 onwards. Mean serum creatinine concentrations remained stable from month 1 after an expected initial postoperative rise (Fig. 1). From month 1 all serum creatinine concentrations were higher in the 2 higher-dosage everolimus groups, but these concentrations were not statistically increased from baseline levels. Consistent with this result, creatinine clearance decreased in all groups and then remained stable from month 1 onward (Table 4). By 28 days posttransplant, creatinine clearance had decreased in the placebo and everolimus-treated groups (placebo,  $29.1 \pm 30.2$  mL/min; everolimus 1 mg,  $21.6 \pm 21$  mL/min; everolimus 2 mg,  $42 \pm 25$  mL/min; and everolimus 4 mg,  $38.2 \pm 33.3$  mL/min; [ $P = 0.13$ ]). By 2 months, creatinine clearance had stabilized in all groups and by month 12 were nearly equivalent (placebo,  $59 \pm 21$  mL/min; everolimus

TABLE 4. Creatinine Clearance

	Placebo	Everolimus 1 mg/day	Everolimus 2 mg/day	Everolimus 4 mg/day	P Value*
Baseline	95.9 ± 35	87.5 ± 25.3	96.7 ± 30.4	96 ± 35.8	
Day 1	95.1 ± 39	86.1 ± 23.5	91.4 ± 33.6	93.2 ± 44	0.49
Day 7	55 ± 6	77.7 ± 11	81.8 ± 6	76.7 ± 4	0.29
Day 14	72.7 ± 22	85 ± 30	82.8 ± 36	71.2 ± 32	0.17
Day 28	68.3 ± 17	71.6 ± 25	63.5 ± 28	62.6 ± 22	0.13
Month 3	58.3 ± 15	64.6 ± 21	60.7 ± 17	63.4 ± 28	0.25
Month 6	58.9 ± 16	68.8 ± 21	60.2 ± 27	62.8 ± 29	0.42
Month 12	59 ± 21	67.3 ± 22	53.7 ± 15	59.1 ± 13	0.15
Month 36		73.9 ± 23	61.5 ± 30	69.9 ± 7	0.44

NOTE: Creatinine clearance was calculated by Cockcroft-Gault formula (mL/min).

\*Based on the Kruskal-Wallis test. There was no statistical difference among groups at any time point.



**Figure 2. Mean total cholesterol concentrations in de novo liver transplant recipients treated with placebo (baseline through month 12) or everolimus 1 mg/day, 2 mg/day or 4 mg/day (baseline through month 36).**

1 mg,  $67.3 \pm 22$  mL/min; everolimus 2 mg,  $53.7 \pm 14.7$  mL/min; everolimus 4 mg,  $59.1 \pm 13$  mL/min;  $P = 0.15$ ) (Table 4).

Mean total cholesterol levels increased from baseline in all treatment groups, with maximum levels reached by month 6 (Fig. 2) [mean values of 5.7, 6.1, 5.8, and 7.3 mmol/L in the placebo, everolimus 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively]. Although changes from baseline were dose-related, there were no significant differences between treatment groups. The incidence of high post-baseline values ( $\geq 9$  mmol/L) was low, but a trend across treatment groups suggested a dose relationship (3%, 7%, 10%, and 16% in the placebo and everolimus 1-mg/day, 2-mg/day and 4-mg/day groups, respectively).

There were also dose-related increases in mean triglyceride levels (2.7, 2.1, 3.1, and 4.1 mmol/L in the placebo and everolimus 1-mg/day, 2-mg/day, and 4-mg/day groups, respectively). Maximum values were reached by about month 6. Mean high-density lipoprotein cholesterol levels increased from baseline in all groups, but these changes were not dose-related. There was an increase overall in low-density lipoprotein cholesterol levels across groups, but this parameter was determined in a small proportion of patients only. Although, cholesterol levels were lower in patients receiv-

ing a 4-mg dose of everolimus, this finding might reflect the fact that many patients in this treatment dropped out prior to completion of the study.

There were no clinically meaningful changes from baseline in any liver function test parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin). Mean albumin values were within the normal range by month 12 and comparable between groups; this is consistent with a functioning liver allograft. There were no treatment-associated changes in any group for electrolytes or metabolic and endocrine parameters; no clinical trends were observed in body weight changes, or changes from baseline in blood pressure or heart rate.

## EFFICACY

There were no significant dose-related or between-group differences in rates of the composite end point of efficacy failure or its individual components (i.e., BPAR, graft loss, death, or loss to follow-up) in the intent-to-treat population (Table 5). Most of the efficacy-related events were recorded before month 12. Although there were lower rates of treated cellular rejection and mortality in the everolimus 2-mg/day and 4-mg/day groups, these did not reach statistical significance ( $P = 0.239$ ) (Table 5). The trend toward lower rates for BPAR was related to everolimus dosage, with rates ranging from 32.1% to 25.8% at 12 months and from 39.3% to 29% at 36 months across increasing everolimus dosages (Table 6). At 12 months, the rate of treated acute rejection with placebo was 40%.

Everolimus exposure was divided into 3 exposure groups of about equal size (tertiles), and efficacy was summarized in Table 6. A logistic regression of logarithmic everolimus exposure vs. efficacy suggests an increased efficacy with increasing everolimus exposure ( $P = 0.0137$ ). The Kaplan-Meier analysis of the time to efficacy event for the 3 everolimus exposure groups gave similar results (log-rank test,  $P = 0.006$ ).

A Cox regression relating logarithmic everolimus and cyclosporine exposure to efficacy suggested, again, an effect of everolimus on efficacy ( $P = 0.0304$ ), while

TABLE 5. Efficacy-related Events (Months 12 and 36)

	Placebo n = 30	Everolimus 1 mg/day n = 28	Everolimus 2 mg/day n = 30	Everolimus 4 mg/day n = 31
<b>Month 12</b>				
Efficacy failure (composite end point)	17 (56.7%)	20 (71.4%)	16 (53.3%)	18 (58.1%)
BPAR	12 (40.0%)	9 (32.1%)	8 (26.7%)	8 (25.8%)
Graft loss	2 (6.7%)	0	4 (13.3%)	1 (3.2%)
Death	5 (16.7%)	5 (17.9%)	1 (3.3%)	4 (12.9%)
Clinically confirmed chronic rejection	1 (3.3%)	0	1 (3.3%)	0
≥2 acute rejection episodes	7 (23.3%)	3 (10.7%)	3 (10.0%)	6 (19.4%)
<b>Month 36</b>				
	n = 30	n = 28	n = 30	n = 31
Efficacy failure (composite end point)	–	21 (75.0%)	18 (60.0%)	19 (61.3%)
BPAR	–	11 (39.3%)	9 (30.0%)	9 (29.0%)
Graft loss	–	0	4 (13.3%)	1 (3.2%)
Death	–	5 (17.9%)	1 (3.3%)	4 (12.9%)
Clinically confirmed chronic rejection	–	0	1 (3.3%)	0
≥2 acute rejection episodes	–	5 (17.9%)	4 (13.3%)	8 (25.8%)

NOTE: *P* = not significant for all efficacy-related events (Pearson chi-square test for equal proportions).

TABLE 6. BPAR by Exposure Level

Placebo	Everolimus Exposure		
	≤3 ng/mL	3-6 ng/mL	>6 ng/mL
11/30 (37%)	8/16 (50%)	4/28 (14%)	2/17 (12%)

NOTE: A logistic regression of logarithmical everolimus exposure vs. efficacy shows an increased efficacy with increasing everolimus exposure (*P* = 0.0137). The Kaplan-Meier analysis of the time to efficacy event for the three everolimus exposure groups gave similar results (log-rank test: *P* = 0.006).

the effect of cyclosporine was not clear (*P* = 0.13). The hazard ratio for a doubling of everolimus exposure was 0.47 (95% confidence interval: 0.24-0.93), and the hazard ratio for a doubling of cyclosporine exposure was 0.47 (95% confidence interval: 0.17-1.23).

All graft losses and most deaths were associated with typical posttransplant complications, not with study medication. Most rejections were mild or moderate (BANFF grades I or II) and not associated with everolimus dosing level.

## DISCUSSION

Everolimus (Certican Novartis, Basel, Switzerland) is an orally active 40-O-(2-hydroxyethyl) derivative of rapamycin, a macrolide antibiotic produced by *Streptomyces hygroscopicus*.<sup>14</sup> It exerts its immunosuppressive effect by blocking interleukin 2 and interleukin 15–driven proliferation of hematopoietic (T and B cells) and nonhematopoietic (vascular smooth muscle cells) cells by inhibiting the activation of p70S6 kinase.<sup>15,16</sup> Inhibition of p70S6 kinase arrests the cell cycle at the G1 phase preventing the progression of the cells into

the S phase. The inhibitory effect of everolimus is mediated by its forming a complex with the immunophilin FK506 binding protein 12, which also binds rapamycin. The 50% inhibitory concentration for everolimus inhibition of FK506 binding to FK506 binding protein 12 is 1.8-2.6 nmol/L and is approximately 3-fold higher than that of sirolimus (0.4-0.9 nmol/L).<sup>25</sup> The present study was performed to determine the long-term tolerability and safety of everolimus at 3 dose levels (1 mg/day, 2 mg/day, and 4 mg/day) over 1 year and after completion of the 12-month double-blind period, patients remaining on active treatment were followed in an open-label treatment protocol for an additional 2 years. The secondary objective was to compare everolimus with placebo in terms of a composite end point of BPAR, graft loss, death, or loss to follow-up.

Adverse events were more frequent in patients receiving higher doses of everolimus, and although not statistically significant, the results suggest that an everolimus dose of 4 mg/day may not be tolerated by liver transplant patients. The safety and tolerability profile reported in this study was comparable to that observed in a previous phase I study in 26 de novo liver transplant recipients.<sup>24</sup> Furthermore, the incidence of adverse events was comparable with those seen in studies of everolimus in renal transplant recipients.<sup>20,21,23,26</sup> Infections were seen in approximately two-thirds of patients overall, which concurs with the 6-month infection rates of 50% to 60% reported by Vitko et al.<sup>23</sup> in their analysis of 2 study populations comprising 493 renal transplant recipients treated with everolimus 1.5 mg/day or 3 mg/day. No serious, life-threatening infections were noted. In particular, there was no increase in the incidence of CMV or other serious viral infections in everolimus-treated patients compared to the control group even in patients taking everolimus at 4 mg/day. Most of the infections noted were bacterial, and these infections were easily treated with a short course of

antibiotics. Of interest, whereas the use of sirolimus in de novo liver transplant patients has been reported to be associated with serious wound infections, impaired wound healing, and wound dehiscence, there was no apparent increase in the incidence of wound infections or dehiscence in patients receiving everolimus even at the 4-mg dose.<sup>11,12</sup> Despite these findings, a higher proportion of patients discontinued study drug in the everolimus groups than with placebo. The causes of discontinuation were multifactorial and included death, serious adverse events including renal dysfunction, lipid disorders, and abnormalities in hematologic parameters, especially a dose-related increase in the incidence of thrombocytopenia. However, the incidence of these individual abnormalities were not statistically different between groups. Thus the reasons for the large dropout rate in the trial overall and especially in the everolimus-treated patients may have reflected lack of familiarity with use of this agent and reluctance of patients to continue therapy under close observation for an additional 2-year period rather than lack of tolerability of the agent.

There were only 3 cases of HAT in the everolimus drug treatment groups, none of which were thought to be drug related. This is an important finding, since administration of the proliferation inhibitor sirolimus in liver transplant recipients has been associated with an increased incidence of HAT,<sup>27</sup> and 2 de novo liver transplant clinical studies, 1 involving cyclosporine-sirolimus and 1 using tacrolimus-sirolimus combination therapy, were discontinued because of the increased incidence of HAT leading to graft loss and death.<sup>8,28</sup> However, a recent study found no association (when compared with historical controls) of sirolimus therapy with HAT.<sup>10</sup>

Everolimus has been associated with thrombocytopenia and leukopenia in cardiac and renal transplant recipients<sup>8,22</sup> and with dose-related reductions in platelet counts in renal transplant recipients.<sup>20,29</sup> We also found a dose-related increase in the incidence of thrombocytopenia, although thrombocytopenia did not result in need for drug discontinuation and no bleeding was attributable to this finding. In addition, the very low rates of malignancy in our study support the observation that proliferation inhibitors appear to prevent tumor progression.<sup>30</sup> Sirolimus and everolimus have demonstrated impressive growth-inhibiting effects in a broad range of human cancers (including breast cancer) in preclinical and early clinical trials, and the likely molecular targets that mediate this effect, together with potential clinical implications, are under investigation.<sup>31</sup>

No clinical laboratory parameters gave cause for significant concern in this study. Serum creatinine levels remained stable from month 1 through month 36, and although higher levels were consistently reported in the everolimus 2-mg/day and 4-mg/day groups than in the other groups, changes from baseline were not significant among treatment groups. Changes in creatinine levels between baseline and posttransplant visits were confounded by the presence of renal dysfunction at the time of transplant and may, in part, reflect the contri-

bution of everolimus to cyclosporine-related nephrotoxicity or other factors not yet identified. The fact that the placebo group also showed a reduction in renal function suggests that this side effect is a manifestation of chronic use of the CNI cyclosporine. Furthermore, creatinine clearance reflected early renal dysfunction, but by 12 months, improvement in creatinine clearance was seen in all study groups and maintained to month 36. Recently, we have reported use of C<sub>2</sub> cyclosporine A monitoring is associated with increased efficacy and decreased toxicity profiles in liver transplant patients.<sup>32</sup> Whether use of cyclosporin A C<sub>2</sub> monitoring would reduce renal toxicity seen with everolimus in combination with cyclosporin A in this trial remains to be investigated. Additionally, use of everolimus with tacrolimus, a combination therapy that has been suggested to have less renal toxicity, might be another approach to reduce the incidence of renal dysfunction.<sup>10</sup>

Although total cholesterol and triglycerides showed dose-related increases, differences were not significantly different between groups. Maximum values for both parameters were reached by month 6. Hypercholesterolemia and hypertriglyceridemia have been reported with everolimus after cardiac and renal transplantation, as is typical for drugs of this class.<sup>8</sup> In general, levels peak after 2 to 3 months of exposure and are not progressive, similar to observations with everolimus in renal transplantation.<sup>9</sup>

There was no relationship between everolimus dosage and the composite end point of efficacy failure or its individual components (death, BPAR, graft loss). Although there was a trend towards lower rates of treated acute rejection episodes in the everolimus treatment groups relative to placebo, larger studies will need to be conducted to confirm this. A logistic regression of logarithmic everolimus exposure vs. efficacy did, however, suggest an increased efficacy with increasing everolimus exposure. Furthermore, there was evidence of a dosage-relationship for treated acute rejection, with greater percentages of patients experiencing such episodes at lower dosages of everolimus (particularly the lowest dosage of 1 mg/day). This trend remained evident throughout the double-blind study period. The mortality rate was low across the study, and no deaths were considered to be treatment related. Similar results relating to efficacy end points have been seen in recent renal transplantation studies with everolimus 1.5 mg/day and 3 mg/day over 6- and 12-month periods.<sup>26,33,34</sup> The data also strongly support data generated from studies in heart and kidney patients that an everolimus trough level of 3 ng/mL must be achieved for efficacy. In addition, although everolimus was well tolerated, there were more adverse events and a higher rate of discontinuation at high doses.<sup>25,35</sup>

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