NOVARTIS

Certican® (everolimus)

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Indication: Heart Transplantation

Briefing Book for Advisory Committee

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List of abbreviations

AE	adverse event
ALT	alanine aminotransferase/glutamic pyruvic transaminase/GPT
AR	acute rejection
AST	aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
AZA	azathioprine
BD	Breslow-Day test
b.i.d.	bis in die/twice a day
BMI	body mass index
BPAR	biopsy proven acute rejection
CAD	coronary artery disease
CAV	cardiac allograft vasculopathy
CDS	Core Data Sheet
CI	confidence interval
CMV	Cytomegalovirus
CRF	Case Report/Record Form
CRI	Chronic Renal Insufficiency
CS&E	Clinical Safety and Epidemiology
CR	Clinical Research
CRO	Contract Research Organization
CsA	Cyclosporine (Neoral®; Novartis)
ECG	Electrocardiogram
ESRD	end stage renal disease
GFR	glomerular filtration rate
HDC	hemodynamic compromise
IEC	Independent Ethics Committee
ISHLT	International Society for Heart and Lung Transplantation
ITT	intention to treat
i.v.	intravenous(ly)
IVUS	intravascular ultrasound
IRB	Institutional Review Board
KM	Kaplan-Meier analysis
LVAD	left ventricular assist device
MACE	major adverse cardiovascular events
MedDRA	medical dictionary for regulatory activities

MIT	maximal intimal thickness
MMF	mycophenolate mofetil (CellCept®; Roche)
mTOR	mammalian target of rapamycin
NNT	number needed to treat
o.d.	omnia die/once a day
p.o.	per os/by mouth/orally
PK	pharmacokinetics
PD	pharmacodynamics
PRA	panel reactive antibody
PSUR	product safety update report
RAD	Novartis designation for its development program for everolimus, used in study numbers and treatment arm designations
REB	Research Ethics Board
SAE	serious adverse event
SOP	Standard Operating Procedure
SMPC	summary of product characteristics
WHO	World Health Organization

1 Executive Summary

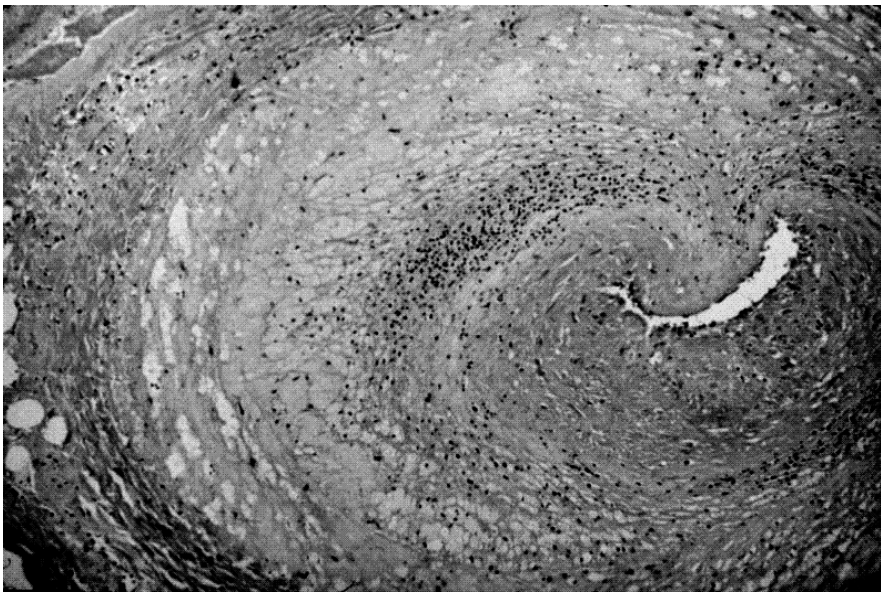
1.1 Unmet medical need

Cardiac transplantation is the established treatment of choice for eligible patients with end-stage congestive heart failure. The two thousand or so heart transplants that occur annually in the USA represent only a small fraction of patients with heart-failure, as donor organs are extremely scarce. The immediate response to transplant is often a dramatic improvement of heart failure symptoms.

Acute cellular allograft rejection (AR)is the most frequent complication following cardiac transplantation. Acute cellular allograft rejection is the immune systems attempt to destroy tissue of foreign origin and is mediated by immune cells called T- lymphocytes. The detailed pathobiology of the rejection process is reviewed in Halloran 2004. There are two agents approved for used to prevent cardiac rejection. One is the calcineurin inhibitor cyclosporine (also referred to as cyclosporin A, CsA or Neoral®). The other is mycophenolate mofetil (MMF, Cellcept®), which is approved for use as an adjunct with CsA. Refer to Halloran 2004, Table 2 for a review of the properties, uses and side effects of these agents. Without chronic lifelong prophylactic immunosuppressive medications, acute rejection would lead to the destruction of the transplanted heart within a matter of days. With lifelong immunosuppressive medications the clinician must remain constantly vigilant for the occurrence of rejection, as acute rejection requiring treatment remains a frequent event after cardiac transplantation and occurs in roughly 40-50% of patients within the first year of transplant. During the first year, patients stabilize and CsA and prednisone are decreased to levels that permit better long term tolerability. Prophylactic immunosuppression with cyclosporine (CsA), in combination with other adjunctive immunosuppressive agents has transformed acute rejection from an imminently life-threatening event to a more manageable occurrence. However, the management of acute rejection requires inpatient hospitalization, the intravenous use of potent immunosuppressive agents, with their attendant side-effects, intensive monitoring and repeated endomyocardial biopsies. Recent advances in histopathological classification of acute rejection using the International Society for Heart and Lung Transplantation (ISHLT) grading system, allow clinicians to differentiate acute rejection episodes of ISHLT≥3A that require immediate attention and treatment, and lesser severity grades, for which expectant management is appropriate. Longer-term, registry data demonstrates that acute rejection of sufficient severity to require treatment is associated with an increased risk for death; the 2005 ISHLT Registry Report indicates there is a significant increase in the relative risk for death over time among patients with a treated rejection surviving to 1 year after transplant. Other expected complications of the treatment of rejection (largely high-dose steroid and lympholytic antibody treatments) include bacterial, viral and opportunistic infection, steroid side-effects (Cushing's syndrome, diabetes, hypertension, acne, weight gain, edema, osteoporosis, glucose-intolerance, avascular necrosis of the femoral head, psychiatric symptoms) an increased risk for post-transplant lymphoproliferative disease (PTLD) and perhaps other malignancies. Therefore, reducing the frequency of acute rejection represents an important near-term clinical endpoint, with long-term implications.

Patients who survive the first year after heart transplant also face the occurrence of accelerated coronary artery disease in the transplanted heart, also known as cardiac allograft vasculopathy (CAV). Immunological and non-immunological perioperative injury to the endothelium allows subendothelial growth-factor enhanced proliferation of fibroblasts and myofibroblasts in the arterial wall, leading to luminal compromise, as shown in Figure 1-1. CAV is a frequent cause of mortality late after transplantation (Taylor 2003), and may result in myocardial impairment and the occurrence of left ventricular dysfunction. The 2005 report of the ISHLT registry indicates that among 1 year transplant survivors with the diagnosis of vasculopathy, there is a 40% increase in the risk for death after 5 years. Unlike other native coronary disease, once CAV occurs, the diffuse nature of vessel involvement limits the potential for successful revascularization. Agents that can reduce the progression of allograft vascular disease should markedly impact the ventricular dysfunction and mortality following cardiac transplantation.

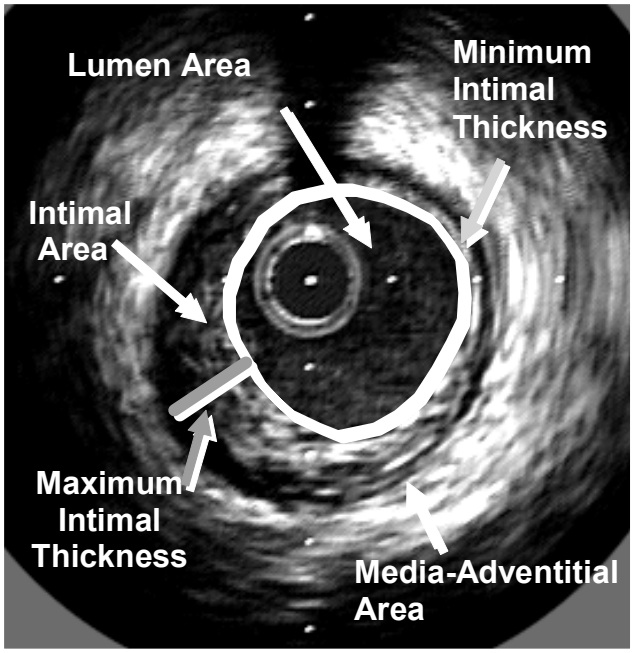
Figure 1-1 Postmortem histopathology of a coronary artery with severe cardiac allograft vasculopathy leading to luminal obliteration.



1.2 Intravascular ultrasound for the detection of CAV

The most sensitive and specific technique for the early detection of CAV is the measurement of intimal thickness by intravascular ultrasound (IVUS) (see Figure 1-2). IVUS is sensitive and specific for the detection of intimal thickness, and can be employed for longitudinal monitoring of the progression and regression of intimal lesions.

Figure 1-2 Key landmarks on IVUS



Several early studies found an association between intimal thickness post-transplant and the subsequent development of angiographic transplant coronary artery disease and mortality (Rickenbacher 1995, Mehra 1995, Kapadia 1999). More recently, a change in maximal intimal thickness of > 0.5 mm from baseline to one year post-transplant (also described as rapidly progressive vasculopathy) has become established as a prognostic measure of CAV (Kobashigawa 2005). Moreover, change in MIT above 0.5 mm in the major coronary arteries at one year portends the increased frequency of major adverse cardiovascular events and death. Overall, the rapid progression in first-year intimal thickness as detected by IVUS appears to represent the cumulative effects of adverse events that ultimately lead to poor clinical outcome. It is for this reason that recent trials and series in heart transplantation by and large employ IVUS to determine the status of intimal disease, and the reason this technique was employed in study B253.

Everolimus is an immunosuppressive agent that is used primarily to prevent acute cellular rejection of transplanted organs. In addition, everolimus and/or its parent compound sirolimus demonstrate an anti-fibroproliferative effect leading to the preservation of normal vascular architecture in preclinical atherosclerotic, mechanical and alloimmune vascular injury models. Both everolimus and sirolimus markedly diminish remodeling of angioplasty injury when employed in drug-eluting intracoronary stents. Oral sirolimus has also demonstrated activity in hypothesis-testing clinical trials in the setting of angioplasty and cardiac transplantation. On the basis of preclinical and clinical data, everolimus is a promising agent for prevention or treatment of allograft vasculopathy.

Everolimus (RAD) Transplantation Development Program

Everolimus is a chemical derivative of sirolimus developed for the suppression of organ rejection. The effectiveness of everolimus to prevent acute rejection has been demonstrated in

a pivotal trial in heart transplantation (B253) and 2 pivotal trials (B201 and B 251) for kidney transplantation. Safety concerns characteristic of everolimus are consistent across indications and include an abnormal lipid phenotype of increased cholesterol and triglyceride with normal or elevated high-density lipoprotein levels, and an increase in bacterial infections. Nephrotoxicity related to CsA exposure is worsened by everolimus exposure. The clinical profile is similar to sirolimus, which is registered worldwide only for kidney transplantation; everolimus shares with sirolimus a decrease in frequency of cytomegalovirus (CMV) infections relative to mycophenolate mofetil (MMF; CellCept®; Roche). Globally the results of these three everolimus pivotal trials, combined with data from three additional clinical trials in kidney transplantation (B156, A2306 and A2307) demonstrating everolimus with low-exposure CsA provides very good renal function and protection from acute renal rejection. These data convinced most health authorities to grant marketing authorization.

1.2.1 Efficacy of everolimus in heart transplantation

The limited number of heart transplant procedures performed worldwide (approximately 4000 vs 30-40,0000 kidney transplantation procedures) profoundly impacts the frequency and size of clinical trials in cardiac transplantation. The blinded randomized clinical trial B253 compared the safety and efficacy of 1.5 mg and 3.0 mg of everolimus versus 1.0 to 3.0 mg of azathioprine per kilogram of body weight per day, in combination with cyclosporine, corticosteroids, and statins, and represents only the 4th registration trial ever attempted in heart transplantation. Study B253 enrolled 634 primary cardiac allograft recipients. The primary analyses for efficacy were performed at months 6, 12 and 24. In addition, available data was analyzed through 48 months.

Efficacy for Acute rejection

The primary endpoint for heart study B253 was efficacy failure (a composite endpoint of the first occurrence of either acute cellular rejection of ISHLT grade ≥ 3A, or rejection associated with hemodynamic compromise, or graft loss, or death, or lost to follow-up) assessed at 6 months, with subsequent evaluations at 12 and 24 months. The results of the primary analysis are provided in Table 1-1.

Table 1-1 Study B253- Primary analysis results for efficacy at 6 months

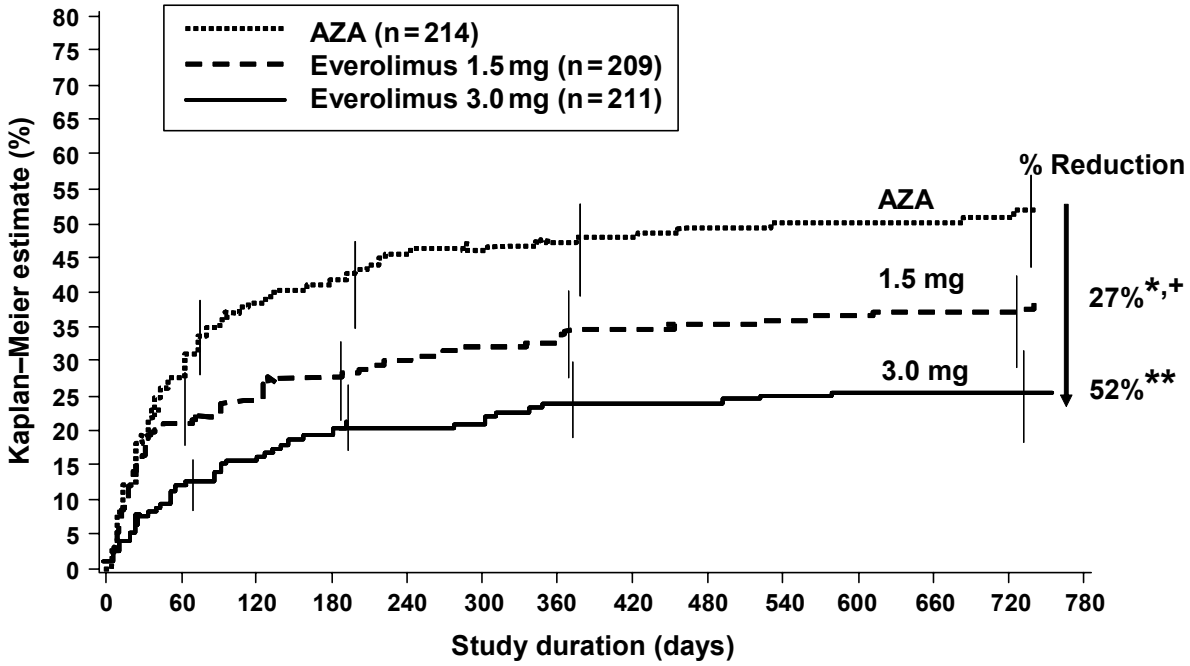
	Patients	Efficacy failure events	% with an event	RR*	P*
AZA	214	100	46.7	–	–
Everolimus 1.5	209	76	36.3	.778	0.031
Everolimus 3.0	211	57	27.0	.578	<.001

* Comparison to AZA

This study met its prospectively defined primary efficacy endpoint for superiority for everolimus of significantly reducing the incidence of efficacy failure. In addition, the components of efficacy failure were compared individually. This beneficial effect on efficacy failure was driven by a significant effect on the prevention of acute rejections, shown in

Figure 1-3. The reduction in acute rejection events was maintained years after the 6 month primary endpoint, indicating rejection episodes were prevented and not merely delayed.

Figure 1-3 Incidence of biopsy proven ISHLT grade ≥ 3 A acute cardiac allograft rejection by treatment arm in Study B253

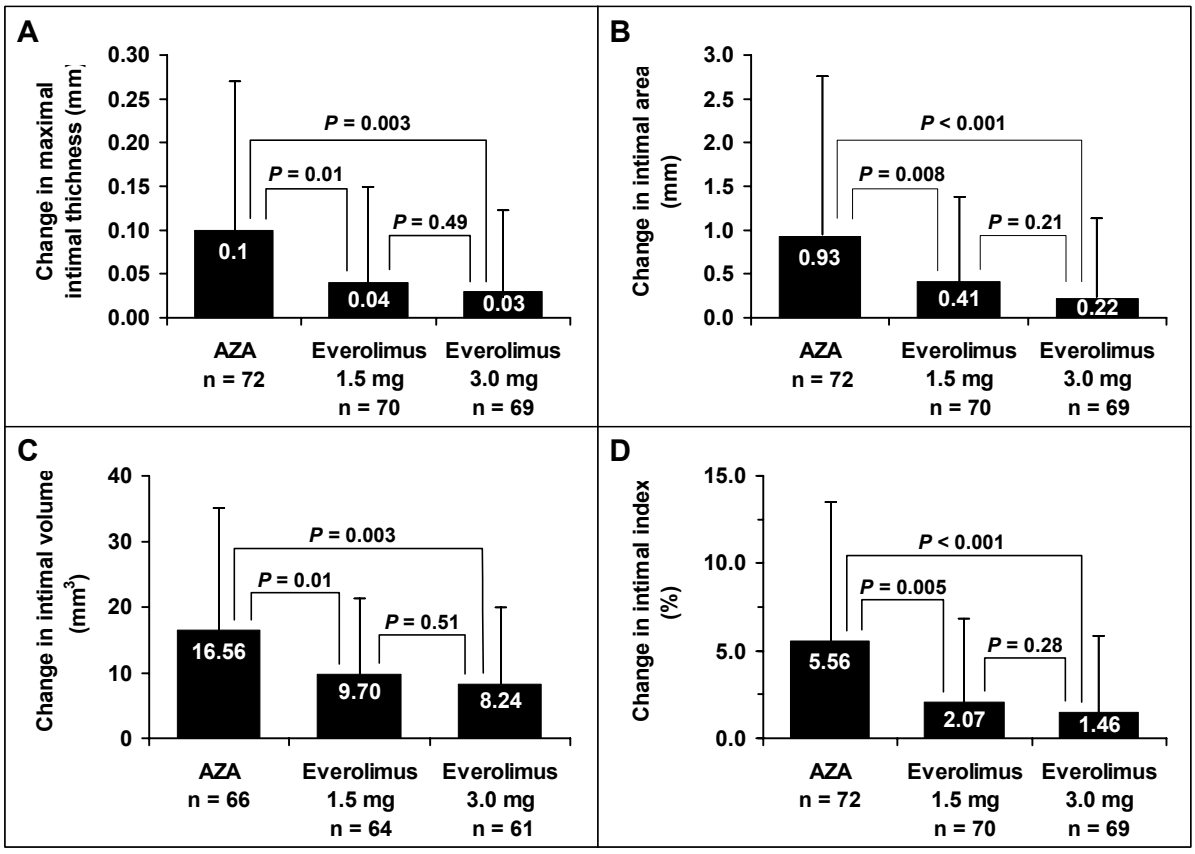


*P = 0.005 vs AZA; **P < 0.001 vs AZA; +P = 0.005 vs everolimus 3.0 mg

Efficacy of everolimus on CAV as measured by IVUS

In addition to quantifying the effect of everolimus on acute rejection, an assessment of the effect of everolimus on allograft vasculopathy was performed using intravascular ultrasound (IVUS) a state of the art methodology for longitudinal evaluation. Despite the known difficulties of performing longitudinal IVUS assessment in the clinically complex cardiac transplant population in the setting of a blinded global multi-center trial, planned comparison of more than 200 patients using intravascular ultrasound (IVUS) was performed at baseline (within 6 weeks of transplant) and at 1 year. Everolimus significantly reduced all measures of intimal thickness 1 year after transplantation as shown in Figure 1-4.

Figure 1-4 **Mean (±SD) Change in Maximal Intimal Thickness (Panel A), Intimal Area (Panel B), Intimal Volume (Panel C), and Intimal Index (Panel D) from Base Line to 12 Months.**



Eisen 2005

This study represents the largest data set to date providing evidence that any pharmacological agent, in man, can significantly blunt the progression of cardiac allograft vasculopathy.

- Per protocol, IVUS examinations were to be performed both at baseline and 12 months in patients that were still on study medication. Ultimately, 70 of the 209 patients (33%) in the 1.5 mg everolimus arm, 69 of the 211 patients (33%) in the 3mg everolimus arm and 72 of the 214 patients (34%) in the AZA arm, had paired baseline and 1-year IVUS tapes that were technically adequate (including at least 11 site matched-slices) for interpretation.
- The strength of this adequately sized, blinded (clinicians, patients, and IVUS reviewers) assessment of treatment effect must be balanced against certain methodological limitations regarding how the IVUS data was obtained. Many patients who did not have matching IVUS studies were excluded by the investigator for clinical reasons such as renal dysfunction or recent rejection episodes, which might make an IVUS procedure unsafe. In other instances patients were excluded for technical reasons not subject to bias. Therefore the potential for bias exists, though biases favoring everolimus were not detected. Specifically, the potential influence of renal function on the selection of patients for performance of IVUS was not confirmed as there was no evidence of higher creatinine clearance among everolimus-treated patients undergoing IVUS. It is interesting to note

that similar numbers of patients in each group had IVUS performed, despite differences in renal function. Consistent with prior studies (Kobashigawa, Starling 2003), logistic regression analysis demonstrated creatinine clearance was not associated with the development of intimal thickness or CAV measured by IVUS (NDA data on file).

1.2.2 Safety of everolimus in heart transplantation

The overall safety profile was similar between the everolimus 1.5 mg and AZA treated patients, with the notable exceptions of more frequent reports of adverse events related to increased lipids or renal impairment in the everolimus treatment groups. Adverse event data demonstrated a dose-related increase in adverse events in everolimus-treated patients. Also notable were:

- Graft and patient survival rate was excellent in all treatment groups, with no significant between-group differences.
- The incidence of nonfatal SAEs and discontinuations of study medication due to AEs was significantly higher in the everolimus 3 mg group compared with the other groups.
- Overall infection rates were comparable between treatment groups in study B253. However, there was a statistically significantly lower incidence rate of viral infections (in particular cytomegalovirus infections) in the everolimus groups than in the AZA group. The incidence of bacterial infections (particularly pneumonia) was significantly higher in the everolimus 3 mg group compared to the AZA group.
- The rate of malignancies was similar across all treatment groups: 8%. 7% and 5% in the everolimus 1.5 and 3mg groups and the AZA group, respectively.
- No changes in adverse event profile were seen in the 24 month analyses.

1.2.2.1 Laboratory abnormalities

- Renal function data: Mean serum creatinine was significantly higher and creatinine clearance significantly lower in both everolimus groups as compared to the AZA group. Patients in the everolimus arms had stable renal function in follow-up between the Month 12 and Month 24 visits. Cox regression analysis showed a strong effect of CsA exposure ($p=0.0004$) on renal events but no effect of everolimus exposure ($p=0.275$). Analyses of CsA reduction during the blinded first 12 month study period showed declining creatinine clearance was reduced in proportion to CsA dose reduction.
- Potentially clinically meaningful decreases in hemoglobin and platelet counts occurred more frequently in the everolimus-treated patients than in the AZA group. Conversely, leukopenia was more frequent in the AZA group.
- Elevations of serum lipids were seen in both everolimus groups, but mean LDL and HDL cholesterol were similar between the everolimus groups and AZA. There was no evidence of a negative clinical impact of lipid elevations during the treatment period. Graft-related major adverse cardiovascular events (MACE) were reduced in patients treated with everolimus followed through 48 months, with no increase in non-graft-related major adverse cardiovascular events.

1.2.2.2 Adverse Events

- Overall, adverse events were more frequent and the discontinuation rate was higher in the group receiving everolimus 3 mg/day than in the everolimus 1.5 mg/day group. This suggests that the 3 mg/day dose was associated with worse tolerability than the 1.5 mg/day dose, despite a demonstration of better efficacy.

1.3 Exposure effect relationships of everolimus and CsA

Exposure-effect relationships for everolimus and CsA in heart transplantation were similar to those demonstrated in adequate and well-controlled pivotal kidney trials B201 (n=588) and B251 (n=584) in renal transplantation. These analyses support that, in the context of adequate exposure to everolimus, increased CsA exposure increases the risk for renal dysfunction (as measured by a decreased creatinine clearance), but does not affect the risk for efficacy failure. That is to say, among patients receiving everolimus in either the heart or kidney transplantation trials, rejection in everolimus-treated patients was lower than comparator and not affected by CsA exposure. Higher CsA exposure increased the probability of renal dysfunction in the everolimus exposed patients. The exposure-effect relationships demonstrated in the pivotal kidney studies were prospectively tested in recent trials in kidney transplantation (A2306 and A2307). Results with reduced CsA in these recent trials were consistent with those predicted by the exposure-response models; prospective lowering of CsA is associated with a better renal safety profile. This provides adequate assurance that reduced CsA exposure in heart transplantation patients treated with everolimus will be accompanied by reduced renal dysfunction while maintaining efficacy.

1.4 Therapeutic drug monitoring

Cardiac transplant patients are a complex patient population to manage. Rarely is heart failure the sole medical condition which clinicians must contend at the time of transplant. As reviewed by Halloran 2004, drugs used to prevent acute rejection have frequent and major toxicities which the clinician must manage in order to keep patients alive and free of rejection. Adjustments to therapy, particularly the dosing of calcineurin inhibitors CsA and tacrolimus, are frequent and routine. Sometimes these adjustments are needed to palliate intercurrent illness (infection, malignancy). Adjustments to the dosing of calcineurin inhibitors are needed to manage nephrotoxicity, hyperlipidemia and diabetes. Sometimes adjustments are made to compensate for the withdrawal of adjunctive agents (Cellcept® or AZA) due to the GI effects, neutropenia, and cytomegalovirus infection. In addition, dose adjustments must be made to allow the use of calcium channel blockers, antibiotics and antifungals, to compensate for the effect these drugs have on the clearance of calcineurin inhibitors. Most commonly, these dose adjustments are made in order to raise or decrease a measured pre-dose whole-blood trough concentration. This practice is referred to as therapeutic drug monitoring.

Based upon the exposure-effect relationships characteristic of everolimus and CsA when coadministered, it would be advisable to monitor the trough concentrations of both agents. Specifically, to prevent acute rejection it would be desirable to alter dosing to achieve an everolimus trough concentration above 3 ng/ml. Once the everolimus trough concentration had been stabilized at or above this level, and the risk for rejection minimized, the clinician can then alter CsA exposure to prevent or reduce nephrotoxicity.

1.5 Summary

Everolimus, at doses of 1.5 mg/day and 3.0 mg/day, met is primary endpoint for superior efficacy at 6 months, and at all subsequent evaluations. The unequivocal demonstration of the superior effect of everolimus to prevent the occurrence of acute rejection in an ITT analysis of an adequate and well controlled trial is unique among immunosuppressive agents. The association of everolimus treatment with delayed progression of cardiac allograft vasculopathy (CAV) has not hitherto been demonstrated for any agent as part of a large clinical trial experience. Benefits of reduced cytomegalovirus and major adverse cardiovascular events are additional important considerations in favor of RAD. The safety concern of an enhancement of CsA nephrotoxicity is an understood and expected outcome of a blinded trial performed prior to the appreciation of the potential for interaction of everolimus with CsA. PK/PD analyses support that CsA exposure has a primary role in this toxicity and suggests flexible CsA-dosing can be undertaken to palliate nephrotoxicity without significant risk of precipitating acute rejection. Other safety events, such as the observed increase in bacterial infections and dyslipidemia, are well understood with this class of agents. These events have not been demonstrated, in heart study B253 or kidney studies B201 and B251, to influence mortality. In addition, there exists drugs to address hypercholesterolemia and infections.

Everolimus is the first adjunctive agent to demonstrate superiority to reduce acute ISHLT grade $\geq$ 3A heart transplant rejection in an adequate and well-controlled study. Acute rejection has a number of associated adverse sequelae. Preventing rejection might benefit patients by reducing the frequency of rejection-associated co-morbidities and improve long term outcomes. A second line of evidence, that of the reduction in allograft vasculopathy, also suggests another avenue for everolimus to improve the longer-term prognosis of patients after heart transplantation. The improvement in the natural history of heart transplantation must be balanced against the increase in nephrotoxicity with the use of routine CsA exposure and everolimus in the blinded trial setting. The PK/PD analysis performed demonstrates that far more flexibility can be accorded clinicians dosing CsA than was mandated in the protocol. How low CsA can be reduced without precipitating rejection has not been definitively determined. Nevertheless, with flexible management of CsA dosing toward the lowest quartile of exposures seen in the heart B253 trial (as is recommended in the European product labeling) unnecessarily high CsA exposure can be avoided, and the risk for nephrotoxicity reduced. Improved renal performance is being achieved in current cohorts of patients dosed with everolimus in clinical practice (Lehmkuhl, unpublished data). So long as risks are appropriately anticipated and recognized, the clinical utility of everolimus can be maximized beyond that which was demonstrated in the trial. The potential prescribers of everolimus are a small cadre of expert clinicians treating heart transplant recipients, who understand the relevant drug interactions and the potential for renal impairment. The achievement of good clinical outcomes will further be aided by educating this small clinical community about the potential for benefit and risk with the use everolimus. Overall, the available data is considered adequate to support the use of everolimus in the US, as it is labeled and used in many other countries.

2 Background on Certican (proposed trademark)

2.1 Regulatory History and Chronology

Specific Food and Drug Administration (FDA) guidelines do not exist for the development of immunosuppressive drugs for the indication of transplantation. However, Novartis has discussed the clinical development and registration requirements for the approval of everolimus, for use in renal and heart transplantation with the Health Authorities, especially the FDA Division of Special Pathogen and Immunologic Drug Products/HFD-590. In the design and management of the clinical program for registration, Novartis met with the FDA face-face and/or discussed in teleconference and attempted to incorporate FDA input into the design of the core studies and other supporting studies in order to provide adequate information for marketing approval. Novartis also provided the safety results from ongoing clinical results to a Data Safety Monitoring Board (DSMB) throughout the core study program. Everolimus has received an approvable NDA status by the FDA whereby the drug is efficacious in both heart and kidney transplantation, however, the FDA has requested additional information to support safe dose recommendations for the combination use of everolimus and cyclosporine.

The following is a brief chronology of the current NDA:

- NDA submissions (kidney and heart) Dec 2002
- Approvable letter #1 for kidney and heart indications Oct 2003
- Major NDA Amendment 1 (“complete response”) Feb 2004
- Approvable letter #2 for kidney and heart indications Aug 2004
- End-of-Review meeting (face-to-face) Nov 2004
- Amendment to support advisory committee meeting for heart transplant only Mar 2005
- New clinical protocols submitted to IND (kidney and heart) Jun, Sept 2005

2.1.1 Global Registration Status

Approval for everolimus has been obtained in a total of 48 countries throughout Europe, Australia, South Africa, the Middle East, Central and South America, the Caribbean and Asia. As of April 2005 there is commercial use in 24 countries. Additional information is located in Section 9 Global Registration status of Certican (everolimus).

2.2 Clinical Development Program

The clinical development program for everolimus was the most comprehensive in transplantation. The global registration (NDA) program was developed to obtain health authority approval for use of everolimus in kidney and heart transplantation. There were a total of 37 trials with 2900 patients exposed to everolimus. The core NDA studies included one (1) pivotal phase 3 study in heart transplantation and seven (7) studies in kidney transplantation in which there were over 1800 patients exposed to everolimus with 220 investigators worldwide. In addition, there were seventeen (17) clinical pharmacology and/or short term clinical studies. See Section 9 for additional information on the full development

program for everolimus in transplantation. See Table 2-1 below for a summary of the core clinical studies.

Table 2-1 Core studies (n=2532 total patients; n=1814 exposed to everolimus)

Organ Type	Study	Description
Heart *de novo* (n = 634)	B253	6, 12, 24, and partial data at 48 months
Kidney *de novo* (n = 1275)	B201, B251, B157	6, 12, and 36 months
(n = 19)	B351	Pediatric Tx; 12 and 24 months
Kidney *de novo* (n= 111)	B156	Reduced-dose Neoral plus Simulect 12 and 36 months
Kidney *de novo* (n = 237)	A2306	Reduced-dose Neoral 6 and 12 months
Kidney *de novo* (n = 256)	A2307	Reduced-dose Neoral plus Simulect 6 and 12 months

Heart transplantation

The clinical efficacy and safety of the everolimus tablet formulation in heart transplantation was evaluated in one double-blind, randomized trial (B253). The NDA included phase 3 data for n=634 de novo heart transplant patients from core study B253 at 6, 12 and 24 months, and partial data at 48 months. Approximately n=420 patients received everolimus and n=210 patients received active comparator (azathioprine).

Kidney transplantation

The primary studies demonstrating clinical efficacy and safety of the everolimus tablet were four renal transplant studies: in two phase 3 double-blind trials (B201, B251) using fixed dose everolimus with standard doses of Neoral (cyclosporine USP) MODIFIED, and, in two open label trials (A2306, A2307) using therapeutic drug monitoring for everolimus with reduced doses of Neoral.

Synopses of pivotal heart (B253), and kidney (B201, B251 using full dose cyclosporine) studies, and studies with reduced dose cyclosporine, (A2306 and A2307) are located in Section 9.3.

3 Heart Transplantation

3.1 Background- Unmet medical need following cardiac transplantation.

Cardiac transplantation has become the established treatment of choice for eligible patients with end-stage congestive heart failure, however, it is only offered to few patients because:

- There is a limited availability of donor organs.
- In cardiac transplantation graft loss equals death (unless a rare re-transplant is done).

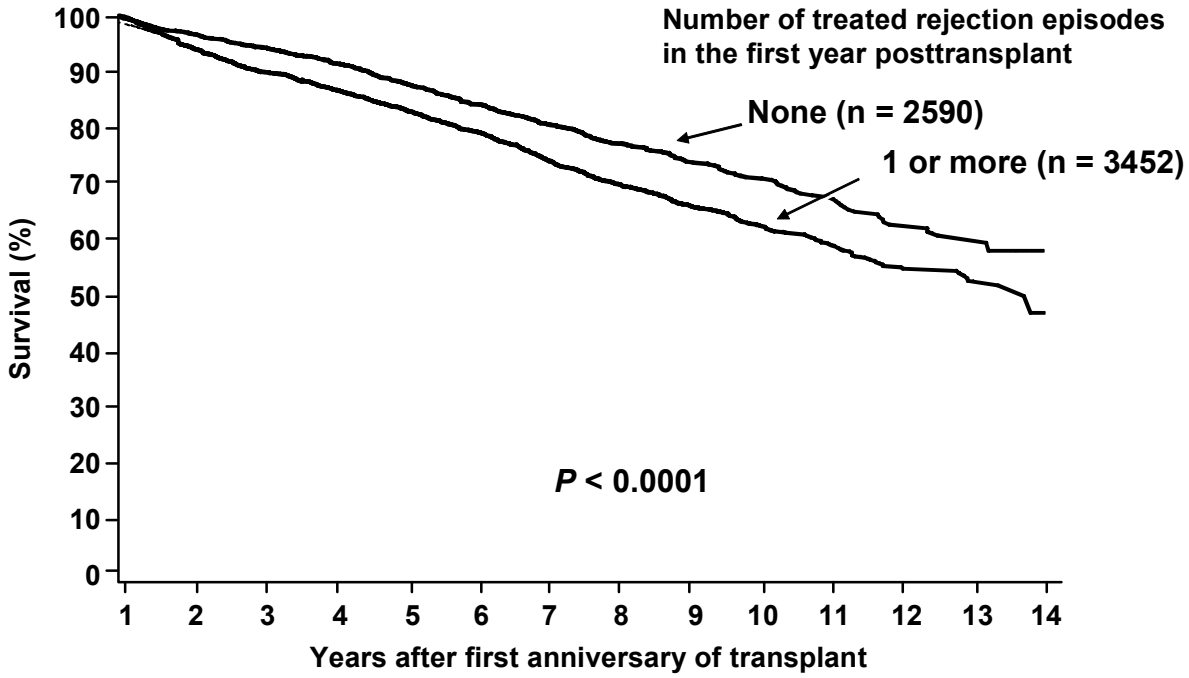
- Survival outcomes in cardiac transplantation are inferior to those in kidney and liver transplantation (UNOS 2003).

3.1.1 Acute rejection

Organ rejection is the immune system's attempt to destroy allogeneic tissue and is most commonly mediated by immune cells called T- lymphocytes, a clinical event with histopathological correlates termed acute cellular rejection (AR). Without the lifelong administration of immunosuppressive drugs following a cardiac transplant, the allogeneic heart would succumb to assault by the recipient immune system in a matter of days or weeks. There are two agents approved for used to prevent cardiac rejection. One is the calcineurin inhibitor cyclosporine (also referred to as cyclosporin A, CsA or Neoral®). The other is mycophenolate mofetil (MMF, Cellcept®), which is approved as an adjunct in heart transplantation for use with CsA. Refer to Halloran 2004, Table 2 for a review of the properties, uses and side effects of these agents. Even in the setting of chronic prophylaxis with these agents, episodes of acute rejection requiring treatment remain a frequent event after cardiac transplantation. Rejection episodes occur in roughly 40-50% of patients treated with CsA and either MMF or azathioprine within the first year of transplant. During the first year patients stabilize, and CsA and prednisone doses are gradually decreased to levels that permit better long term tolerability. The use of chronic prophylactic immunosuppression with CsA and adjunctive agents has transformed acute rejection from an imminently life-threatening event to a manageable event. Recent advances in histopathological classification of acute rejection using the International Society for Heart and Lung Transplantation (ISHLT) grading system, allow clinicians to differentiate acute rejection episodes of ISHLT≥3A that require immediate attention and treatment, and lesser severity grades, for which expectant management is appropriate. Uncomplicated acute rejection may engender little immediate risk to patients, yet its treatment is not without complications and longer-term complications of acute rejection may limit patient and graft survival. The patient with acute rejection often requires hospitalization and intensive monitoring. Other expected complications of the treatment of rejection (largely high-dose steroid and lympholytic antibody treatments) are bacterial, viral and opportunistic infection, steroid side-effects (Cushing's syndrome, diabetes, hypertension, acne, weight gain, edema, osteoporosis, glucose-intolerance, avascular necrosis of the femoral head, psychiatric symptoms), an increased risk for post-transplant lymphoproliferative disease (PTLD) and perhaps other malignancies.

The deleterious effects of rejection and its treatment can be observed in the longer term as well. Registry data demonstrates the association of acute rejection of sufficient severity to require treatment with an increased risk for death. Figure 3-1 is an analysis by the Cardiac Transplantation Research Database (CTRD) and demonstrates the correlation between treated rejection and worsened survival. A similar prognosis for patients with treated acute rejection is noted in the 2005 ISHLT Registry Report. The analyses of this dataset indicates that rejection is associated with a significant increase in the relative risk for death after 5 years.

Figure 3-1 Association of Acute Rejection and Survival. CTRD 1990 – 2003



3.1.2 Cardiac Allograft Vasculopathy (CAV)

Coronary artery disease in the transplanted heart, also known as cardiac allograft vasculopathy (CAV), is a major cause of mortality late after transplantation (Taylor 2003). It affects up to 80% of all heart transplant recipients within 5 years of surgery. Immunological and non-immunological perioperative injury to the endothelium allows subendothelial growth-factor enhanced proliferation of fibroblast and myofibroblasts in the arterial wall, leading to luminal compromise, as shown in Figure 3-1. The transplanted heart is denervated, and for many patients, the first presentation of compromised coronary circulation due to allograft vasculopathy is sudden death. The 2005 report of the ISHLT registry indicates that among 1 year transplant survivors with the diagnosis of vasculopathy, there is a 40% increase in the risk for death after 5 years. For other patients CAV may result in myocardial impairment and symptoms of left ventricular dysfunction, leading to congestive heart failure and an impaired quality of life.

The diffuse nature of vessel involvement limits the potential for successful revascularization, hence the emphasis on developing pharmaceutical agents to prevent progression of deleterious vascular remodeling, rather than treatments following its occurrence. Unfortunately there is no established treatment for CAV. Re-transplantation is uncommonly performed due to the limited organ supply as well as the poor outcomes seen with re-transplantation.

3.1.3 Therapeutic Options

Currently approved therapies for the prophylaxis of acute cellular rejection in heart transplant recipients are limited to CsA and MMF, which are administered together. About 50% of

patients receive unapproved agents or combinations (tacrolimus, azathioprine, sirolimus) of immunosuppressive agents, as many patients cannot be optimally managed using only CsA and MMF. An effect on IVUS-defined CAV has never been prospectively demonstrated for currently approved agents.

3.2 Clinical Study B253

3.2.1 Introduction

Study B253 was a randomized placebo controlled double-blind study to define the efficacy and safety of everolimus administered as part of a regimen of CsA and steroids. The primary efficacy objective was to demonstrate superiority vs. an AZA comparator. As part of an adequate and well-controlled safety and efficacy trial for registration of everolimus (B253), a prospectively planned study of allograft vasculopathy was performed using state of the art methodology and longitudinal evaluation. The everolimus B253 study provides the most robust evidence to date that an orally administered mTOR inhibitor can both prevent acute cellular rejection and significantly blunt the progression of cardiac allograft vasculopathy, the greatest unmet need in cardiac transplantation. These two outcomes should come as no surprise, as these results are consistent with the hypothesis-generating clinical and preclinical material discussed earlier. That is, both everolimus and the related agent sirolimus are active in preclinical rejection, atherosclerotic, mechanical and alloimmune vascular injury models as well as in the clinical setting.

3.2.2 Design

RAD study B253 was designed to assess the safety and efficacy of two doses of everolimus compared to azathioprine in *de novo* heart transplant recipients as measured by the incidence of a composite endpoint of death, graft loss/re-transplant, biopsy proven acute rejection episode ISHLT $\geq$ Grade 3A or any clinically suspected acute rejection episode associated with hemodynamic compromise in the first 6 months post-transplant. In addition, a prospective IVUS substudy was designed to evaluate the impact of chronic everolimus administration on measures of cardiac allograft vasculopathy.

Study B253 was designed as a two-year prospective, multi-center, randomized, double-blind, double-dummy, active controlled, safety and efficacy study of 634 *de novo* heart transplant recipients among 55 centers in North America, South Africa, Australia and Europe. Three hundred and thirty eight of the patients were enrolled in 29 North American centers. Patients were randomized to one of three treatment groups (209, 211, and 214 in the everolimus 1.5 and 3.0 mg groups and the AZA group, respectively) as part of a triple immunosuppressive therapy utilizing Neoral and steroids. The study was subsequently unblinded after all patients completed 12 months to evaluate renal impairment in some patients treated with the CsA and everolimus combination.

3.2.3 Dose Selection and Rationale

The selection of everolimus doses for clinical studies was initially based upon *in vitro* and *in vivo* primate data for efficacy. Further selection of the two doses of everolimus tested in phase 3 were based on the safety, tolerability, and pharmacokinetic results from kidney transplantation Studies RADB 154 and RADW 102. The anticipated everolimus drug

exposures were similar to the exposures reported for sirolimus in pivotal trials in renal transplantation.

Tapering CsA dosing was guided by exposure ranges. The exposures chosen for the study reflected a consensus across the investigators regarding ranges that would both accommodate their clinical practices, as well as provide an adequate degree of immunosuppression to patients randomized to the AZA comparator group. In accordance to standard practice, the highest exposures to CsA occurred in the month following transplantation, with subsequent reductions over time. Thus, the regimens tested all had a tapering exposure to CsA, administered with either everolimus, administered at 1.5 mg/day or 3.0 mg/day in divided doses, or AZA.

3.2.4 Choice of Comparator

Azathioprine was chosen as the comparator for this trial as it was then the most prevalent standard of care at the time.

3.2.5 Patient Population

Main inclusion criteria:

- Male and female patients,
- 16-65 years of age in North America and 18-65 years of age in Europe
- scheduled to undergo their first orthotopic heart transplantation,

Main exclusion criteria:

- Evidence of active infection at the time of transplant
- Use of experimental agents at the time of transplant

3.2.6 Efficacy evaluation

All efficacy analyses were conducted on data from the ITT population and included data obtained after discontinuation of study medication. The ITT population consisted of all randomized patients.

Evaluation of primary efficacy endpoint

The primary efficacy endpoint was the composite endpoint of efficacy failure (defined as acute rejection of ISHLT grade ≥3A, acute rejection associated with HDC, graft loss, death or lost to follow-up) evaluated at 6 months. The hypothesis tested was that either everolimus arm was superior to AZA. To account for multiplicity of testing, the multiple comparisons of everolimus with AZA were made using the Bonferroni and modified Bonferroni (Hochberg) procedures to maintain the overall type I error rate of 0.05.

The primary efficacy endpoint was evaluated using Z-test statistics. Based on these results, 2-sided $100(1-\alpha')\%$ confidence intervals for the difference (RAD – AZA) in rate of efficacy failure was constructed, where $\alpha' = 0.05$ and 0.025. As supplementary analyses, the Kaplan-

Meier (KM) estimates of the probability of experiencing efficacy failure were compared using the Log-rank test to take into account the time to an event.

Evaluation of secondary efficacy endpoints

Secondary efficacy variables were assessed in a similar way as the primary efficacy endpoint. Secondary efficacy variables included:

- Efficacy failure within 12 and 24 months, as well as its single components at Month 6, 12 And 24
- Treated acute rejections at 6, 12 and 24 months
- Graft loss or death at 12 and 24 months
- Antibody treated acute rejections at 6.12 and 24 months
- Chronic rejection at 12 and 24 months (chronic rejection refers to vasculopathy demonstrated by IVUS).

3.2.6.1 Intravascular Ultrasound (IVUS)

Methods and protocol specified analyses

To assess coronary artery intimal proliferation in study RAD B253, intravascular ultrasound imaging (IVUS) was performed during the first 6 weeks post-transplant (Baseline) and at Months 12 and 24 post-transplant. IVUS was performed using an automated, mechanical pullback through the left anterior descending artery (LAD) at a rate of 0.5mm per second with up to 40 slices. The vessel of primary interest for this study was the left anterior descending coronary artery (LAD). If the LAD could not be interrogated for technical reasons, the next vessel that would be examined would be the left circumflex artery. If either vessel could not be studied, the final choice was the right coronary artery. Following the first IVUS study, if the vessel which was interrogated at the previous assessment was not accessible, then IVUS imaging was to be discontinued in this patient, and a tape was not submitted to the IVUS core laboratory for interpretation**.** Submitted tapes of the IVUS studies were evaluated by a central laboratory at the Cleveland Clinic. The reviewers were blind to treatment assignment. The test parameters to be measured were: minimum/maximum diameter and circumference of lumen and vessel and minimum/maximum intimal thickness.

The population used for the 12-month IVUS analysis was a subset of the ITT population who had 2 matched full studies. Matching in the full study required a minimum of 11 matched images from the baseline to the 12-month visit .

The primary IVUS efficacy variable was the change in average maximum intimal thickness (MIT) from Baseline to Month 12. Comparisons between treatment groups were made by means of 3 pairwise Wilcoxon rank sum tests. The main secondary IVUS efficacy variable was the incidence of allograft vasculopathy (chronic rejection), which was defined as at least 0.5mm increase from baseline in MIT in at least one slice of an automated pullback sequence. The incidence of allograft vasculopathy was compared between treatment groups by performing 3 pairwise Fisher's exact tests.

There were limited data available upon which to base sample size assumptions for IVUS. A minimum number of 78 patients per treatment arm with evaluable IVUS images both at Baseline and Month 12 was needed, to detect, with a power of 80% at significance level of 2.5% (two-sided t-test), a between treatment group difference of 0.2 mm in the primary IVUS parameter (change in average maximum intimal thickness from Baseline at Month 12), assuming a (common) standard deviation of the primary IVUS parameter of 0.4 mm.

Eligibility criteria for IVUS

All patients enrolled in centers performing IVUS were eligible to participate in the study. If severe vasculopathy developed during the course of this study the operator was encouraged to complete IVUS imaging unless this procedure would pose a significant risk to the patient. Vessels of a diameter <2.0 mm were generally agreed to be too small to allow safe imaging.

3.2.7 Safety evaluation

Safety variables included adverse events, serious adverse events, infections, electrocardiogram, safety laboratory tests (hematology, urinalysis, biochemistry, endocrinology) and vital signs. Safety variables were analyzed in the safety population by descriptive statistics, and pairwise between-group comparisons were made using the Wilcoxon rank sum test or Fisher's exact test. The safety population consisted of all randomized patients who received at least one dose of study medication and have at least one safety/tolerability assessment.

3.2.8 Pharmacokinetic evaluations

Serial steady-state everolimus and cyclosporine trough concentrations were obtained at each scheduled visit over the first 12 months post-transplant. There were a total of 2,704 everolimus trough blood levels in 410 patients and 3,892 cyclosporine troughs from 602 patients (399 receiving everolimus and 203 receiving azathioprine).

3.2.9 Statistical methods

3.2.9.1 Rational for selecting efficacy variables

The composite endpoint of acute rejection of ISHLT grade ≥3A, acute rejection associated with HDC, graft loss, death or lost to follow-up was proposed in Study B253 and accepted by FDA for the following reasons:

- Lower grades of acute rejection often have a clinically benign course, and are observed for progression rather than treated.
- Grade 3A may progress to hemodynamic compromise or graft loss if untreated. There is consensus among transplant physicians that this degree of rejection requires treatment.

3.2.9.2 Sample size and power calculations

The sample size estimate of 210 per treatment arm was planned based upon:

- Type I error rate of 2.5%, two sided comparison.

- Efficacy failure rate: 30% for Certican and 45% for AZA
- 80% power in claiming superiority of Certican over AZA
- Calculation based on Fisher's exact test (implemented by nQuery).

3.2.10 Exploratory Analyses for efficacy and safety relationships

3.2.10.1 Exposure-efficacy relationships

The exposure-efficacy relationship was assessed for heart transplant study B253 using the Cox proportional hazard regression model to relate both everolimus and CsA trough levels (calculated as average C_{min} up to event) to the risk of biopsy-proven acute rejections (BPAR). The Cox proportional hazard regression model was also used to derive appropriate dosing recommendations for CsA, which is dosed primarily to achieve defined trough level concentrations, as was performed in RAD study B253. After establishing a strong relationship based on the Cox model, median-effect analysis was applied to hypothesize on the magnitude of the minimal effective concentration. The robustness of this estimate was assessed exploring the observed incidence rates within incremental 1 ng/ml trough level ranges, and further using a hazard ratio analysis on BPAR vs. a single steady-state C_{min} value at Day 7.

The effect of everolimus exposure on BPAR was further explored for subgroups of patients.

3.2.10.2 Exposure-safety relationships

Exposure safety relationships were examined to refine safe dosing recommendations for CsA, which is primarily dosed according to trough concentration. In addition, the relationship between everolimus exposure and various safety parameters was analyzed similarly (using the Cox proportional hazard regression model and median-effect analysis) to explore the upper and lower range of everolimus trough levels for heart transplantation. It is known from the renal and heart transplant study reports that subjects with fixed-dosing of 1.5 mg/day overall had better safety than those on the 3.0 mg/day everolimus arm. As most of the patients on the 1.5 mg/day everolimus regimen had average trough levels below 8 ng/mL, the patients with average trough levels "3-8 ng/mL" (defined as $\geq$ 3 ng/mL and < 8 ng/mL) were analyzed.

In order to explore the relationship between drug exposure and renal function, Cox regression was used to model the renal events defined as the first occurrence of a creatinine value $\geq$ 200µmol/L (2.2mg/dL). The data of the first month (day 30) were not included to reduce the confounding effects of postoperative acute tubular necrosis and prerenal azotemia on the assessment of renal function.

The two factors describing exposure (log-transformed) were included: the geometric means of everolimus and CsA C_{min} until the first occurrence of a creatinine value greater than 200µmol/L, or else censored on Day 225. The creatinine value at day 30 was used as a covariate. Creatinine clearance was another variable explored in this model.

In an attempt to determine the upper range for various safety parameters, a median-effect analysis of everolimus C_{min} vs safety events up to Day 450 was done, using the "notable" criteria for hypercholesterolemia ($\geq$6.2 mmol/L), hypertriglyceridemia ($\geq$5.6 mmol/L), leukocytopenia ($\leq 2.8 \times 10^9$/L), and thrombocytopenia ($\leq 75 \times 10^9$/L), endocrine parameters and infections.

3.2.10.3 Additional exploratory exposure-outcome analyses

Additional exposure-outcome analyses were submitted to FDA in subsequent NDA updates. In addition a longitudinal mixed-effect modeling of CsA reduction on renal improvement was performed.

3.2.11 Exploratory analysis of IVUS data for bias and sensitivity

Study B253 demonstrated superiority of everolimus over AZA for the primary efficacy endpoint. In addition, everolimus patients studied by IVUS met predefined endpoints indicative of graft vasculopathy at a lower rate than AZA patients. The magnitude of the differences between everolimus and AZA are larger than previously demonstrated with other agents in clinical trials. These data were based on N=211 patients who had evaluable IVUS data matched at baseline and at 12 months. However, the data collection could be potentially affected by methodological issues including study center compliance, catheter recall, unevaluable tapes, prospective design as an on-therapy evaluation and the selective exclusion of patients (by blinded investigators) due to the fact that the intravenous contrast used in the IVUS procedure might be poorly tolerated by some patients. These and other reasons led to matched baseline and 12 months IVUS data being available in 1/3 of the ITT population. One therefore cannot definitively state that the 12 month IVUS population was representative of the original study population. These issues could confound the interpretation of IVUS results demonstrated in the otherwise adequate well-controlled blinded phase 3 heart study B253. Therefore the effect of the following additional aspects of the IVUS data were investigated to explore the potential impact of bias upon the interpretation of the IVUS results:

- Distributions of patient disposition and patterns of the reasons for IVUS data loss among the treatment groups.
- Comparability of the 12-month IVUS population to the original study population as well as the population without IVUS.
- The balance of the three treatment groups in the 12-month IVUS population with respect to baseline characteristics and post-baseline measurements (i.e. adverse events/infections, etc.) and with respect to anything that might lead to differential loss of IVUS patients.
- Impact of treatment-related outcomes (i.e. efficacy failure, renal function, etc.) on selecting the 12-month IVUS population to check for potential patient selection bias.
- Robustness of the IVUS treatment difference to alternative assumptions and imputations for data loss.
- These additional IVUS data analyses were submitted in March 2005 in the NDA Amendment.

4 Results of Study B253

4.1 Efficacy Results

4.1.1 Demographic and Baseline Disease Characteristics

Comparisons across the three treatment arms are presented in Table 4-1 and show demographic characteristics were comparable, with no statistically significant differences. In all groups, the majority of patients were male and Caucasian. Three hundred and thirty eight of these patients were treated at 29 North American sites.

Table 4-1 Baseline demographics by treatment group (B253 ITT population)

Demographic variable	Category/ summary statistics	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Age (years)	Mean (±SD)	51.2 (±11.2)	52.1 (±10.8)	50.5 (±11.5)
	Range	(18 - 68)	(16 - 69)	(18 - 69)
Age group, n (%)	< 50 yrs	81 (38.8%)	73 (34.6%)	78 (36.4%)
	≥ 50 yrs	128 (61.2%)	138 (65.4%)	136 (63.6%)
Sex, n (%)	Male n (%)	166 (79.4%)	171 (81.0%)	182 (85.0%)
	Female	43 (20.6%)	40 (19.0%)	32 (15.0%)
Race, n(%)	Caucasian	181 (86.6%)	192 (91.0%)	193 (90.2%)
	Black	21 (10.0%)	11 (5.2%)	13 (6.1%)
	Oriental	2 (1.0%)	3 (1.4%)	3 (1.4%)
	Other	5 (2.4%)	5 (2.4%)	5 (2.3%)
Weight (kg)	Mean (±SD)	76.2 (±15.4)	77.6 (±15.1)	77.0 (±14.9)
Height (cm)	Mean (±SD)	173.3 (±10.4)	172.5 (±8.8)	172.8 (±9.3)
Diabetes History	n (%)	35 (16.7%)	49 (23.2%)	36 (16.8%)
Baseline Cholesterol mmol/L	Mean (±SD)	3.1 (0 .92)	3.2 (0.83)	3.1(0.91)
Baseline Triglyceride mmol/L	Mean (±SD)	1.2(0.73)	1.1 (0.64)	1.2 (0.86)
PRA, n(%)	0%	172 (82.3%)	175 (82.9%)	182 (85.0%)
	1% - 20%	32 (15.3%)	33 (15.6%)	28 (13.1%)
	21% -50%	1 (0.5%)	1 (0.5%)	1 (0.5%)
	51% -100%	1 (0.5%)	0	0
Etiology of end-stage heart disease, n(%)	Idiopathic cardiomyopathy	100 (47.8%)	98 (46.4%)	115 (53.7%)
	Coronary artery disease	78 (37.3%)	84 (39.8%)	68 (31.8%)
	Congenital heart disease	3 (1.4%)	1 (0.5%)	7 (3.3%)
	Myocarditis	3 (1.4%)	2 (0.9%)	3 (1.4%)
	Valvular heart disease	6 (2.9%)	8 (3.8%)	6 (2.8%)
	Other	19 (9.1%)	18 (8.5%)	15 (7.0%)

Donor background characteristics

Donor background characteristics (Table 4-2) were collected to assess balance between the treatment groups of factors that might exert efficacy or safety effects.

Table 4-2 Selected transplant-related baseline characteristics (B253 ITT population)

Characteristics	Category summary statistics	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Donor age (years)	Mean (±SD)	32.5 (±12.5)	34.1 (±12.9)	33.6 (±13.2)
Male donors	n (%)	138 (66.0%)	138 (65.4%)	147 (68.7%)
Female donors	n (%)	69 (33.0%)	73 (34.6%)	66 (30.8%)
Caucasian donors	n (%)	171 (81.8%)	174 (82.5%)	174 (81.3%)
Black donors	n (%)	12 (5.7%)	13 (6.2%)	12 (5.6%)
Oriental donors	n (%)	0	2 (0.9%)	1 (0.5)
Others	n (%)	25 (12.0%)	22 (10.4%)	26 (12.1%)
CMV mismatch (serology), n (%)	CMV: donor+/recipient –	36 (17.2)	48 (22.7%)	37 (17.3%)
Cold ischemia time (hrs)	Mean (± SD)	2.9 (± 1.1)	3.2 (± 1.1)[a]	3.0 (± 1.1)

Note: Numbers in a specific category do not necessarily add up to the total number of patients in this group
[a] Statistically significant difference between the everolimus 3 mg and 1.5 mg group (p=0.009)

The donor characteristics of age, gender and race were comparable between treatment groups. The most prevalent causes for end stage heart failure were idiopathic cardiomyopathy and coronary artery disease.

Positive results for Hepatitis B surface antigen, hepatitis C or HIV tests in recipients were seldom observed and were comparable between groups. The incidence of high PRA (overall and by category) was similar between groups.

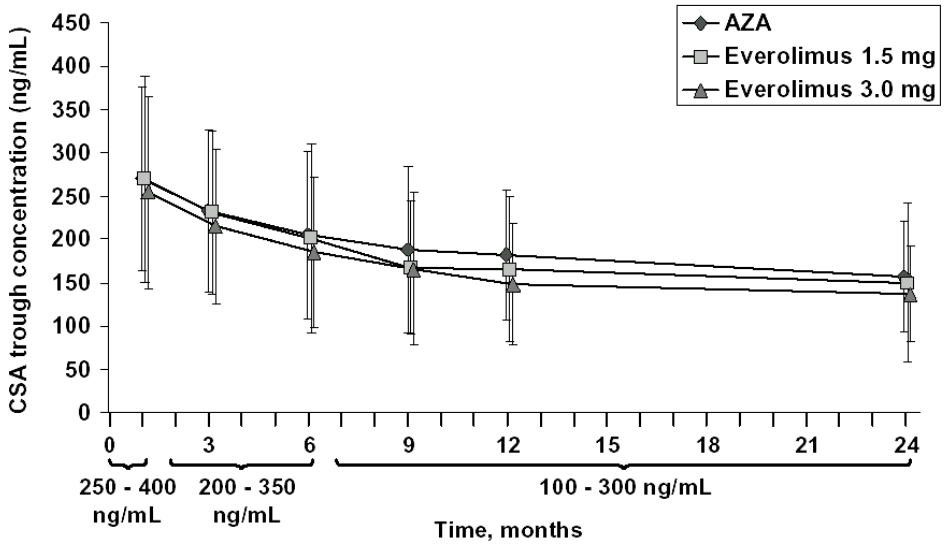
The incidence of CMV-seronegative patients having received a graft from CMV-seropositive donors, considered as high-risk patients for the subsequent development of cytomegalovirus infection, was approximately 5% higher in the everolimus 3 mg group than in both other groups. The differences between groups were not statistically significant. No clinically relevant difference in cold ischemia time was observed between groups, although the difference between the everolimus 3 and 1.5 mg group was statistically significant.

Pre-study diabetes occurred more frequently in the everolimus 3 mg group than in the other groups, but the difference was not statistically significant.

4.1.2 CsA dosing

The protocol dosing of CsA called for reduction in CsA exposure over time. As shown in Figure 4-1 protocol specified CsA dosing was adhered to and most patients had CsA levels in protocol specified ranges at most visits.

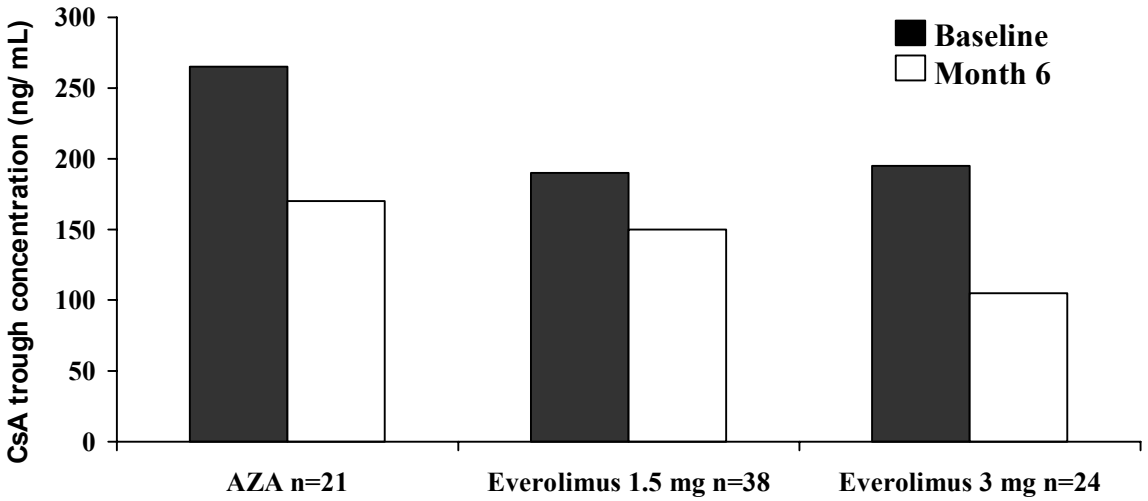
Figure 4-1 Mean (±SD) CsA trough exposure by visit- heart transplant study RAD B253



Renal Amendment

After all patients completed the 12 month portion of study, treatment assignments were unblinded to detect renal impairment in some patients treated with the CsA/everolimus combination. The protocol was amended to allow prospective CsA reduction and 6 month evaluation. Eligible patients were those with both renal impairment and a CsA blood trough concentration greater than 100 ng/ml. Many patients had either acceptable renal function, or had already reduced CsA exposure to 100 ng/ml, so only a minority of the remaining patients were eligible to be studied under this dosing amendment, shown in Figure 4-2. No patient had a baseline visit earlier than month 21 following the transplant. Of those entering the amendment, 13 patients receiving 1.5 mg/day everolimus, 6 patients receiving 3.0 mg/day everolimus, and 7 patients receiving AZA had a baseline renal amendment visit prior to month 24.

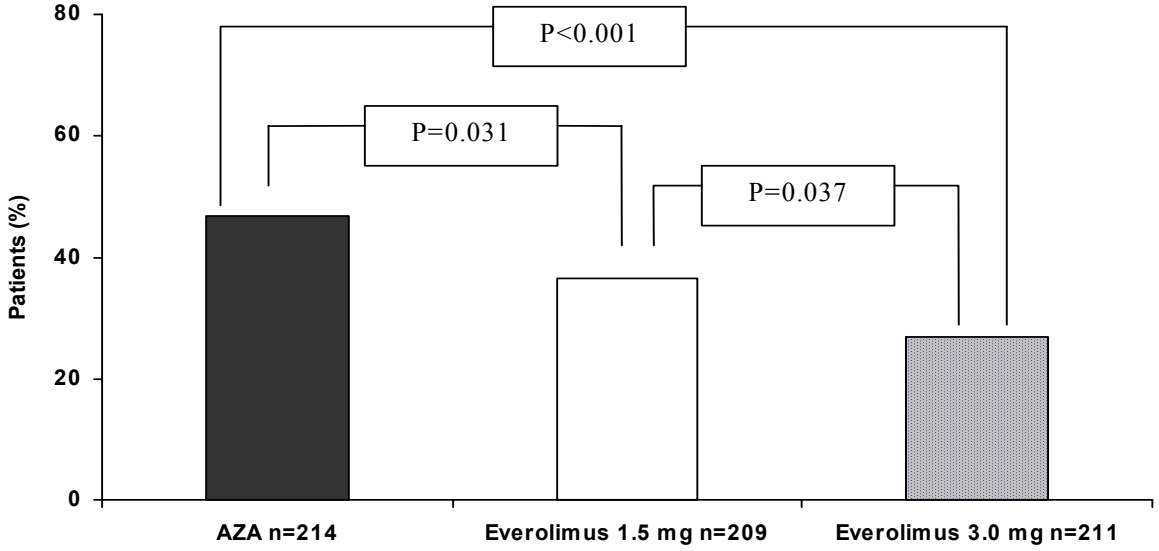
Figure 4-2 Mean CsA trough concentrations among patients in study RAD B253 at amendment baseline and amendment 6 month visit



4.1.3 Primary Efficacy Variable

Both doses of everolimus met the primary endpoint by demonstrating superior efficacy as compared to azathioprine at 6 months after transplant, shown in Figure 4-3. Also, efficacy failure was significantly lower in the everolimus 3 mg group as compared to the everolimus 1.5 mg group.

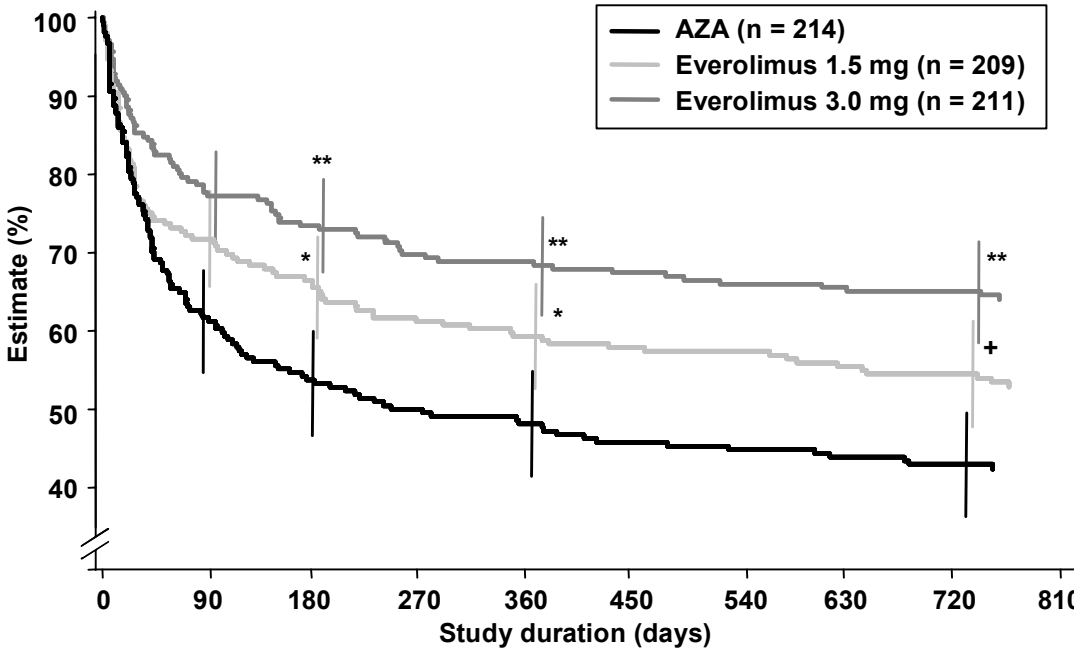
Figure 4-3 Incidence of efficacy failure at 6 months



In addition:

- The incidence of efficacy failure at Month 12, and of acute rejection episodes of ISHLT ≥ grade 3A at Months 6 and 12, were significantly lower in both everolimus dose groups compared with the AZA group (Table 4-3). Data from the on-treatment analysis supported the conclusions observed in the ITT analysis. Comparisons of efficacy of everolimus 1.5 mg/day or 3.0 mg/day were still significant if AZA patients lost to follow-up were not treated as efficacy failures.
- The results of the pairwise comparison of simple event rates of efficacy failure (Z-test) are summarized in Table 4-4, and indicated that both doses of everolimus were superior to AZA. Individual event rates were also analyzed by center at Months 6 and 12 using CMH and BD tests. The CMH test showed similar results as other analyses, and the BD test confirmed homogeneity between centers for all parameters.
- The Z-test results were supported by the comparisons of K-M estimates (Figure 4-4) of the probability of experiencing efficacy failure using the log-rank test.

Figure 4-4 **Kaplan-Meier estimates of the event free survival for efficacy failure (biopsy-proven acute rejection of ≥ grade 3A, rejection associated with hemodynamic compromise, graft loss, death or loss to follow-up)**



AZA	**131**	**114**	**103**	**85**
Everolimus 1.5 mg	**150**	**137**	**124**	**100**
Everolimus 3.0 mg	**163**	**155**	**144**	**124**

****P* < 0.05 vs AZA; ***P* < 0.001, 3.0 mg vs AZA; + *P* = 0.02, 1.5 mg vs AZA.**
95% CIs and number of patients at risk shown at 3, 6, 12, and 24 months.

Table 4-3 Number (%) of patients with efficacy related events at 6, 12 and 24 months – Heart study B253 for Everolimus (Ever) and AZA

	Ever 1.5 mg (N=209)	Ever 3 mg (N=211)	AZA (N=214)	p-value
Efficacy failure (Month 6)	76 (36.4%)	57 (27.0%)	100 (46.7%)	**0.031** [a] **<0.001** [b] **0.037** [c]
Acute rejection of ISHLT ≥ grade 3A	58 (27.8%)	40 (19.0%)	89 (41.6%)	**0.003** [a] **<0.001** [b] **0.032** [c]
Acute rejection associated with HDC	14 (6.7%)	11 (5.2%)	16 (7.5%)	n.s.
Graft loss	4 (1.9%)	8 (3.8%)	6 (2.8%)	n.s.
Death	13 (6.2%)	14 (6.6%)	12 (5.6%)	n.s.
Loss to follow up	0	0	1 (0.5%)	n.s.
Antibody-treated acute rejection episode of ISHLT ≥ grade ≥3A or associated with HDC	12 (5.7%)	6 (2.8%)	14 (6.5%)	n.s.
Efficacy failure (Month 12)	87 (41.6%)	68 (32.2%)	113 (52.8%)	**0.020** [a] **<0.001** [b] **0.045** [c]
Acute rejection of ISHLT ≥ grade 3A	64 (30.6%)	45 (21.3%)	98 (45.8%)	**0.001** [a] **<0.001** [b] **0.029** [c]
Acute rejection associated with HDC	17 (8.1%)	14 (6.6%)	23 (10.7%)	n.s.
Graft loss	7 (3.3%)	11 (5.2%)	10 (4.7%)	n.s.
Death	18 (8.6%)	24 (11.4%)	17 (7.9%)	n.s.
Loss to follow up	0	0	2 (0.9%)	n.s.
Antibody-treated acute rejection episode of ISHLT ≥ grade 3A or associated with HDC	15 (7.2%)	7 (3.3%)	15 (7.0%)	n.s.
Efficacy failure (Month 24)	96 (45.9%)	76 (36.0%)	123 (57.5%)	**0.016** [a] **<0.001** [b] **0.038** [c]
Acute rejection of ISHLT ≥ grade 3A	73 (34.9%)	48 (22.7%)	103 (48.1%)	**0.005** [a] **<0.001** [b] **0.005** [c]
Acute rejection associated with HDC	19 (9.1%)	17 (8.1%)	28 (13.1%)	n.s.
Graft loss	10 (4.8%)	14 (6.6%)	13 (6.1%)	n.s.
Death	21 (10.0%)	29 (13.7%)	24 (11.2%)	n.s.
Lost to follow up	0	0	2 (0.9%)	n.s.
Antibody-treated acute rejection episode of ISHLT ≥ grade 3A or associated w/ HDC	15 (7.2%)	9 (4.3%)	18 (8.4%)	n.s./NA

Notes:
1. Components of efficacy failure are not mutually exclusive, except for the category 'loss to follow-up.'
2. Loss to follow-up for the primary efficacy variable includes all patients who had no efficacy evaluations after Days 154 and 329 who had not experienced acute rejection of ISHLT ≥ grade 3A or associated with HDC, graft loss or death for the 6- and 12-month analyses, respectively.
3. All events other than loss to follow up are included up to the defined cutoff at Day 194 (6 months) and Day 381 (12 months), and day 810 (24 months)

a: Ever 1.5 mg vs. AZA; b: Ever 3 mg vs. AZA, c: Ever 1.5 mg vs. Ever 3 mg (pairwise Z-test, $p \leq 0.05$).

Table 4-4 Analysis of the primary composite efficacy variable and the composite supporting efficacy variable (graft loss/death/lost to follow-up): Z-test based confidence intervals (ITT population - 12-month analysis) – Heart study B253 for Everolimus (Ever) and AZA.

	Ever 1.5 mg (N=209)	Ever 3 mg (N=211)	AZA (N=214)
Primary composite efficacy variable (Month 6)			
Number of patients with event (%)	76 (36.4%)	57 (27.0%)	100 (46.7%)
Differences in rates (Ever – AZA)	-10.3%	-19.7%	
95% CI (Ever – AZA)	(-19.6, -1.0)	(-28.7, -11.0)	
Z-test p-value (2-sided) for no difference	**0.031**	**<0.001**	
Primary efficacy variable (Month 12)			
Number of patients with event (%)	87 (41.6%)	68 (32.2%)	113 (52.8%)
Differences in rates (Ever – AZA)	-11.2%	-20.6%	
95% CI (Ever – AZA)	(-20.7, -1.7)	(-29.8, -11.0)	
Z-test p-value (2-sided) for no difference	**0.020**	**<0.001**	
Primary efficacy variable (Month 24)			
Number of patients with event (%)	96 (45.9%)	76 (36.0%)	123 (57.5%)
Differences in rates (Ever – AZA)	-11.6%	-21.5%	
95% CI (Ever – AZA)	(-21.1, -2.1)	(-30.8, -12)	
Z-test p-value (2-sided) for no difference	**0.016**	**<0.001**	
Graft loss/death/lost to follow-up (Month 6)			
Number of patients with event (%)	13 (6.2%)	14 (6.6%)	14 (6.5%)
Differences in rates (Ever – AZA)	-0.3%	-0.1%	
95% CI (Ever – AZA)	**(-5.0, 4.3)**	**(-4.6, 4.8)**	
Z-test p-value (2-sided) for no difference	0.999	0.969	
Graft loss/death/lost to follow-up (Month 12)			
Number of patients with event (%)	18 (8.6%)	24 (11.4%)	19 ((8.9%)
Differences in rates (Ever – AZA)	-0.3%	2.5%	
95% CI (Ever – AZA)	**(-5.7, 5.1)**	**(-3.2, 8.2)**	
Z-test p-value (2-sided) for no difference	0.999	0.394	
Graft loss/death/lost to follow-up (Month 24)			
Number of patients with event (%)	21 (10.0%)	29 (13.7%)	26 (12.1%)
Differences in rates (Ever – AZA)	-2.1%	1.6%	
95% CI (Ever – AZA)	**(-8.1, 3.9)**	**(-4.8, 8.0)**	
Z-test p-value (2-sided) for no difference	0.999	0.624	

Note:
1. Loss to follow-up for efficacy failure includes all patients who had no efficacy evaluations after Days 154, 329 and 365 who had not experienced efficacy failure for the 6-, 12- and 24-month analyses, respectively.
2. Cutoff dates for all efficacy events other than loss to follow-up were Days 194, 381 and 450 for the 6-, 12- and 24-month analyses, respectively.

Explanatory analyses- Analysis of risk factors for acute rejection

The influence of multiple pre- and post-transplant risk and protective factors on the incidence of acute cardiac allograft rejection ISHLT ≥ grade 3A among 634 patients randomized to either everolimus or azathioprine (AZA) was analyzed. Univariate logistic regression modeling of effect on acute rejection was performed for risk factors: PRA, donor age,

recipient age, recipient coronary artery disease (CD), body mass index (BMI), cold ischemia time (IT), use of LVAD, and hypertension. Of these, the use of LVAD was significantly associated with acute cardiac allograft rejection ISHLT ≥ grade 3A (p=0.002, odds ratio [OR] = 3.2, 95% CI 11.5-6.7). There were only a small number of LVAD-users randomized into each treatment group, and the diagnosis of rejection among LVAD-users was not significantly associated with treatment.

4.1.4 Graft Loss and Death

Graft loss and death data are summarized in Table 4-4. The incidence of graft loss at Months 6 and 12 was low, and comparable between groups. (Note: there were no graft losses with re-transplants. Reports of deaths were examined for any terms indicating cardiac events or unexplained sudden death [3 cases], and these were retrospectively termed as graft losses.) The incidence of patients who died within 6 and 12 months after initial dose of study medication was higher in the everolimus 3 mg group compared to both other groups, but the between-group difference was not significant. At one year, the 95% confidence interval for the difference in the rates of graft loss/death and lost to follow up was (-5.6, 5.1) for the comparison of RAD 1.5mg vs. AZA and (-3.2, 8.2) for the comparison of RAD 3.0 mg vs AZA. The 97.5% confidence interval for the difference in the rates of graft loss/death and lost to follow up was (-6.4, 5.9) for the comparison of RAD 1.5mg vs. AZA and (-4.1, 9.1) for the comparison of RAD 3.0 mg vs. AZA. As the 95% confidence interval for the difference in rates between everolimus arms and AZA is below 10, both groups of everolimus can be considered noninferior to AZA in terms of death. Noninferiority was maintained through 24 months.

4.1.5 Secondary Efficacy Endpoints

Overall, significantly fewer acute rejection episodes of ISHLT ≥ grade 3A occurred in both everolimus groups at 6 and at 12 months as compared to azathioprine; everolimus 3 mg was superior to everolimus 1.5 mg.

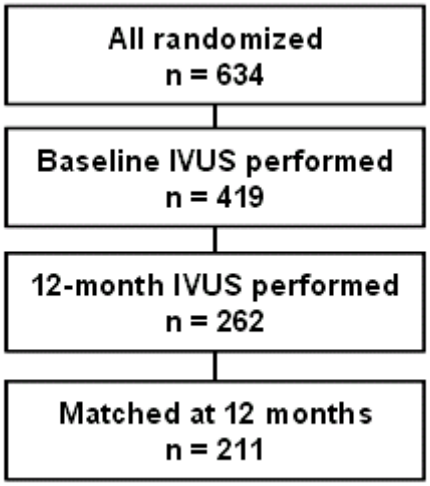
- Individual secondary efficacy events are summarized in Table 4-3. The incidence of patients who experienced an acute rejection of ISHLT ≥ grade 3A within 6 and 12 months after initial dose of study medication was significantly lower in both everolimus groups compared with AZA. Acute rejection was inversely related to everolimus dose level, with a significant between-group difference. By Month 12, the incidence of patients with ≥ 2 acute rejections of ISHLT ≥ grade 3A was 8%, 7% and 14% in the everolimus 1.5 and 3 mg groups and the AZA group, respectively.
- Patients who experienced antibody-treated acute rejection episode of ISHLT ≥ grade 3A or rejection associated with hemodynamic compromise have more severe rejection than those rejections diagnosed through histopathology alone. The incidence of patients who experienced antibody-treated acute rejection episode of ISHLT ≥ grade 3A or associated with hemodynamic compromise within 6 and 12 months after initial dose of study medication was lower in the everolimus 3 mg group compared to both other groups. The difference between the everolimus 3 mg and AZA groups was significant at Month 6.

K-M estimates supported the results observed with the Z-tests. Individual event rates were also analyzed by center at Months 6 and 12 using CMH and BD tests. The CMH test showed similar results as for the other analyses described above, and the BD test confirmed homogeneity between centers for all parameters.

4.1.6 IVUS Results

The IVUS measurements were obtained under blinded conditions at the site, and the core laboratory remained blinded to treatment assignment throughout the analysis. Of the 634 randomized patients in study B253, ultimately 211 had paired IVUS tapes from baseline and 12 months of adequate technical quality to allow for paired analysis. Disposition of the study population that receive IVUS is summarized in Figure 4-5.

Figure 4-5 Disposition of IVUS patients



Details about those patients who did not have matched IVUS analyses are provided in Table 4-5. Similar numbers of patients had no baseline exam among the 3 treatment groups. This often related to issues at the sites that rendering it not possible to perform IVUS according to the standardized protocol. Thus, the sample of 419 patients with a baseline examination was unlikely to be biased. The most common reason for one of these patients not having a matching one-year examination was discontinuation of study medication. The rate of discontinuation of treatment was nearly identical between the everolimus 1.5 mg and AZA groups (30% vs. 29%), whereas the discontinuation rate was somewhat higher in the everolimus 3 mg group at 40%. Finally, of the 262 patients with paired baseline and 12-month examinations, there were 51 IVUS examintions in which 11 segments could not be matched, leaving 211 studies for the 12 month analysis (Figure 4-5). Furthermore, the patients included in the IVUS analyses were equally distributed across the treatment groups, who were generally comparable with respect to demographic and background characteristics (Appendix A). These patients provided a representative sample of the randomized groups large enough to explore clinical and statistical associations.

Table 4-5 The reasons paired IVUS studies were not obtained or evaluated

		Everolimus		
	AZA	1.5 mg	3.0 mg	Total
No baseline	62	68	65	195
Technical issues	7	9	5	21
D/C study, death, AEs	28	25	30	83
Due to renal problems	4	16	12	32
IVUS tape not analyzable	26	8	17	51
Administrative problems	10	8	6	24
No consent	1	4	6	11
Unknown	4	1	1	6
Total	142	139	142	423

The primary IVUS efficacy variables were the change in average maximum intimal thickness from baseline to Month 12 and the incidence of allograft vasculopathy (defined as ≥ 0.5 mm increase from baseline in maximum intimal thickness). The IVUS landmark maximum intimal thickness, which is the basis of both endpoints, is demonstrated in Figure 1-2. In both everolimus dose groups, the change in average maximum intimal thickness from baseline to Month 12, as well as the incidence of allograft vasculopathy, was significantly smaller compared with the AZA group (Table 4-6). An association with everolimus dose level was also observed for both variables.

Table 4-6 Change in average maximum intimal thickness from baseline and incidence of allograft vasculopathy (IVUS population – 12-month analysis) – Heart study B253

	RAD 1.5 mg (N=70)	RAD 3 mg (N=69)	AZA (N=72)	p-value
Change (mm) in average maximum intimal thickness from baseline to Month 12				
				0.014[a] **0.003[b]**
Mean	0.04	0.03	0.10	0.491[c]
Range	(-0.36, 0.27)	(-0.20, 0.25)	(-0.44, 0.74)	
Allograft vasculopathy (largest maximum intimal thickness increase ≥ 0.5 mm) n(%)				
				0.045[a] **0.010[b]**
≥ 0.5 mm	25 (35.7%)	21 (30.4%)	38 (52.8%)	0.590[c]
< 0.5 mm	45 (64.3%)	48 (69.6%)	34 (47.2%)	

a: RAD 1.5 mg vs. AZA; b: RAD 3.0 mg vs. AZA; c: RAD 1.5 mg vs. RAD 3 mg (pairwise comparisons of treatment groups using Wilcoxon's rank sum test for continuous variables and Fisher's exact test for categorical variables, $p \leq 0.05$).

4.1.7 Exploration of the Influence of Demographic Factors on IVUS results

Methodology

Tabulation of patient disposition and reasons for IVUS data loss

The ITT study population was N=634 of which N=211 patients had matched-IVUS data at 12 months (IVUS matched for eleven 1mm segments at both baseline and 12 months, thus, forming the 12 months matched-IVUS population). Patient disposition and reasons for IVUS data loss was tabulated by frequency tables among the three treatment groups. The pattern of reasons for IVUS data loss was examined carefully between the groups to see if the degree of data loss was substantially different among the groups.

Comparison of baseline characteristics and selected post-baseline safety variables

For the assessment of comparability, treatment groups were compared in the 12-month IVUS population with respect to baseline characteristics. The 12-month IVUS population was also compared to three other populations (the ITT study population, patients with only a baseline IVUS, and the 12-month on-treatment population). The comparison was done by side-by-side presentation of all baseline information selected post-baseline safety variables using descriptive statistics (n (%) for binary variables and mean/median/SD for continuous variables).

Assessment of potential biases in selecting the 12-month matched-IVUS patient population

The possibility exists that decisions that led to exclusion from the 12-month IVUS population could be biased by a variety of factors. In order to exclude as many of these sources of bias as possible, the following important clinical aspects were investigated carefully with respect to the patients' 12-month IVUS status (with or without IVUS data):

- baseline characteristics,
- post-baseline measurements (treatment-related outcomes) with respect to anything that might lead to differential loss of IVUS data, such as adverse events, premature discontinuation of study medication biopsy-proven acute rejection, renal function and CsA trough level, etc.

The impact of each of the above factors on IVUS status was assessed as follows:

- Summary statistics of baseline characteristics according to IVUS status and treatment group to identify those baseline factors that could influence exclusion from the 12-month IVUS population.
- A logistic regression model was used for binary variables (such as acute rejection) with covariates: matched-IVUS status (yes/no) at 12 months, indicator variables for two treatment comparisons (everolimus vs. AZA), and interaction terms of IVUS status by treatment. The odds ratio of exposure to the risk factor variable for matched IVUS patients relative to non-IVUS patients was estimated as the measurement of association of the risk factor and IVUS status. Comparisons were made across the three treatment groups to determine if the everolimus arms were comparable to AZA in the way they were selected for the IVUS population. The comparisons were done by checking if there was any treatment by selection process interaction in the logistical regression model. A

statistically significant interaction (at 0.10 level) suggests there was a selection process either in favor of or against everolimus arms, depending upon the direction of the interaction.

- The generalized linear model procedure was used for continuous response variables, such as renal function and CsA trough level, with covariates: matched IVUS status (yes/no) at 12 months, indicator variables for two treatment comparisons (everolimus vs. AZA), interaction terms of IVUS status by treatment

Sensitivity analyses to assess the robustness of the positive IVUS result

- Two different ways of imputing missing IVUS data were used to assess the robustness of the positive 12-month IVUS results: firstly, assigning IVUS data from the AZA arm to patients with missing IVUS data at 12 months and secondly, assigning worst IVUS outcome to all patients with missing IVUS data at 12 months.

Results

There were methodological concerns regarding the collection of the intravascular ultrasound (IVUS) data. Therefore, characteristics of populations were compared for factors which could conceivably bias the outcome of the IVUS study. Recipient factors included: weight, age, age distribution above and below 50 years, gender, race, height, creatinine clearance, coronary artery disease as a reason for transplant and diabetes. Donor factors included cold ischemia time, donor age and donor gender. Notable differences among the study groups included:

- PRA >20% tended to be rare amongst the differing subpopulations, and non-existent among those with matched IVUS studies.
- Recipient coronary artery disease (CAD) prevalence was highest in the everolimus 3 mg group (all populations), a characteristic known to be a risk factor for subsequent CAV.
- Baseline calculated creatinine clearance at or below 29 ml/min was most frequent in the everolimus 1.5 mg arm.
- Baseline diabetes (a recipient risk factor associated with vasculopathy) was overrepresented in all of the everolimus 3.0 mg populations, and reached 35% of the 12 month IVUS population of evaluable patients.
- The number of black patients in the 12 month IVUS population varied and was highest in the everolimus 1.5 mg/day population.

Despite the fact that recipient characteristics (recipient CAD, and baseline diabetes) were weighted against the everolimus 3 mg group, this group exhibited the least progression of intimal thickness during the first year.

We also determine whether the above risk factors plus acute cardiac allograft rejection ISHLT ≥ grade 3A, mean CsA trough, mean steroid dose, creatinine level>200umol/mL, triglyceride ≥ 4.5 mmol/L, cholesterol ≥ 6.2 mmol/L, cytomegalovirus infection, statin, ACE inhibitor, or calcium channel blockers influenced the subsequent occurrence of IVUS-defined cardiac allograft vasculopathy (CAV) at one year. This endpoint was studied because of its demonstrated association with survival and major adverse cardiovascular events at 5 years after transplant. IVUS-defined CAV was an increase from baseline in maximal intimal thickness of 0.5 mm or more in any segment. Among the 211 patients with baseline and 1 year

IVUS, there were too few patients with cytomegalovirus infection or without statin use to determine whether these factors affected CAV. The potential impact of choice of statin (HMG-CoA reductase inhibitors) was also investigated, and no influence of the choice of statin treatment on the change in maximum intimal thickness or the incidence of allograft vasculopathy was detected. Occurrence of at least one acute cardiac allograft rejection ISHLT ≥ grade 3A was not a risk factor for the occurrence of CAV. Of the risk factors analyzed donor age >50 y (p=0.005, OR=3.6, CI=1.5-9.0) was associated with increased risk of CAV as was recipient coronary disease (p=0.04, OR=1.83, CI=1.02-3.25). Interestingly, recipient metabolic risk factors did not influence CAV. The treatment effect of everolimus persisted in analyses adjusted for risk factors.

4.1.8 Assessment of potential selection bias in the matched-IVUS population

Events leading to exclusion from IVUS procedure occurred while patients and investigators were blinded to treatment assignments. The decision of investigators not to perform IVUS may have reflected baseline characteristics and the patient's medical status. This selection could have biased the results in the matched-IVUS subpopulation. Therefore, the following baseline and post-baseline measurements were investigated to determine if the impact of the selection process favored everolimus arms:

- Baseline variables: recipient age, recipient gender, recipient race, donor age and gender, GFR < 29 mL/min/1.73m2, diabetes, hypertension, BMI > 33, PRA and CMV donor/recipient mismatch
- Post-baseline variables (at 12 months):
  - biopsy-proven-acute rejection (BPAR) of ISHLT grade ≥3A,
  - biopsy-proven-acute rejection with hemodynamic compromise
  - treated acute rejection
  - renal function (CrCl),
  - total cholesterol,
  - triglyceride,
  - post-transplant diabetes mellitus
  - statin use

The assessments indicated that the impact of the most baseline and post-baseline variables on matched-IVUS selection was similar among the three treatment groups. Difference identified (baseline diabetes and renal function), were biased in favor of the comparator.

Detailed assessments of IVUS selection bias can be found in section 8.2 Appendix A "Additional IVUS data analyses". In the same Appendix, the results of sensitivity analyses for "missingness" of IVUS values were also summarized. All sensitivity analyses supported the primary analysis.

4.2 Exposure and effect relationships for everolimus

4.2.1 Overview of Pharmacokinetics of Everolimus (ADME)

Everolimus pharmacokinetics have been characterized after oral administration of single and multiple doses to adult kidney and heart transplant patients, pediatric kidney transplant patients, hepatically-impaired patients, and healthy subjects.

Absorption

After oral dosing, peak everolimus concentrations occur 1 to 2 h post dose. Over the dose range of 0.5 to 2 mg bid, everolimus Cmax and AUC are dose proportional in transplant patients at steady-state.

Food Effect

In 24 healthy subjects, a high-fat breakfast (44.5 g fat) reduced everolimus C_{max} by 60%, delayed t_{max} by a median 1.3 hours, and reduced AUC by 16% compared with a fasting administration. To minimize variability, everolimus should be taken consistently with or without food.

Distribution

The blood-to-plasma ratio of everolimus is concentration dependent ranging from 17% to 73% over the range of 5 to 5000 ng/mL. Plasma protein binding is approximately 74% in healthy subjects and in patients with moderate hepatic impairment. The apparent distribution volume associated with the terminal phase (Vz/F) from a single-dose pharmacokinetic study in maintenance renal transplant patients is 342 $\pm$ 107 L (range 128 to 589 L).

Metabolism

Everolimus is a substrate of CYP3A4 and P-glycoprotein. The main metabolic pathways identified in man were monohydroxylations and O-dealkylations. Two main metabolites were formed by hydrolysis of the cyclic lactone. Everolimus was the main circulating component in blood. None of the main metabolites contribute significantly to the immunosuppressive activity of everolimus.

The bioavailability of everolimus was significantly increased by co- administration of cyclosporin. In a single-dose study in healthy subjects, cyclosporin for microemulsion (Neoral) increased everolimus AUC by 168 % (range, 46 % to 365 %) and Cmax by 82 % (range, 25 % to 158 %) compared with administration of everolimus alone. Certican had a clinically minor influence on cyclosporine pharmacokinetics in heart and renal transplant patients receiving cyclosporine for microemulsion.

Excretion

After a single dose of radiolabeled everolimus was given to transplant patients receiving cyclosporine, the majority (80%) of radioactivity was recovered from the feces and only a minor amount (5%) was excreted in urine. Parent drug was not detected in urine and feces.

4.2.2 Drug Interactions

Everolimus is mainly metabolized by CYP3A4 in the liver, to some extent in the intestinal wall and is a substrate for the multidrug efflux pump, P-glycoprotein. Therefore, absorption and subsequent elimination of systemically absorbed everolimus may be influenced by medicinal products that affect CYP3A4 and/or P-glycoprotein. Concurrent treatment with strong 3A4-inhibitors and inducers is not recommended. Inhibitors of P-glycoprotein may decrease the efflux of everolimus from intestinal cells and increase everolimus blood concentrations. *In vitro*, everolimus was a competitive inhibitor of CYP3A4 and of CYP2D6, potentially increasing the concentrations of medicinal products eliminated by these enzymes. Thus, caution should be exercised when co-administering everolimus with 3A4- and 2D6 substrates with a narrow therapeutic index. All *in vivo* interaction studies were conducted without concomitant cyclosporine. Pharmacokinetic interactions between everolimus and concomitantly administered drugs are discussed below. Drug interaction studies have not been conducted with drugs other than those described below.

Cyclosporine (CYP3A4/PgP inhibitor and CYP3A4 substrate)***:*** The bioavailability of everolimus was significantly increased by co-administration of cyclosporine**.** In a single-dose study in healthy subjects, Neoral increased everolimus AUC by 168 % (range, 46 % to 365 %) and C_{max} by 82 % (range, 25 % to 158 %) compared with administration of everolimus alone. Dose adjustment of everolimus might be needed if the cyclosporine dose is altered. Everolimus had a clinically minor influence on cyclosporine pharmacokinetics in transplant patients receiving Neoral.

Rifampicin (CYP3A4 inducer): Pre-treatment of healthy subjects with multiple-dose rifampicin followed by a single dose of everolimus increased everolimus clearance nearly 3-fold, and decreased C_{max} by 58 % and AUC by 63 %. Combination with rifampicin is not recommended

Ketoconazole (CYP3A4/PgP inhibitor): Multiple-dose ketoconazole administration to healthy volunteers significantly increased everolimus Cmax, AUC, and half-life by 3.9-fold, 15-fold, and 89%. It is recommended that strong inhibitors of CYP3A4 (e.g. ketoconazole, itraconazole, voriconazole, clarithromycin, telithromycin, ritonavir) not be coadministered with everolimus

Erythromycin (CYP3A4/PgP inhibitor): Multiple-dose erythromycin administration to healthy volunteers significantly increased everolimus Cmax, AUC, and half-life by 2.0-fold, 4.4-fold, and 39%. If erythromycin is coadministered, everolimus blood levels should be monitored and a dose adjustment made as necessary.

Verapamil (CYP3A4/PgP inhibitor): Multiple-dose verapamil administration to healthy volunteers significantly increased everolimus Cmax and AUC by 2.3-fold and 3.5-fold. Everolimus half-life was not changed. If verapamil is coadministered, everolimus blood levels should be monitored and a dose adjustment made as necessary.

Atorvastatin (CYP3A4-substrate) **and pravastatin** (PgP-substrate)***:*** Single-dose administration of everolimus with either atorvastatin or pravastatin to healthy subjects did not influence the pharmacokinetics of atorvastatin, pravastatin and everolimus, as well as total HMG-CoA reductase bioreactivity in plasma a to a clinically relevant extent. However, these results cannot be extrapolated to other HMG-CoA reductase inhibitors. Patients should be

monitored for the development of rhabdomyolysis and other adverse events as described in the respective labeling for these products.

Other possible interactions: Moderate inhibitors of CYP3A4 and PgP may increase everolimus blood levels (e.g. **antifungal substances:** fluconazole; **macrolide antibiotics**; **calcium channel blockers:** nicardipine, diltiazem**; protease inhibitors:** nelfinavir, indinavir, amprenavir**.** Inducers of CYP3A4 may increase the metabolism of everolimus and decrease everolimus blood levels (e.g. St. John's Wort **(**Hypericum perforatum**))**; **anticonvulsants:** carbamazepine, phenobarbital, phenytoin; **non-nucleoside RT inhibitors:** efavirenz, nevirapine).

Vaccination: Immunosuppressants may affect response to vaccination and vaccination during treatment with everolimus may be less effective. The use of live vaccines should be avoided.

4.2.3 Pharmacokinetics in kidney and heart transplant patient

Kidney transplantation

The combination of everolimus in conjunction with CsA was studied in transplant patients.

Pharmacokinetics are comparable for kidney and heart transplant patients receiving everolimus twice daily simultaneously with cyclosporine, USP MODIFIED. Steady-state is reached by day 4 with an accumulation in blood levels of 2- to 3-fold compared with the exposure after the first dose. In kidney transplantation time-to-peak-concentration occurs at 1 to 2 h post dose. C_{max} averages 11.1 ± 4.6 and 20.3 ± 8.0 ng/mL and AUC averages 75 ± 31 and 131 ± 59 ng.h/mL at 0.75 and 1.5 mg b.i.d., respectively. Predose trough blood concentrations (C_{min}) average 4.1 ± 2.1 and 7.1 ± 4.6 ng/mL at 0.75 and 1.5 mg b.i.d., respectively. Everolimus exposure remains stable over time in the first post-transplant year. C_{min} is significantly correlated with AUC yielding a correlation coefficient between 0.86 and 0.94. Based on a population pharmacokinetic analysis oral clearance (CL/F) is 8.8 L/h (27 % interpatient variation) and the central distribution volume (Vc/F) is 110 L (36 % interpatient variation). Residual variability in blood concentration is 31 %. The elimination half-life is 28 $\pm$ 7h.

Heart transplantation

There was a total of 2,328 everolimus trough concentrations from 410 patients (5.6 ± 2.0 samples per patient) and 129 everolimus steady-state profiles from 55 patients. Data from the profiles are summarized below in Table 4-7.

Table 4-7 Synopsis of Everolimus pharmacokinetics – Heart study B253

Parameter	0.75 mg bid			1.5 mg bid		
	Month 2	Month 3	Month 6	Month 2	Month 3	Month 6
N	22	23	20	20	20	14
$C_{min, b}^{ss}$ (ng/ml)	4.7 ± 2.6	4.9 ± 3.9	4.5 ± 2.4	10.0 ±4.3	10.2 ± 5.2	9.8 ± 5.6
T_{max} (h)	2 (1 – 5)	2 (1 – 5)	2 (1 – 5)	2 (1 – 5)	2 (0 – 5)	2 (1 – 5)
$C_{max, b}^{ss}$ (ng/ml)	10.2 ± 3.8	9.9 ± 4.3	10.5 ± 4.8	19.9 ± 8.6	18.6 ± 6.8	21.8 ± 12.4
$AUC_{t, b}^{ss}$(ng.h/ml)	79 ± 30	82 ± 43	80 ± 39	80 ± 39	158 ± 60	164 ± 87
$C_{avg. b}^{ss}$ (ng/ml)	6.6 ± 2.5	6.9 ±3.6	6.7 ± 3.3	13.3 ± 5.3	13.1 ± 5.0	13.7 ± 7.2
PTF (%)	89 ± 36	77 ± 40	96 ± 67	77 ± 35	70 ± 40	85 ± 32

Values a mean ± sd except for tmax which is median (range).

Data are from the 119 profiles of patients who remained on the initially assigned dose at the respective visit.

4.2.4 Everolimus and CsA exposure relationships in study B253

Everolimus trough levels were stable in the first year post-transplant and averaged 5.2 ± 3.8 and 9.4 ± 6.3 ng/mL in patients treated with 1.5 and 3 mg/day, respectively. Trough levels correlated with area under the concentration-time curve (AUC), yielding a correlation coefficient of 0.90 as assessed in a subset of 43 patients in whom AUC profiles were obtained at months 2, 3, and 6.

Cyclosporine trough levels were similar among the three treatment groups over 6 months post-transplant; however, 15–20% lower doses of cyclosporine were used in everolimus-treated patients, compared with azathioprine-treated patients. At months 9 and 12, relative to azathioprine, cyclosporine trough levels were 10–20% lower in patients receiving everolimus 1.5 and 3 mg/day. These differences are probably due to cyclosporine dose adjustments made in response to increased serum creatinine in some everolimus-treated patients.

4.2.5 Exposure-efficacy (acute rejection) in heart study (B253)

Overview

Everolimus was dosed by daily fixed doses in the clinical trials. In heart transplantation, everolimus trough concentrations were significantly related to freedom from rejection ($r = 0.93$, $p = 0.02$). The minimal effective trough level of everolimus was 3 ng/mL and efficacy was strongly correlated with everolimus exposure and for the two week period post-transplant was also related to cyclosporine exposure. At everolimus levels > 3 ng/mL, biopsy-proven acute rejections significantly decreased. Various types of analyses (such as, Cox regression analysis including the influence of both everolimus and CsA, time-to-event analyses, median-effect, incremental exposure analyses and supplemental analyses using the Cmin around Day 7) have consistently supported the effectiveness of everolimus exposures > 3 ng/mL.

The impact of low CsA trough level on efficacy was assessed by using the median-effects analyses to see if the primary efficacy failure rate at 12 months is associated with low CsA level as well as providing the descriptive statistics of efficacy failure rates by the CsA level

quartile ranges. Renal dysfunction events (defined as exceeding various threshold values for creatinine and creatinine clearance) were also analyzed against the CsA level quartile ranges.

The key findings of these analyses include the following:

- In heart transplantation, the efficacy failure rates in the everolimus arms were not significantly different at 1 year across the range of average CsA levels. However, there does appear to be a greater incidence of efficacy failure associated with lower CsA levels in the AZA treatment group.
- On the other hand, in everolimus cohorts, CsA exposure also correlated with freedom from rejection during the first two weeks after heart transplantation, but at subsequent time periods no relationship with acute rejection was demonstrated.

4.2.6 Cohort Analysis by everolimus trough levels in heart study (B253)

Incremental everolimus C_{min} ranges

To better understand the decreasing slope of the C_{min}-BPAR relationship (Figure 4-7, figure 4-8) , and to assess the robustness of the lower and upper range of the therapeutic window, incremental trough level ranges ≥ 3 ng/mL were investigated (Table 4-8).

Table 4-8 Efficacy events by average everolimus trough level Day 1 - 450 (B253)

Everolimus trough levels *	BPAR ≥ grade 3A (ISHLT)	Graft Loss	Death
< 3 ng/mL	30/68 (44%)	3/65 (5%)	7/65 (11%)
3 – 4 ng/mL	16/49 (33%)	1/47 (2%)	3/47 (6%)
4 – 5 ng/mL	8/43 (19%)	3/50 (6%)	6/50 (12%)
5 – 6 ng/mL	11/50 (22%)	3/52 (6%)	6/52 (12%)
6 – 7 ng/mL	7/37 (19%)	1/42 (2%)	2/42 (5%)
7 – 8 ng/mL	10/42 (24%)	1/40 (3%)	2/40 (5%)
8 - 9 ng/mL	6/28 (21%)	1/28 (4%)	2/28 (7%)
9 – 10 ng/mL	3/20 (15%)	1/20 (5%)	3/20 (15%)
≥ 10 ng/mL	11/67 (16%)	2/67 (3%)	8/67 (12%)
All on RAD	111/420 (26%)	19/420 (5%)	43/420 (10%)
RAD 3–8 ng/mL	52/221 (24%)	9/231 (4%)	19/231 (8%)
≥ 8 ng/mL	20/115 (17%)	4/115 (3%)	13/115 (11%)
AZA	99/214 (46%)	10/214 (5%)	18/214 (8%)

* Average trough levels including trough levels prior to Day 7 up to event or else censored at Day 450
x-y ng/mL stands for ≥ x ng/mL and < y ng/mL.

The analysis on BPAR shows that the minimal effective level is 3 ng/mL (or slightly higher). Higher levels beyond 8 ng/mL appear to further reduce the risk of BPAR; however, numbers are too small to draw strong conclusions. No relationship was detected for graft loss and the death rates.

Time to early occurring biopsy-proven acute rejection episodes

It is known from the renal and heart transplant study reports that subjects with fixed-dosing of 1.5 mg/day overall had better safety than those on the 3 mg/day everolimus arm. As most of the patients on the 1.5 mg/day everolimus regimen had average trough levels below 8 ng/mL, the patients with average trough levels "3-8 ng/mL" (defined as ≥ 3 ng/mL and < 8 ng/mL) were analyzed. To explore the distribution of BPAR over time, Kaplan-Meier (K-M) plots (Figure 4-6) of the percent of patients free of rejection by trough levels <3, 3-8, and ≥8 ng/mL was done. The K-M estimates between the three everolimus trough level groups are statistically significant (log-rank test, p=0.0001. A Cox proportional hazard model further indicated that the risk of having an acute rejection was 2.5-fold higher in patients whose trough levels were <3 ng/mL relative to those whose trough levels were 3-8 ng/mL (p≤0.0001).

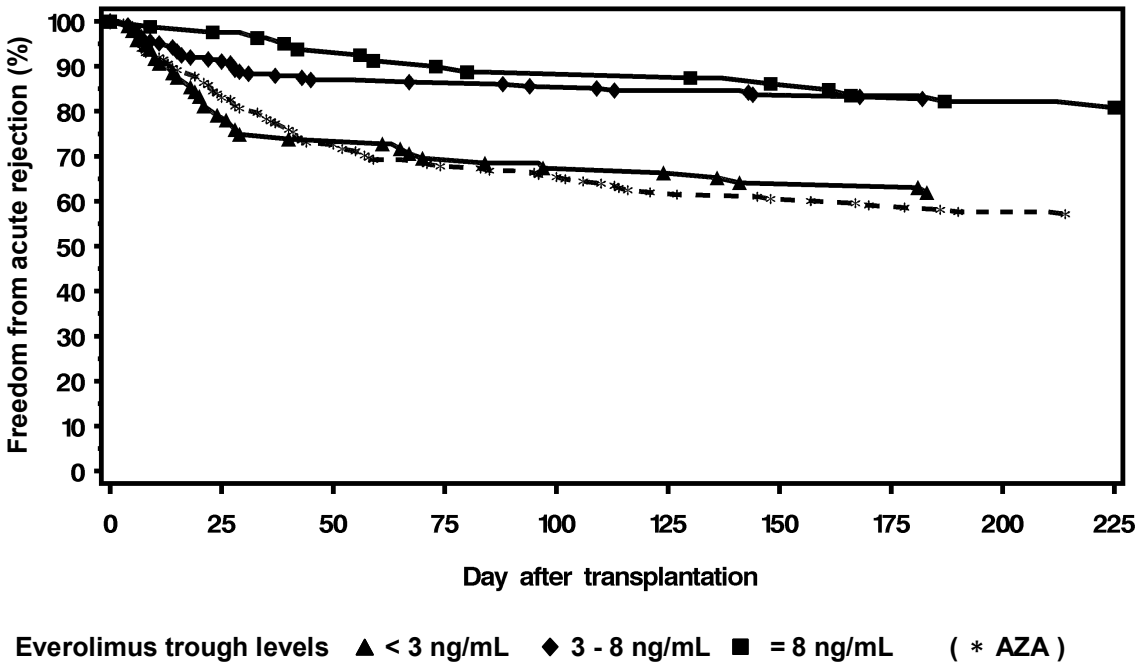
Trough levels ≥8 ng/mL numerically appear to further reduce the risk during the initial 60 days post-transplant (Table 4-9), however, patients in the two trough level groups 3-8 ng/mL and ≥ 8 ng/mL experienced the same risk of BPAR (relative risk =1.04, p=0.90) up to Day 225. This is also depicted in the Figure 4-6.

Table 4-9 Cumulative likelihood of early BPAR ISHLT ≥ grade 3A (B253)

Time to BPAR	Day 6	Day 12	Day 30	Day 60	Day 180
RAD < 3 ng/mL	4.1%	9.3%	25.2%	26.2%	35.9%
RAD 3-8 ng/mL	2.7%	4.9%	11.2%	13.1%	16.8%
RAD ≥ 8 ng/mL	0%	1.2%	2.5%	8.8%	16.5%
AZA	1.9%	8.5%	19.4%	30.8%	41.4%

Based on Kaplan-Meier estimates up to Day 225.

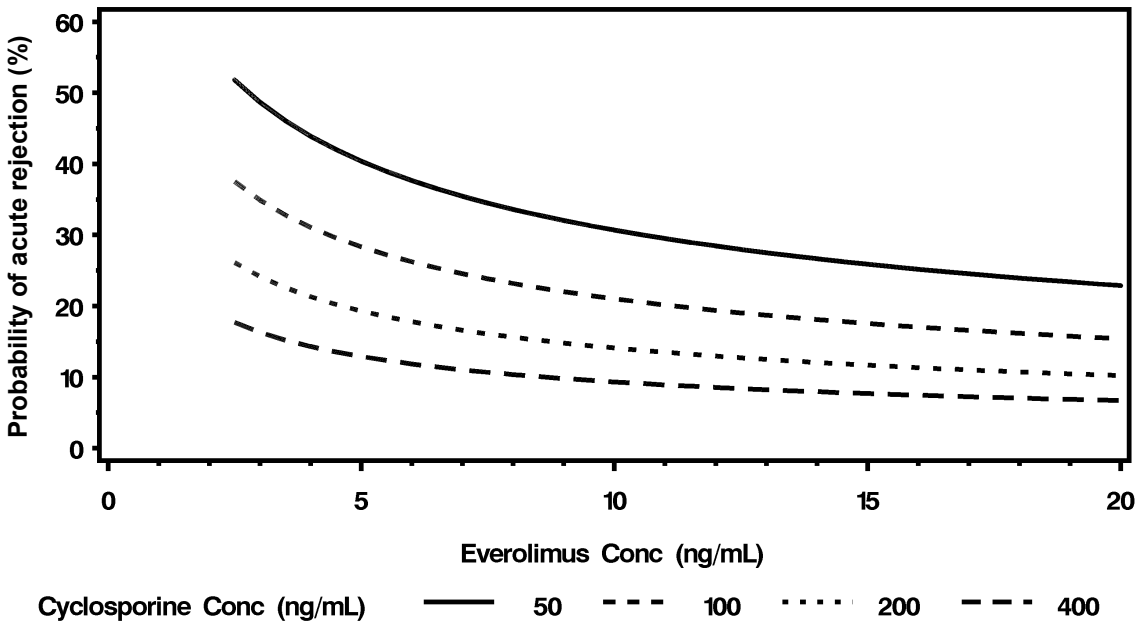
Figure 4-6 Kaplan-Meier plots (Day 1 – 225): Percent of patients free of BPAR ISHLT ≥ grade 3A (B253)



4.2.7 Exposure-Efficacy relationships for CsA in heart study (B253)

In Figure 4-7, the probability of a BPAR from Day 1 to 225 is plotted against everolimus exposure for selected, fixed CsA trough levels. The Cox regression analysis showed a strong effect of increasing everolimus (p=0.0023) and CsA exposure (p=0.0003) on freedom from BPAR.

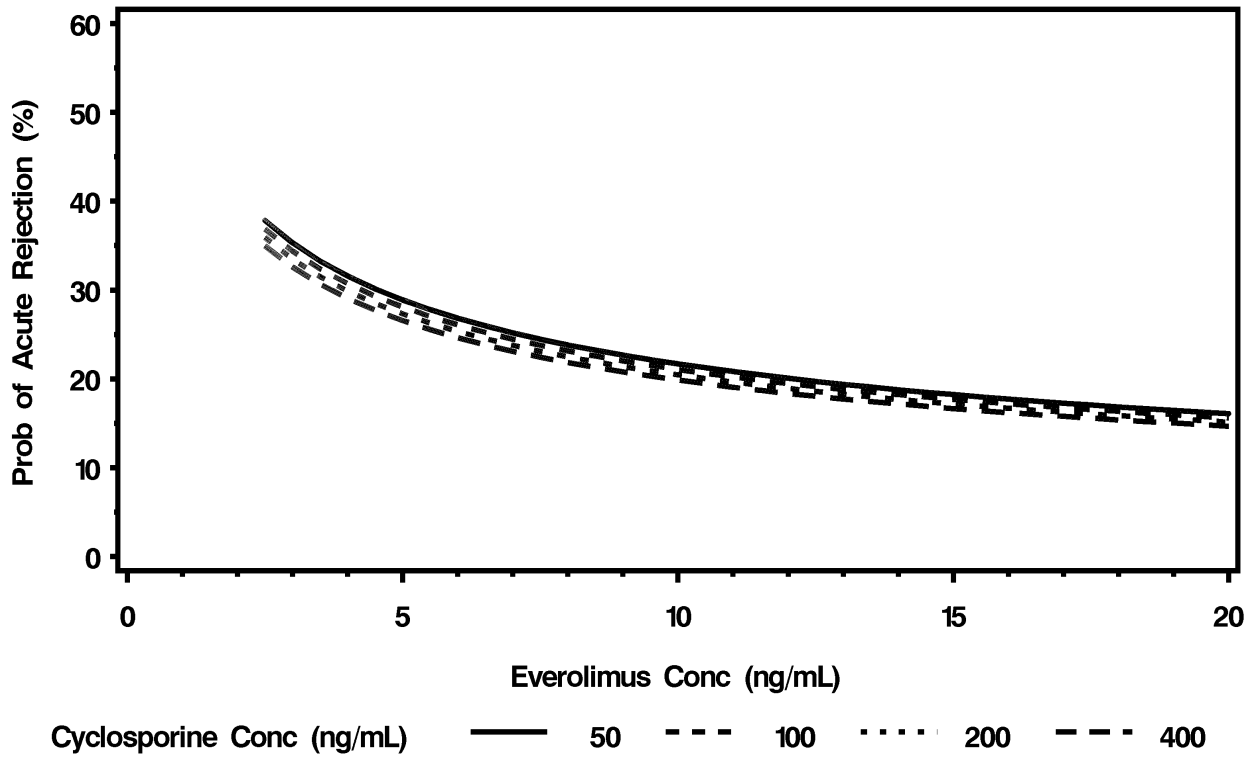
Figure 4-7 Probability of BPAR ISHLT ≥ grade 3A (Day 1-225) as a function of simultaneous everolimus and CsA trough levels (B253)



Cox proportional hazard regression model for BPAR (Day 15-225)

Fifty two BPAR events were seen during the first 14 days post-transplantation (19, 11 and 22 on 1.5 mg/day, 3 mg/day everolimus and AZA, respectively. A second Cox regression model (Day 15-225) was performed (Figure 4-8) to investigate the influence of immunosuppressive treatment from day 15 onwards. This latter Cox regression analysis again showed a strong effect of increasing everolimus C_{min} on freedom from BPAR (p=0.013), however, CsA exposure (p=0.909) was no longer correlated with outcome. Contrasting Figure 4-7 with Figure 4-8 suggests that CsA exposure may mainly be influential and beneficial in the initial post-transplant period, while everolimus exposure apparently shows a more robust correlation with BPAR. Due to smaller number of events in the time period 15-225 as compared to the time period 1-225, the estimates of the effects of CsA and everolimus are certainly less precise.

Figure 4-8 Probability of BPAR ISHLT ≥ grade 3A (Day 15-225) as a function of simultaneous everolimus and CsA trough levels (B253)



4.2.8 Quartile analysis in heart study (B253)

The analyses described below utilized the study B253 data to assess whether further evidence can be obtained to demonstrate that lower CsA levels in the presence of everolimus were not associated with loss of efficacy.

The methodology described applies to the study populations from study B253. Patients were grouped in quartiles according to average CsA levels over the time periods Days 1-28, and later intervals reflecting inflection points for CsA tapering according to the protocol. The CsA levels from all patients in each study were used to determine the percentiles (25^{th}, 50^{th}, and 75^{th}) that defined the quartiles for efficacy analysis. In addition, these values were given for each treatment group. The risk of efficacy failure (the composite endpoint of rejection, death, graft loss, and loss to follow-up) was then provided for each CsA exposure quartile.

As depicted below in Table 4-10, Table 4-11, Table 4-12, and Table 4-13, in study B253, after day 28 the efficacy failure rates in the everolimus (RAD) arms were not significantly different during each of the time intervals across the range of CsA quartiles. However, after day 28 it appeared the lowest incidence of efficacy failure was consistently associated with the highest CsA quartile in the AZA treatment group.

Table 4-10 CsA levels (ng/mL) and rate of biopsy-proven acute rejection by CsA quartile range from Day 1- Month 1(Day 28) – Study B253

Treatment group	AR% (<25th)	25th percentile	AR% (25th–50th)	50th percentile	AR% (50th – 75th)	75th percentile	AR% (≥75th)
Overall		168		215		264	
RAD 1.5 mg	20.4	162	18.0	202	26.0	268	8.0
RAD 3 mg	13.7	168	3.9	214	5.9	261	9.6
AZA	21.6	176	25.5	221	15.7	265	13.7

These findings were also confirmed in the analysis of average CsA levels given over the first year post-transplantation (Table 5-9 and Figure 5-7).

Table 4-11 CsA levels (ng/mL) and rate of biopsy-proven acute rejection by CsA quartile range from Month 2 (Day 29) – Month 3 (Day 90) – Study B253

Treatment group	AR% (<25th)	25th percentile	AR% (25th–50th)	50th percentile	AR% (50th – 75th)	75th percentile	AR% (≥75th)
Overall		192		240		295	
RAD 1.5 mg	2.3	198	9.1	248	7.0	300	4.5
RAD 3 mg	9.5	185	7.1	232	11.9	291	4.7
AZA	13.6	193	26.7	242	26.7	295	8.9

Table 4-12 CsA levels (ng/mL) and percentage with acute rejection by percentile from Month 4 (Day 91) – Month 6 (Day 180) – Study B253

Treatment group	AR% (<25th)	25th percentile	AR% (25th–50th)	50th percentile	AR% (50th – 75th)	75th percentile	AR% (≥75th)
Overall		156		204		257	
RAD 1.5 mg	9.1	156	4.3	208	0.0	257	17.4
RAD 3 mg	5.3	149	0.0	190	10.5	240	0.0
AZA	8.7	170	4.2	214	16.7	259	8.3

Table 4-13 CsA levels (ng/mL) and percentage with acute rejection by percentile from Month 7 (Day 181) – Month 12 (Day 360) - Study B253

	AR% (<25th)	25th percentile	AR% (25th-50th)	50th percentile	AR% (50th-75th)	75th percentile	AR% (≥75th)
Overall		129		170		214	
RAD 1.5 mg	3.0	125	0.0	170	12.1	213	5.9
RAD 3 mg	0.0	125	0.0	158	3.2	207	0.0
AZA	2.9	138	11.8	185	14.7	228	2.9

4.2.9 Exposure-threshold for IVUS outcomes in heart study (B253)

Everolimus

The power of exposure-effect analyses of the IVUS population are limited because of the small sample size. However, exploratory analyses were performed to detect if there was an exposure threshold for everolimus (everolimus Cmin) at which the frequency of IVUS-defined CAV (a change in intimal thickness ≥0.5mm) is reduced. As shown in Table 4-14, there is a numerical decrease in the frequency of change in intimal thickness ≥0.5 mm at a threshold mean everolimus exposure of 5 ng/mL, which becomes a significant decrease in the frequency of vasculopathy at an exposure thresholds of 6 ng/mL and above. Examination of mean maximal intimal thickness also supports the 6 ng/mL exposure threshold (Table 4-15). The ability to demonstrate an exposure threshold within the everolimus groups further supports the argument that the effect of everolimus on intimal thickness reflects a pharmacological effect.

Table 4-14 Odds ratio for CAV (change in intimal thickness ≥0.5mm) for IVUS patients at ascending threshold values of mean everolimus Cmin (B253 12 month data)

RAD C_{min}	Vasculopathy rate ≥ cut-off vs. < cut-off	Estimate of odds ratio	95% CI	p-value
≥2 vs. <2	43/130 (33%) vs. 3/8 (38%)	0.824	0.188, 3.609	0.797
≥3 vs. <3	40 /118 (34%) vs. 6/20 (30%)	1.197	0.427, 3.35	0.732
≥4 vs. <4	33/103 (32%) vs. 13/35 (37%)	0.798	0.358, 1.777	0.580
≥5 vs. <5	25/87 (29%) vs. 21/51 (41%)	0.576	0.279, 1.19	0.136
≥6 vs. <6	16/67 (24%) vs. 30/71 (42%)	0.429	0.206, 0.892	0.02
≥7 vs. <7	11/57 (19%) vs. 35/81 (43%)	0.314	0.142, 0.693	0.004
≥8 vs. <8	7/46 (15%) vs. 39/92 (42%)	0.244	0.099, 0.603	0.002
≥9 vs. <9	6/39 (15%) vs. 40/99 (40%)	0.268	0.103, 0.699	0.0007
≥10 vs. <10	4/30 (13%) vs. 42/108 (39%)	0.242	0.079, 0.742	0.013
≥11 vs. <11	2/23 (9%) vs. 44/115 (38%)	0.154	0.034, 0.688	0.014

Table 4-15 Mean Maximal Intimal Thickness (MIT) for IVUS patients at ascending threshold values of mean everolimus Cmin (B253 12 month data) data)

RAD C_{min}	Mean (SD)	p-value
≥2 vs. <2	0.45 (0.25) vs. 0.44 (0.19)	0.897
≥3 vs. <3	0.46 (0.25) vs. 0.38 (0.19)	0.229
≥4 vs. <4	0.45(0.26) vs. 0.43(0.20)	0.757
≥5 vs. <5	0.43 (0.22) vs. 0.48 (0.28)	0.250
≥6 vs. <6	0.40 (0.211) vs. 0.49 (0.27)	0.042
≥7 vs. <7	0.39 (0.22) vs. 0.48 (0.26)	0.027
≥8 vs. <8	0.38 (0.22)vs. 0.48 (0.25)	0.017
≥9 vs. <9	0.38 (0.22) vs. 0.47 (0.25)	0.057
≥10 vs. <10	0.35 (0.17) vs. 0.47 (0.25)	0.019
≥11 vs. <11	0.35 (0.17)vs. 0.47 (0.25)	0.033

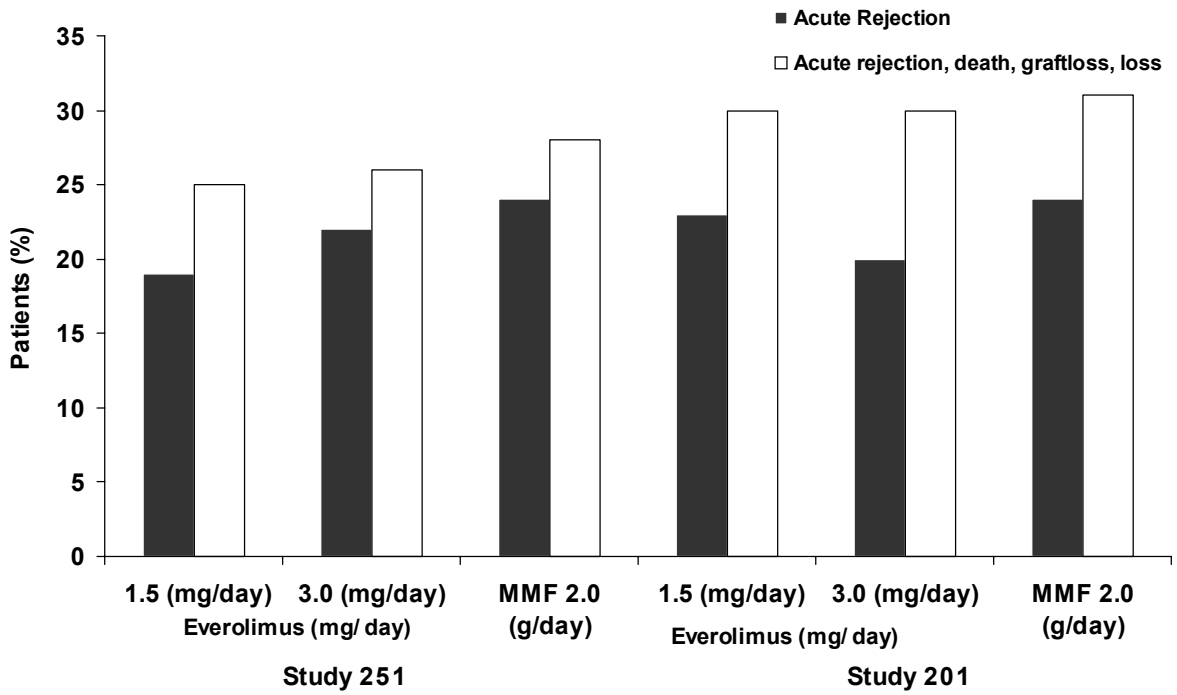
Cyclosporine

Linear regression analyses of 12 month everolimus and CsA exposure (log-transformed) effect on allograft vasculopathy provided evidence of an effect of everolimus exposure on allograft vasculopathy (p=0.0207), while no relationship to CsA exposure was seen (p=0.8626). Similar relationships were seen with logistic regression of CsA and everolimus exposure over the 1st 6 months of the study.

4.2.10 Exposure-effect in kidney transplantation

Results at 12-months from two large, double-blind, randomized, parallel-group, multicenter studies (B201 and B251) indicated that everolimus given as fixed doses of 1.5 or 3.0 mg/day was equivalent in efficacy to MMF 2.0 g/day in kidney transplant patients receiving triple immunosuppressive therapy with conventionally dosed CsA and corticosteroids (Vitko et al., 2003; Lorber 2005). See Figure 4-9.

Figure 4-9 Efficacy at 12 months in kidney transplantation: everolimus studies B201 and B251



The exposure–efficacy analyses of these kidney studies produced similar results to the analysis for the heart study B253. Specifically ,the analyses demonstrated that the risk of experiencing BPAR or graft loss was significantly decreased with everolimus trough levels ≥ 3 ng/mL. Analyses of rejection by 1 ng/ml exposure interval (which is free from model assumptions) also confirmed that the minimum effective level of everolimus is approximately 3 ng/mL. The results are robust when looking at early events, or events over time up to 450 days. Cox regression modeling for two different time windows post-transplantation (Days 1–30 and Days 1–225) was used to assess the simultaneous influence of CsA and everolimus on efficacy. Both regressions showed a highly significant effect of everolimus exposure on BPAR, whereas the presence of CsA in the conventional therapeutic range did not demonstrate a significant effect. Furthermore, the risk of BPAR was found to be 3.4-fold higher with everolimus trough levels below 3 ng/mL.

4.3 Summary of Efficacy Assessments- Heart Transplantation

The incidence of death and graft loss was low in all treatment arms. This study met its primary efficacy objective. The primary analysis of efficacy was supported by all secondary analyses and other exploratory analyses:

- Everolimus, at doses of 1.5 and 3 mg/day in combination with CsA and corticosteroids, was superior to AZA (1-3 mg/kg/day) as measured by the incidence of efficacy failure at Months 6, 12 and 24. In particular, significantly fewer acute rejection episodes occurred in

both everolimus groups, and in a dose-related manner. Everolimus 3.0 mg/day was also superior to everolimus 1.5 mg/day.

- Overall, 12-month graft and patient survival rate was good in all treatment groups, with no statistically significant differences between groups.
- Modeling data from the heart study B253 suggests that improved efficacy outcomes are likely when TDM of everolimus Cmin ≥ 3 ng/mL. Measuring everolimus blood trough concentrations for the purpose of guiding dosing will be integral to assuring low rates of rejection are achieved, and to permit the optimal management of CsA.
- CsA exposure after the first two weeks post-transplantation was not associated with efficacy failure. This supports the concept of CsA reduction in the maintenance phase.
- Both everolimus doses were superior to AZA in preventing allograft vasculopathy, an important contributor for long-term outcome, as measured by changes in maximum intimal thickness.
- Intimal thickness (CAV) was reduced in everolimus arms, in both a dose and concentration-dependent manner.

5 Safety Results

Overview

The focus of the safety assessment is the blinded study period through month 12. Additional analyses of longer-term data are also provided. The following conclusions are based on data from one large, adequate, well-controlled study (B253) in de novo heart transplant recipients:

- Graft and patient survival rate was excellent in all treatment groups, with no significant between-group differences.
- The incidence of nonfatal SAEs and discontinuations of study medication due to AEs was significantly higher in the everolimus 3 mg group compared with the other groups.
- The overall infection rates were comparable between groups. However, there was a significantly lower incidence of viral infections (particularly cytomegalovirus infections) in the everolimus groups than in the AZA group. The incidence of bacterial infections (particularly pneumonia) was significantly higher in the everolimus 3 mg group compared with the AZA group.
- Decreases in mean hemoglobin and platelet counts occurred more frequently in everolimus treated patients than in the AZA group, and were associated with everolimus dose level. Conversely, leukopenia was more frequent in the AZA group.
- Elevations of serum lipids were greater in both everolimus groups; however, mean LDL and HDL were not significantly different between groups. Mean triglycerides were elevated in both everolimus groups compared with the AZA group.
- Treatment with everolimus and CsA was associated with significant increases in mean serum creatinine and decreases in creatinine clearance relative to AZA/CsA treatment .
- The Cox regression analysis showed a strong effect of CsA exposure (p=0.0004) on renal events but no effect of everolimus exposure (p=0.275).

- No clinically relevant trends in vital signs or ECG parameters were observed.
- The rate of malignancies was similar across all treatment groups.
- No changes in adverse event profile were seen in the 24 month analyses.
- Subgroup analyses showed no differences in safety profile with age, sex or race.
- Long-term safety data from patients with up to 4 years of exposure (not shown) did not reveal any new safety concerns associated with the longer duration of exposure. The data indicate that the drug is well tolerated in the target population at a dose of 1.5 mg/day.

5.1 Exposure to Certican

The duration of exposure was similar in the everolimus 1.5 mg and AZA groups, and lower in the everolimus 3 mg group after 90 days because more patients discontinued in this group (Table 5-1). The average daily dose of everolimus or AZA was close to the prospectively planned dose in all groups with no substantial reductions over time; average values for the everolimus 1.5 mg, everolimus 3 mg and AZA groups were 1.3 mg, 2.5 mg and 1.7 mg/kg, respectively.

Table 5-1 Duration of exposure to study medication (ITT population – 12-month analysis) – Heart study B253

Duration of exposure[1]	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Any exposure	209 (100%)	211 (100%)	214 (100%)
≥ 7 days	202 (96.7%)	206 (97.6%)	208 (97.2%)
≥ 28 days	192 (91.9%)	192 (91.0%)	202 (94.4%)
≥ 90 days	182 (87.1%)	171 (81.0%)	180 (84.1%)
≥ 180 days	169 (80.9%)	156 (73.9%)	173 (80.8%)
≥ 360 days	151 (72.2%)	135 (64.0%)	157 (73.4%)
> 450 days	147 (70.3%)	127 (60.2%)	153 (71.5%)

Note: Patients may be counted in more than one duration category.
[1] Includes periods of temporary interruption of study drug.

Long-term analysis – 24 months

The majority of patients received the study medication for >810 days (61% each in the everolimus 1.5 mg and AZA groups and 51% in the everolimus 3 mg group). At Month 24, mean average daily doses of study medication were close to planned doses in the everolimus 1.5 mg group (1.3 mg) and the AZA group (1.5 mg/kg), and below planned doses in the everolimus 3 mg group (2.3 mg).

Extension data – 48 months

Fifty percent of patients in the everolimus 1.5 mg and AZA groups and 42% of patients in the everolimus 3.0 mg group received study medication for >1170 days and slightly less than half of patients (47%, 40% and 46% in the everolimus 1.5 mg, everolimus 3 mg and AZA groups, respectively) received study medication for >1530 days. At Month 48, mean average daily doses of study medication were close to planned doses in the everolimus 1.5 mg and AZA

groups (1.6 mg and 1.2 mg/kg, respectively), and below planned doses in the everolimus 3 mg group (2.4 mg).

5.2 Patient Disposition

The rates of discontinuation of study medication prior to Month 12 were similar for the everolimus 1.5 mg and AZA groups and higher for the everolimus 3 mg group (Table 5-2). The most common reason for discontinuation of study medication was AEs in all treatment groups, with the highest incidence rate reported in the everolimus 3 mg group. However, premature discontinuations of study medication because of unsatisfactory therapeutic effect were less frequently observed in the everolimus 3 mg group compared with both other groups.

Table 5-2 Patient disposition (ITT population - 12-month analysis) – Heart study B253

Patient disposition	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Randomized (ITT population)	209 (100%)	211 (100%)	214 (100%)
Completed 12 months treatment[1]	147 (70.3%)	127 (60.2%)	153 (71.5%)
Discontinued treatment prior to 12 months[1]	62 (29.7%)	84 (39.8%)	61 (28.5%)
Reason for treatment discontinuation[2]			
Adverse event(s)	33 (15.8%)	46 (21.8%)	28 (13.1%)
Abnormal laboratory value(s)	4 (1.9%)	14 (6.6%)	8 (3.7%)
Abnormal test procedure 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%)
Lost to follow-up	0	0	1 (0.5%)
Withdrawal of consent	5 (2.4%)	9 (4.3%)	2 (0.9%)
Discontinued study prior to 12 months[1]	19 (9.1%)	24 (11.4%)	21 (9.8%)
Reason for discontinuing study[3]			
Death	19 (9.1%)	24 (11.4%)	18 (8.4%)
Withdrawal of consent	0	0	2 (0.9%)
Lost to follow-up	0	0	1 (0.5%)

Note: The cutoff date for 12-month disposition data was Day 450.
[1] Time window for 12-month visit was Days 312 to 450.
[2] As indicated by the investigator on the End of Treatment CRF.
[3] As indicated by the investigator on the Study Completion CRF.

Long-term analysis – 24 months

The rates of discontinuation of study medication prior to Month 24 were again similar for the everolimus 1.5 mg and AZA groups and higher for the everolimus 3 mg group (Table 5-3). The most common reason for discontinuation of study medication was AEs in all treatment groups, with the highest incidence rate reported in the everolimus 3 mg group. Premature discontinuations of study medication because of lack of efficacy were less frequently observed in the everolimus 3 mg group compared with both other groups.

Table 5-3 Patient disposition (ITT population - 24-month analysis) – Heart study B253

Patient disposition	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Randomized (ITT population)	209 (100%)	211 (100%)	214 (100%)
Completed 24 months treatment[1]	127 (60.8%)	107 (50.7%)	131 (61.2%)
Discontinued treatment prior to 24 months[2]	82 (39.2%)	104 (49.3%)	83 (38.8%)
Reason for treatment discontinuation			
Adverse event(s)	43 (20.6%)	58 (27.5%)	40 (18.7%)
Abnormal laboratory value(s)	9 (4.3%)	18 (8.5%)	10 (4.7%)
Unsatisfactory therapeutic effect	15 (7.2%)	3 (1.4%)	18 (8.4%)
Protocol violation	2 (1.0%)	4 (1.9%)	2 (0.9%)
Withdrawn consent	6 (2.9%)	11 (5.2%)	3 (1.4%)
Lost to follow-up	0	1 (0.5%)	2 (0.9%)
Administrative problems	0	0	1 (0.5%)
Death	7 (3.3%)	9 (4.3%)	7 (3.3%)
Discontinued study prior to 24 months[3]	23 (11.0%)	33 (15.6%)	31 (14.5%)
Reason for discontinuing study			
Withdrawn consent	2 (1.0%)	3 (1.4%)	5 (2.3%)
Lost to follow-up	0	1 (0.5%)	2 (0.9%)
Death	21 (10.0%)	29 (13.7%)	24 (11.2%)

[1] Patients discontinuing study medication or study after Day 810 are considered 'still on study medication or study.'
[2] Reasons as listed on the End of Treatment CRF.
[3] Reasons as listed on the Study Completion CRF.

Kaplan-Meier analysis was performed for the time to discontinuation and overall, the rates of attrition among the study arms were not significantly different.

5.3 Adverse Events

5.3.1 Overall Adverse Events

The study was blinded until the last patient had completed the 12-month visit and the 12-month data base had been locked. Thus all AEs up to at least 12 months were reported by the investigators in a blinded fashion.

Adverse events were reported while the patient was on study medication, serious AEs while on study medication and during the 30 days after discontinuation of study medication. Information on death and malignancies were reported for the full 24-month period regardless of the duration of study treatment.

Pivotal heart study (B253)

All but 2 patients reported at least one AE, and hypertension was the most frequent AE in all groups. The incidence of anemia and thrombocytopenia was higher in both everolimus dose groups than in the AZA group, and was dose related. The incidence of leukopenia was higher in the AZA group compared with both everolimus groups.

The most frequent AEs that differed by more than 5% from AZA in any everolimus arm are summarized in Table 5-4.

AEs reported with at least a 10% difference between the everolimus 1.5 and 3 mg groups and the AZA group, respectively, included anemia NOS, thrombocytopenia, renal impairment NOS and cytomegalovirus infection. Everolimus dose-related increases (at least 5% difference between the 1.5 and 3 mg groups, respectively) were observed for the following AEs: thrombocytopenia, fatigue, hypokalemia (10% vs. 15% each), pain NOS and back pain.

Table 5-4 Cumulative Incidence of Adverse Events with ≥5 percent points difference between everolimus 1.5 or 3 mg and azathioprine through 12 months – Heart study B253

Adverse Event	RAD 1.5mg (N=209)	RAD 3mg (N=211)	AZA (N=214)
Anaemia NOS			
Incidence - %	30.6	40.8	25.7
Absolute difference from AZA	4.9	15.1	-
(95% CI)	(-3.6 to 13.5)	(6.2 to 23.9)	
	p = 0.260	p = 0.0008	
Leukopenia NOS			
Incidence - %	20.1	20.4	27.1
Absolute difference from AZA	-7.0	-6.7	-
(95% CI)	(-15.1 to 1.1)	(-14.8 to 1.3)	
	p = 0.088	p = 0.102	
Thrombocytopenia			
Incidence - %	10.0	17.1	7.5
Absolute difference from AZA	2.6	9.6	-
(95% CI)	(-2.8 to 8.0)	(3.4 to 15.8)	
	p = 0.349	p = 0.002	
Pericardial effusion			
Incidence - %	22.5	22.7	16.4
Absolute difference from AZA	6.1	6.4	-
(95% CI)	(-1.4 to 13.7)	(-1.1 to 13.9)	
	p = 0.110	p = 0.095	
Diarrhoea NOS			
Incidence - %	18.2	19.9	13.6
Absolute difference from AZA	4.6	6.4	-
(95% CI)	(-2.3 to 11.6)	(-0.7 to 13.4)	
	p = 0.192	p = 0.078	
Incisional hernia NOS			
Incidence - %	8.6	3.8	1.9
Absolute difference from AZA	6.7	1.9	-
(95% CI)	(2.5 to 11.0)	(-1.2 to 5.1)	
	p = 0.002	p = 0.232	
Nausea			
Incidence - %	24.4	28.0	30.8
Absolute difference from AZA	-6.4	-2.9	-
(95% CI)	(-14.9 to 2.1)	(-11.5 to 5.8)	
	p = 0.138	p = 0.515	

Adverse Event	RAD 1.5mg (N=209)	RAD 3mg (N=211)	AZA (N=214)
Chest pain			
Incidence - %	16.7	12.3	10.7
Absolute difference from AZA	6.0	1.6	-
(95% CI)	(-0.5 to 12.5)	(-4.5 to 7.6)	
	p = 0.073	p = 0.611	
Fatigue			
Incidence - %	9.6	15.2	15.0
Absolute difference from AZA	-5.4	0.2	-
(95% CI)	(-11.6 to 0.8)	(-6.6 to 7.0)	
	p = 0.090	p = 0.951	
Oedema lower limb			
Incidence - %	31.1	28.4	25.7
Absolute difference from AZA	5.4	2.7	-
(95% CI)	(-3.2 to 14.0)	(-5.7 to 11.2)	
	p = 0.218	p = 0.526	
Pain NOS			
Incidence - %	4.8	10.4	4.7
Absolute difference from AZA	0.1	5.8	-
(95% CI)	(-3.9 to 4.2)	(0.8 to 10.8)	
	p = 0.957	p = 0.024	
Cytomegalovirus infection			
Incidence - %	7.7	7.6	21.5
Absolute difference from AZA	-13.8	-13.9	-
(95% CI)	(-20.4 to -7.3)	(-20.5 to -7.4)	
	p = 0.000	p = 0.000	
Nasopharyngitis			
Incidence - %	6.7	9.0	3.7
Absolute difference from AZA	3.0	5.3	-
(95% CI)	(-1.3 to 7.2)	(0.6 to 9.9)	
	p = 0.171	p = 0.025	
Pneumonia (any)			
Incidence - %	14.8	15.6	6.1
Absolute difference from AZA	8.8	9.6	-
(95% CI)	(3.0 to 14.5)	(3.7 to 15.4)	
	p = 0.003	p = 0.0014	
Blood creatinine increased			
Incidence - %	10.0	8.1	4.2
Absolute difference from AZA	5.8	3.9	-
(95% CI)	(1.0 to 10.7)	(-0.7 to 8.4)	
	p = 0.019	p = 0.097	
Diabetes mellitus NOS			
Incidence - %	4.8	7.1	1.9
Absolute difference from AZA	2.9	5.2	-
(95% CI)	(-0.5 to 6.3)	(1.3 to 9.2)	
	p = 0.094	p = 0.009	

Adverse Event	RAD 1.5mg (N=209)	RAD 3mg (N=211)	AZA (N=214)
Hyperlipidaemia NOS			
Incidence - %	14.4	13.7	5.1
Absolute difference from AZA	9.2	8.6	-
(95% CI)	(3.6 to 14.8)	(3.1 to 14.1)	
	p = 0.0013	p = 0.0022	
Arthralgia			
Incidence - %	11.0	5.7	12.6
Absolute difference from AZA	-1.6	-6.9	-
(95% CI)	(-7.8 to 4.5)	(-12.4 to -1.5)	
	p = 0.607	p = 0.0125	
Back pain			
Incidence - %	11.5	19.4	13.1
Absolute difference from AZA	-1.6	6.3	-
(95% CI)	(-7.9 to 4.7)	(-0.6 to 13.3)	
	p = 0.616	p = 0.075	
Muscle cramps			
Incidence - %	13.4	9.5	15.4
Absolute difference from AZA	-2.0	-5.9	-
(95% CI)	(-8.7 to 4.7)	(-12.2 to 0.3)	
	p = 0.553	p = 0.062	
Myalgia			
Incidence - %	6.2	6.6	1.4
Absolute difference from AZA	4.8	5.2	-
(95% CI)	(1.2 to 8.5)	(1.5 to 8.9)	
	p = 0.009	p = 0.0057	
Renal impairment NOS			
Incidence - %	24.9	27.0	16.4
Absolute difference from AZA	8.5	10.7	-
(95% CI)	(0.8 to 16.2)	(2.9 to 18.4)	
	p = 0.029	p = 0.007	
Dyspnoea NOS			
Incidence - %	14.8	19.0	13.1
Absolute difference from AZA	1.7	5.9	-
(95% CI)	(-4.9 to 8.4)	(-1.1 to 12.8)	
	p = 0.604	p = 0.098	
Epistaxis			
Incidence - %	2.9	10.9	1.4
Absolute difference from AZA	1.5	9.5	-
(95% CI)	(-1.3 to 4.2)	(5.0 to 14.0)	
	p = 0.296	p = 0.000	
Hypotension NOS			
Incidence - %	6.7	2.8	9.3
Absolute difference from AZA	-2.6	-6.5	-
(95% CI)	(-7.8 to 2.5)	(-11.0 to -2.0)	
	p = 0.315	p = 0.005	

Infrequently reported (<3%) adverse events of interest from the heart study B253 included pneumonitis, hemolytic uremic syndrome (HUS), and liver test abnormal. No new safety relationships were uncovered in the 24 month analysis.

5.3.2 Serious Adverse Events

The incidence of nonfatal SAEs (including serious infections) was higher in the everolimus 3 mg group compared with the other groups. At least a 5% between-group difference was observed for the 4 nonfatal SAEs: anemia NOS, pericardial effusion, any pneumonia and cytomegalovirus infection. The most commonly affected system organ classes and the most common nonfatal SAEs (≥ 5% in any group) are summarized in Table 5-5.

Table 5-5 Number (%) of patients reporting nonfatal SAEs (≥ 5% in any group) (safety population – 12-month analysis) – Heart study B253

MedDRA system organ class Preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Any non-fatal SAE	135 (64.6%)	149 (70.6%)	129 (60.3%)
Blood & lymphatic system disorders	13 (6.2%)	30 (14.2%)	14 (6.5%)
Anemia NOS	4 (1.9%)	15 (7.1%)	6 (2.8%)
Cardiac disorders	42 (20.1%)	42 (19.9%)	33 (15.4%)
Pericardial effusion	20 (9.6%)	16 (7.6%)	6 (2.8%)
Gastrointestinal disorders	27 (12.9%)	29 (13.7%)	21 (9.8%)
General disorders & admin. site conditions	17 (8.1%)	28 (13.3%)	26 (12.1%)
Pyrexia	6 (2.9%)	14 (6.6%)	12 (5.6%)
Infections & infestations	45 (21.5%)	72 (34.1%)	52 (24.3%)
Cytomegalovirus infection	3 (1.4%)	6 (2.8%)	19 (8.9%)
Pneumonia (any)	19 (9.1%)	27 (12.8%)	11 (5.1%)
Injury & poisoning	3 (1.4%)	11 (5.2%)	4 (1.9%)
Investigations	12 (5.7%)	8 (3.8%)	14 (6.5%)
Metabolism & nutrition disorders	12 (5.7%)	24 (11.4%)	19 (8.9%)
Musculoskeletal, connective tiss. & bone disorders	3 (1.4%)	12 (5.7%)	6 (2.8%)
Neoplasms (benign & malignant)	12 (5.7%)	8 (3.8%)	6 (2.8%)
Nervous system disorders	13 (6.2%)	17 (8.1%)	21 (9.8%)
Renal & urinary disorders	22 (10.5%)	27 (12.8%)	16 (7.5%)
Renal impairment NOS	5 (2.4%)	11 (5.2%)	6 (2.8%)
Respiratory, thoracic & mediastinal disorders	27 (12.9%)	29 (13.7%)	14 (6.5%)
Surgical & medical procedures	13 (6.2%)	11 (5.2%)	11 (5.1%)
Vascular disorders	22 (10.5%)	17 (8.1%)	13 (6.1%)

Note: Cutoff date for nonfatal SAEs was Day 450.

Long-term safety population (24-month analysis)

Between Days 1 to 810, the most frequent nonfatal SAEs (≥ 5% of patients in any group) are summarized in Table 5-6.

Table 5-6 Number (%) of patients reporting common nonfatal serious adverse events, including infections (≥ 5% in any group) (safety population – 24-month analysis) – Heart study B253

MedDRA Preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Any nonfatal SAE	150 (71.8%)	162 (76.8%)	140 (65.4%)
Pericardial effusion	22 (10.5%)	16 (7.6%)	6 (2.8%)
Pneumonia (any)	28 (13.4%)	33 (15.6%)	11 (5.1%)
Dyspnea NOS	11 (5.3%)	6 (2.8%)	4 (1.9%)
Renal failure acute	11 (5.3%)	10 (4.7%)	9 (4.2%)
Pyrexia	10 (4.8%)	17 (8.1%)	12 (5.6%)
Renal impairment NOS	7 (3.3%)	16 (7.6%)	6 (2.8%)
Anemia NOS	6 (2.9%)	17 (8.1%)	8 (3.7%)
Cytomegalovirus infection	3 (1.4%)	5 (2.4%)	19 (8.9%)

Between Days 1 to 810, the incidence of serious infections was similar in the everolimus 1.5 mg and AZA groups (31% and 27%, respectively), and significantly higher in the everolimus 3 mg group (43%). Serious infections reported by at least 5% of patients in any group were any pneumonia (13%, 16% and 5% in the everolimus 1.5 and 3 mg and the AZA groups, respectively) and cytomegalovirus infection (9% in the AZA group).

5.3.3 Deaths

The incidence of deaths was similar in all groups (9%, 11%, and 8% in the everolimus 1.5 and 3 mg groups and the AZA group, respectively). Primary causes for death reported for at least 2 patients in any group are summarized in Table 5-7.

Table 5-7 Incidence of deaths (primary cause) (≥ 2 patients in any group) (safety population – 12-month analysis) – Heart study B253

MedDRA Preferred term	Ever 1.5 mg (N=209)	Ever 3 mg (N=211)	AZA (N=214)
Cardiac disorders	3 (1.4%)	3 (1.4%)	2 (0.9%)
Multiorgan failure	2 (1.0%)	3 (1.4%)	1 (0.5%)
Graft rejection	0	0	2 (0.9%)
Heart transplant rejection	2 (1.0%)	0	2 (0.9%)
Pneumonia (any)	0	2 (0.9%)	0
Sepsis NOS	2 (0.5%)	1 (0.3%)	0
Respiratory failure (exc neonatal)	2 (1.0%)	0	0
Intracranial hemorrhage NOS	1 (0.5%)	2 (0.9%)	0

Long-term safety population (24-month analysis)

Between Days 1 to 810, the incidence of deaths was similar in all groups (21 patients [10%], 29 patients [14%], and 24 patients [11%] in the everolimus 1.5 and 3 mg groups and the AZA group, respectively). Primary causes of death reported for at least 2 patients in any group are summarized in Table 5-8 below.

Table 5-8 Primary reasons for death reported in ≥ 2 patients in any group (safety population) (Months 0 to 24) – Heart study B253

MedDRA Preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Any death	21 (10%)	29 (13.7%)	24 (11.2%)
Sepsis NOS	3 (1.4%)	1 (0.5%)	4 (1.9%)
Multi-organ failure	2 (1.0%)	3 (1.4%)	1 (0.5%)
Heart transplant rejection [1]	2 (1.0%)	0	2 (0.9%)
Transplant rejection [1]	0	1 (0.5%)0	2 (0.9%)
Pneumonia (any)	0	3 (1.4%)	1 (0.5%)
Intracranial hemorrhage NOS	1 (0.5%)	2 (0.9%)	0
Respiratory failure	2 (1.0%)	0	0

[1] There was an inconsistency in coding, and both of these terms that describe rejection are included in the database. These terms were mutually exclusive, and each patient was included under one term only.

The incidence of death due to infection summarizing all available patient data is shown in Table 5-9.

Table 5-9 Deaths due to infection (safety population) (Months 0 to 24) – Heart study B253

MedDRA Preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Deaths due to infections & infestations	4 (1.9%)	6 (2.8%)	7 (3.3%)
Fungal infection NOS	0	1 (0.5%)	0
Lung infection NOS	0	0	1 (0.5%)
Pneumocystis Carinii pneumonia	0	1 (0.5%)	0
Pneumonia (any)	0	3 (1.4%)	1 (0.5%)
Sepsis NOS	3 (1.4%)	1 (0.5%)	4 (1.9%)
Sepsis secondary	0	1 (0.5%)	0
Septic shock	1 (0.5%)	0	1 (0.5%)

5.3.4 Adverse events of special interest

Major adverse cardiac events, infections, wound healing and malignancies are discussed in more detail in Sections 5.5.3 through 5.5.6.

5.4 Clinical Laboratory Evaluations

Hematology

Laboratory abnormalities for hematological parameters of interest, based on notable criteria, are summarized in Table 5-10.

Table 5-10 Number of patients with hematological abnormalities based on notable ranges (safety population, 0-12 months) – Heart study B253

Laboratory test	Notable (abnormal) values		RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Hemoglobin	low	<7.0 g/dL x 10^9/L	6 (2.9%)	16 (7.7%)[c]	8 (3.8%)
Leukocytes (total)	low	<2.8 x 10^9/L	16 (7.8%)[a]	26 (12.4%)[b]	43 (20.2%)
	high	>16 x 10^9/L	71 (34.5%)	78 (37.3%)	87 (40.8%)
Neutrophils	low	<1.5 x 10^9/L	3 (1.5%)[a]	6 (2.9%)[b]	17 (8.0%)
Platelet count	low	Day 1 to Day 28: ≤50 x 10^9/L after Week 4: ≤75 x 10^9/L	15 (7.3%)	16 (7.7%)	9 (4.2%)

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg (p<0.05, based on 95% binomial confidence intervals).

Long-term safety population (24-month analysis)

Significant between-group differences for laboratory abnormalities based on notable criteria were observed for hemoglobin, total neutrophil and leukocyte counts, as shown in Table 5-11.

Table 5-11 Number of patients with hematological abnormalities based on notable ranges (safety population, 0-24 months) – Heart study B253

Laboratory test	Notable (abnormal) values		RAD 1.5 mg (N=206)	RAD 3 mg (N=209)	AZA (N=213)
Hemoglobin	low	<7.0 g/dL	7 (3.4%)	17 (8.1%)[c]	9 (4.2%)
Leukocytes (total)	low	<2.8 x 10^9/L	15 (7.3%)[a]	29 (13.9%)[b]	48 (22.3%)
Neutrophils	low	<1.5 x 10^9/L	3 (1.5%)[a]	6 (2.9%)[b, c]	19 (8.9%)

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg (p<0.05, based on 95% confidence intervals).

Chemistry

Mean total bilirubin was slightly above the upper level of normal (ULN) at baseline in all groups. Mean values were within the normal range in all groups from Month 1 onwards, but values in the AZA group were slightly higher than in both everolimus groups at all time points. Decreases from baseline were significantly larger for both everolimus groups compared with the AZA group at all visits from Week 1 onwards. Notably high values (≥34.2 µmol/L) were lowest in the everolimus 1.5 mg group and highest in the AZA group, with no significant between-group differences.

A small, transient post-transplant increase in mean ALT/SGPT was observed in all groups. Thereafter, mean ALT/SGPT decreased over time in all groups, but were within the normal range from Month 2 onwards in all groups. Changes in ALT/SGPT from baseline were significantly different between both everolimus groups and AZA at most post-baseline visits, possibly due to the distinctly higher mean baseline value in the AZA group. The incidence of notably high values (≥3 x ULN) was higher in both everolimus groups compared with the AZA group, but no significant between-group differences were observed.

Similarly to ALT/SGPT, mean AST/SGOT decreased from relatively high baseline values, and was within the normal range as early as Week 1 in all groups. At several time points, changes in AST/SGOT from baseline were significantly different between the everolimus

3 mg group and the AZA group, but the higher mean baseline value in the AZA group might have partly contributed to this finding. The incidence of notably high values (≥3 x ULN after week 2) was comparable for the everolimus 1.5 and AZA groups, and was higher in the everolimus 3 mg group, and the difference between the two everolimus dose groups was significant.

Mean alkaline phosphatase was increased from baseline in all groups. Mean values were generally higher in both everolimus groups compared with the AZA group, and differences in changes from baseline reached statistical significance more frequently for each everolimus group compared with the AZA group. Notably high values (≥3 x ULN) were infrequently observed, but were slightly higher in the everolimus 3 mg group than in both other groups.

For adult male patients, a low testosterone value was defined as < 10 nmol/L (< 50 years) or < 7 nmol/L (≥ 50 years), a high FSH value was defined as > 8 IU/L (both age groups) and a high LH value was defined as > 12 IU/L (both age groups). Overall, there was a significant difference at Months 6 and 12 in endocrine parameters between everolimus- and AZA-treated male patients, with lower testosterone levels and elevated gonadotropins (LH and FSH) in both everolimus groups compared with the AZA group. Laboratory-defined hypogonadism in adult males – defined as both low (age-adjusted) testosterone and LH > 15 IU/L – was higher in both everolimus groups compared with the AZA group.

Laboratory abnormalities for selected biochemical parameters of interest, based on notable criteria, are summarized in Table 5-12.

Table 5-12 Number of patients with biochemistry abnormalities based on notable ranges (safety population, 0-12 months) – Heart study B253

Laboratory test	Notable (abnormal) values		RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
SGOT (AST)	High	≥3 x ULN after Week 2	5 (2.6%)	14 (7.0%)[c]	7 (3.4%)
SGPT (ALT)	High	≥3 x ULN after Week 2	37 (18.0%)	40 (19.0%)	26 (12.1%)
Bilirubin (total)	High	>34.2 µmol/L	33 (16.0%)[a]	51 (24.2%)[c]	63 (29.4%)
Alkaline phosphatase	High	≥3 x ULN	5 (2.4%)	11 (5.2%)	5 (2.3%)
Creatinine	High	30% increase from Baseline	144 (69.6%)[a]	158 (74.9%)[b]	117 (54.7%)
Cholesterol total	High	≥9.1mmol/L	23 (11.1%) [a]	28 (13.3%) [b]	7 (3.3%)
Triglycerides	High	≥8.5mmol/L	7 (3.4%) [a]	8 (3.8%) [b]	1 (0.5%)
Glucose (fasting)	High	<2.5 mmol/L	6 (2.9%)	4 (1.9%)	4 (1.9%)
	Low	>13.9 mmol/L	23 (11.2%)	37 (17.5%)	38 (17.8%)
Amylase	High	≥2 x ULN	31 (15.0%)	47 (22.3%)	44 (20.6%)
Uric acid	High	≥714µmol/L (male), ≥535µmol/L (female)	50 (24.3%)	56 (26.5%)	46 (21.5%)
Hypogonadism *	High	Definition see text	7/133 (5.3%)[a]	10/122 (8.2%)[b]	1/148 (0.7%)

* Definition of hypogonadism, see text. The denominator is the number of patients with testosterone and LH data at Month 12. Patients with low testosterone and LH data at Month 6, but without assessments at Month 12, were also considered as having hypogonadism.

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg ($p<0.05$, based on 95% confidence intervals).

5.4.1 Clinical laboratory parameters of special interest

Creatinine, creatinine clearance and serum lipids are discussed in more detail in Section 5.5.1 and 5.5.2.

5.5 Safety Topics of Special Interest

5.5.1 Creatinine and creatinine clearance (CrCl)

On-treatment creatinine values are summarized by visit in Table 5-13.

Mean creatinine was increased from baseline levels in all groups; however, mean creatinine levels were consistently higher in both everolimus groups than in the AZA group at all visits. The changes from baseline were significantly different between the everolimus 3 mg and AZA groups from Week 1 to Month 12, and between the everolimus 1.5 mg and AZA groups from Months 2 to 12. Notably high creatinine values were more frequently observed in both everolimus groups compared with the AZA group, and were related to everolimus dose level (see also Table 5-12).

Table 5-13 Creatinine: Summary statistics by visit window (safety population, 0-12 months) – Heart study B253

	Mean (mean change from Baseline)							
Variable	**N**	**Month 1**	**N**	**Month 3**	**N**	**Month 6**	**N**	**Month 12**
Creatinine (μmol/L)								
RAD 1.5 mg	188	144 (7)	165	164 (24)[a]	152	177 (40)[a]	137	181 (45)[a]
RAD 3.0 mg	188	150 (16)[b,c]	151	164 (28)[b]	151	177 (47)[b]	131	188 (57)[b]
AZA	197	129 (-5)	163	135 (2)	163	150 (17)	149	147 (14)

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg (p<0.05, Wilcoxon rank sum test).

Corresponding to the described creatinine characteristics, mean creatinine clearance (Cockcroft-Gault formula; Table 5-14) was decreased from baseline in both everolimus groups; however, in the AZA group, almost no change from baseline was observed. The changes from baseline were significantly different between the everolimus 3 mg and AZA groups as early as Week 2 to Month 12, and between the everolimus 1.5 mg and AZA groups from Months 3 to 12.

Table 5-14 Creatinine clearance (Cockcroft-Gault): Summary statistics by visit window (safety population, 0-12 months) – Heart study B253

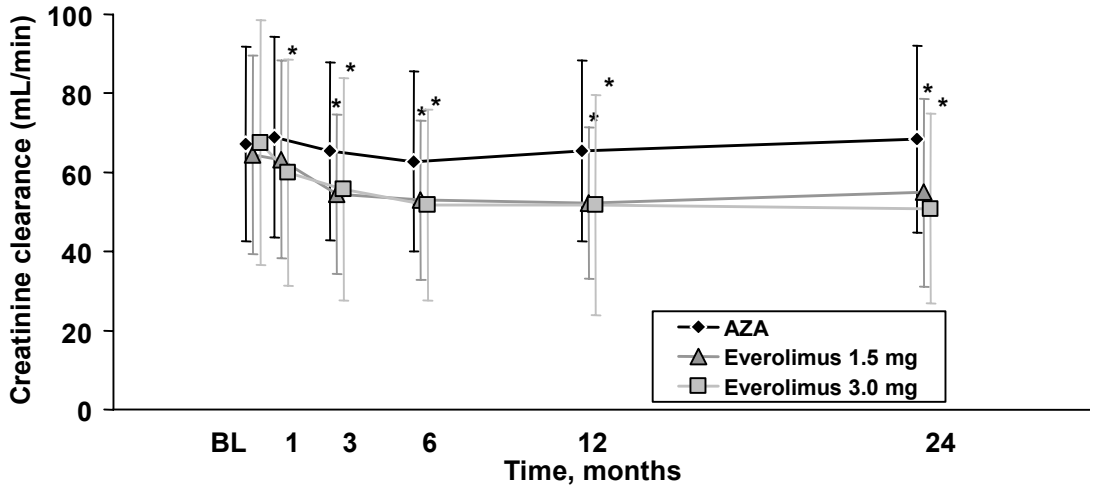
	Mean (mean change from Baseline)							
Variable	**N**	**Month 1**	**N**	**Month 3**	**N**	**Month 6**	**N**	**Month 12**
Creatinine clearance (m//minL)								
RAD 1.5 mg	188	63.6 (-2.6)	165	54.6 (-11.4)[a]	152	53.2 (-13.1)[a]	137	52.1 (-14.9)[a]
RAD 3.0 mg	188	60.1 (-8.7)[b,c]	151	55.8 (-12.6)[b]	151	51.9 (-19.1)[b]	131	51.9 (-18.5)[b]
AZA	197	69.0 (1.2)	163	65.3 (-2.3)	163	62.5 (-4.6)	149	65.3 (-2.6)

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg (p<0.05, Wilcoxon rank sum test).

Figure 5-1 shows creatinine clearance that included values obtained after discontinuation of study medication. Similar results were obtained in an on-therapy analysis of creatinine and creatinine clearance.

Taken together, the findings on creatinine and creatinine clearance show an effect of the combination of everolimus and CsA on renal function.

Figure 5-1 Study B253: Creatinine Clearance over time



	BL	1	3	6	12	24
AZA, n	205	201	166	167	152	122
Everolimus 1.5 mg, n	194	193	169	156	140	116
Everolimus 3.0 mg, n	206	190	153	152	132	100

***P* < 0.001**
Error bars = Standard deviation.

5.5.2 Lipids

The prevalent use of statin medications reflected protocol requirement. Although the number of patients who received HMG-CoA reductase inhibitors was high (approximately 90% in all groups), mean total cholesterol increased in all groups. Mean values as well as the changes from baseline were generally greater in both everolimus groups compared to the AZA group from Week 2 to Month 12, but remained within normal ranges throughout the study. The impact of study medications affected all groups and was stable beyond study visits. From Week 2 to Month 12, the differences in changes from baseline were significant for both everolimus groups compared with the AZA group (Table 5-15).

Table 5-15 Lipid variables: Change from baseline by visit-window (safety population, 0-12 months) – Heart study B253

Variable	Mean (mean change from Baseline)							
	N	Month 1	N	Month 3	N	Month 6	N	Month 12
Total cholesterol (mmol/L)								
RAD 1.5 mg	188	5.4 (2.3)[a]	165	6.1 (3.0)[a]	151	6.0 (2.9)[a]	137	5.7 (2.6)[a]
RAD 3.0 mg	188	5.5 (2.4)[b]	151	6.2 (3.0)[b]	151	6.2 (3.0)[b]	131	5.8 (2.6)[b]
AZA	197	5.0 (1.9)	163	5.5 (2.3)	162	5.4 (2.2)	149	5.2 (2.0)
LDL cholesterol (mmol/L)								
RAD 1.5 mg	180	2.8 (1.2)	148	3.2 (1.6)	120	3.2 (1.5)	117	3.1 (1.4)
RAD 3.0 mg	170	2.9 (1.1)	126	3.2 (1.5)	121	3.2 (1.5)	108	3.0 (1.3)
AZA	188	2.7 (1.0)	155	3.1 (1.4)	148	3.0 (1.3)	138	2.9 (1.2)
HDL cholesterol (mmol/L)								
RAD 1.5 mg	188	1.7 (0.8)	165	1.7 (0.8)[a]	151	1.5 (0.6)	137	1.3 (0.4)
RAD 3.0 mg	187	1.6 (0.7)	150	1.6 (0.7)[b]	150	1.5 (0.6)	130	1.3 (0.4)
AZA	197	1.6 (0.7)	163	1.5 (0.5)	162	1.4 (0.5)	149	1.3 (0.4)
Triglycerides (mmol/L)								
RAD 1.5 mg	188	2.0 (0.8)[a]	165	2.6 (1.4)[a]	151	3.1 (1.9)[a]	137	3.1 (1.9)[a]
RAD 3.0 mg	188	2.2 (1.0)[b]	151	2.8 (1.7)[b]	150	3.1 (2.0)[b]	131	3.0 (1.8)[b]
AZA	197	1.7 (0.4)	163	2.0 (0.8)	162	2.1 (0.9)	149	2.1 (0.9)

a: RAD 1.5 mg vs AZA; b: RAD 3 mg vs AZA; and c: RAD 1.5 mg vs RAD 3 mg ($p<0.05$, Wilcoxon rank sum test).

The incidence of notably high post-baseline total cholesterol was similar in both everolimus groups (11% and 13% in the 1.5 and 3 mg groups) and significantly lower in the AZA group (3%) (Table 5-12). Hypercholesterolemia was reported as an AE in 10%, 10% and 7% of patients in the everolimus 1.5 and 3 mg groups and the AZA group, respectively; hyperlipidemia NOS was reported as an AE in 14%, 14% and 5% of patients.

Distinct elevations from baseline were also observed for mean LDL and HDL, but mean values were comparable in all treatment groups at all visits (Table 5-15). Although differences in changes from baseline in mean HDL were significant at a few visits for both everolimus groups compared with the AZA group, no consistent treatment-related changes were observed. Notably high LDL values were more frequent in both everolimus groups.

Mean triglycerides increased in all groups, and mean values as well as changes from baseline were consistently larger in both everolimus groups compared with the AZA group from Week 2 to Month 12 Table 5-15). Mean values in both everolimus groups were above the ULN (2.3 mmol/L) from Months 2 to 12, the differences in changes from baseline were significant compared with the AZA group. The overall incidence of notably high triglyceride values was low, but significantly higher in both everolimus groups compared with the AZA group (Table 5-12). Hypertriglyceridemia was reported as an AE in 4%, 6% and 3% of patients in the everolimus 1.5 and 3 mg groups, and the AZA group, respectively

The distribution of lipid parameters according to cut-offs based upon the NCEP guidelines showed similar relationships to those described above.

An important predictor of cardiovascular risk is the LDL/HDL ratio, and no association to treatment was observed.

The results of the IVUS examinations and long term follow-up for major adverse cardiovascular events (noted below) suggest an overall favorable vascular effect of everolimus exposure. Hence, the vascular impact of the observed dyslipidemic effect may be outweighed by the effects of everolimus on vascular smooth muscle.

5.5.3 Major adverse cardiovascular events (MACE)

To further explore whether lipid abnormalities would be reflected in an increase in vascular disease, the adverse event database was reviewed for the incidence of major adverse cardiovascular events, defined as graft related and non-graft-related, and also according to whether these were fatal or nonfatal events. Adverse events for coronary disease were also captured in this analysis, as the prognosis in heart transplant recipients is more grave than native coronary disease.

Graft related major adverse cardiovascular events was defined as:

- acute myocardial infarction
- congestive heart failure
- percutaneous cardiac intervention
- coronary artery bypass grafting
- implantable cardiac defibrillator
- ventricular fibrillation / ventricular tachycardia
- angina pectoris
- sudden death.

Non-graft related major adverse cardiovascular events consisted of:

- transient ischemic attack
- cerebrovascular accident
- peripheral vascular disease.

Patients provided major adverse cardiovascular events information during the period they received study medication, as part of routine adverse event reporting, There was a trend toward a lower incidence of all graft-related major adverse cardiovascular among patients treated with everolimus when considering data from baseline through month 48, with no increase in non-graft related major adverse cardiovascular events (Table 5-16).

Table 5-16 Cumulative summary of patients with any major adverse cardiovascular events (MACE) (safety population at 48 months*) – Heart study B253

Category of MACE	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Total MACE	49 (23.4%)	48 (22.7%)	56 (26.2%)
Graft related	31 (14.8%)	38 (18.0%)	44 (20.6%)
Non-graft related	22 (10.5%)	12 (5.7%)	16 (7.5%)
Non-fatal MACE	44 (21.1%)	41 (19.4%)	52 (24.3%)
Graft related	27 (12.9%)	33 (15.6%)	40 (18.7%)
Non-graft related	20 (9.6%)	10 (4.7%)	16 (7.5%)
Fatal MACE	7 (3.3%)	9 (4.3%)	10 (4.7%)
Graft related	5 (2.4%)	6 (2.8%)	9 (4.2%)
Non-graft related	2 (1.0%)	3 (1.4%)	1 (0.5%)

* Patients provided MACE information as long as they were on study medication.

In order to look at major adverse cardiovascular events unrelated to perioperative complications (due technical issues, preexisting conditions such as pulmonary hypertension, complications related to bypass or anticoagulation, transplant coronary disease, etc.) major adverse cardiovascular events after Month 1 were analyzed.

The results in Table 5-17, show that the everolimus groups had fewer graft-related, nonfatal graft-related, and fatal graft-related major adverse cardiovascular events than the AZA group. The difference between the everolimus 1.5 mg and AZA groups was significant for non-fatal graft-related major adverse cardiovascular events and approached significance for all graft-related major adverse cardiovascular events. A KM analysis of the time to nonfatal graft related MACE showed significantly better event-free survival for the everolimus 1.5 mg arm.

Table 5-17 Cumulative incidence of graft-related MACE from Day 28 to Month 48 – Heart study B253

Category of MACE	RAD 1.5 mg (N=202)		RAD 3 mg (N=206)		AZA (N=211)
	n (%)	p-value[1]	n (%)	p-value[1]	n (%)
All Graft-related MACE*	19 (9.4%)	0.074	25 (12.4%)	0.324	33 (15.6%)
Nonfatal graft-related MACE*	15 (7.4%)	0.039	20 (9.7%)	0.225	29 (13.7%)
Fatal Graft-related MACE*	5 (2.4%)	0.417	6 (2.9%)	0.601	9 (4.3%)

[1]Fisher exact test
* Patients provided MACE information as long as they were on study medication.

5.5.4 Infections

Most infections occurred in the early post transplant period with few new infections occurring in the second year of follow-up. As shown in Table 5-19, the incidence of infections through 12 months was similar in the everolimus 1.5 and AZA groups and higher in the everolimus 3 mg group. The incidence of any pneumonias was higher in the everolimus 1.5 and 3 mg groups compared with the AZA group; however, the incidence of cytomegalovirus infection was higher in the AZA group compared with both everolimus groups.

Table 5-18 Infections reported in at least 10% of patients in any group through month 12 – Heart study B253

MedDRA Preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Any infection	151 (72.2%)	162 (76.8%)	150 (70.1%)
Cytomegalovirus infection	16 (7.7%)	16 (7.6%)	46 (21.5%)
Herpex simplex	17 (8.1%)	12 (5.7%)	22 (10.3%)
Pneumonia (any)	31 (14.9%)	33 (15.6%)	13 (6.1%)
Upper respiratory tract infection NOS	16 (7.7)	18 (8.5%)	23 (10.7%)
Urinary tract infection NOS	18 (8.6%)	27 (12.8%)	21 (9.8%)

Five cases of *P. carinii* infections were reported (one patient each in the everolimus 1.5 mg and AZA groups and 3 patients in the everolimus 3 mg group). No new safety relationships were revealed in the 24 month analysis.

5.5.5 Wound Healing

Impaired wound healing has been noted as a complication of treatment with sirolimus. In kidney transplantation, an increased incidence of lymphocele has been associated with everolimus or sirolimus treatment as compared to either MMF or AZA. To further investigate and the implications of this finding for heart transplant patients, the 24-month safety data of heart transplant study B253 was surveyed for MedDRA terms which directly or indirectly may signal impaired wound healing:

- postoperative wound complications NOS,
- postoperative wound secretion, and
- postoperative hernia were included in this search; however, postoperative infections and postoperative hematoma/ erythema were excluded.

These terms are not mutually exclusive; and patients may appear in more than one row in the Table 5-19.

Table 5-19 Adverse events potentially associated with impaired wound healing (24-month analysis) – Heart study B253

MedDRA preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Impaired healing	0	1 (0.5%)	0
Lymphocele	10 (4.8%)	9 (4.3%)	2 (0.9%)
Pericardial effusion	48 (23.0%)	49 (23.2%)	36 (16.8%)
Cardiac tamponade	6 (2.9%)	10 (4.7%)	3 (1.4%)
Postoperative hernia	1 (0.5%)	0	1 (0.5%)

MedDRA preferred term	RAD 1.5 mg (N=209)	RAD 3 mg (N=211)	AZA (N=214)
Postoperative wound complication NOS	8 (3.8%)	8 (3.8%)	10 (4.7%)
Wound complication	0	2 (0.9%)	0
Wound dehiscence	5 (2.4%)	6 (2.8%)	2 (0.9%)
Wound secretion	1 (0.5%)	1 (0.5%)	0
Wound drainage	0	4 (1.9%)	0

The increase in pericardial effusion and (inguinal) lymphocele detected among everolimus treatment groups is consistent with an effect on wound healing

5.5.6 Malignancy

Pivotal heart study (B253)

The incidence of malignancies was similar in all groups (7%, 6% and 4% in the everolimus 1.5 and 3 mg groups and the AZA group, respectively). Post-transplant lymphoproliferative disease was reported for 3 patients each in the everolimus 1.5 mg and AZA groups and 4 patients in the everolimus 3 mg group.

Long-term safety population (24-month analysis)

Between Days 1 to 810, the incidence of malignancies was similar in all groups (8% each). The most common malignancies were associated with the skin (10, 5, and 6 patients in the 1.5 mg and 3 mg groups and the AZA group, respectively). No new cases of post-transplant lymphoproliferative disease were reported.

5.6 Exposure-Safety Relationships

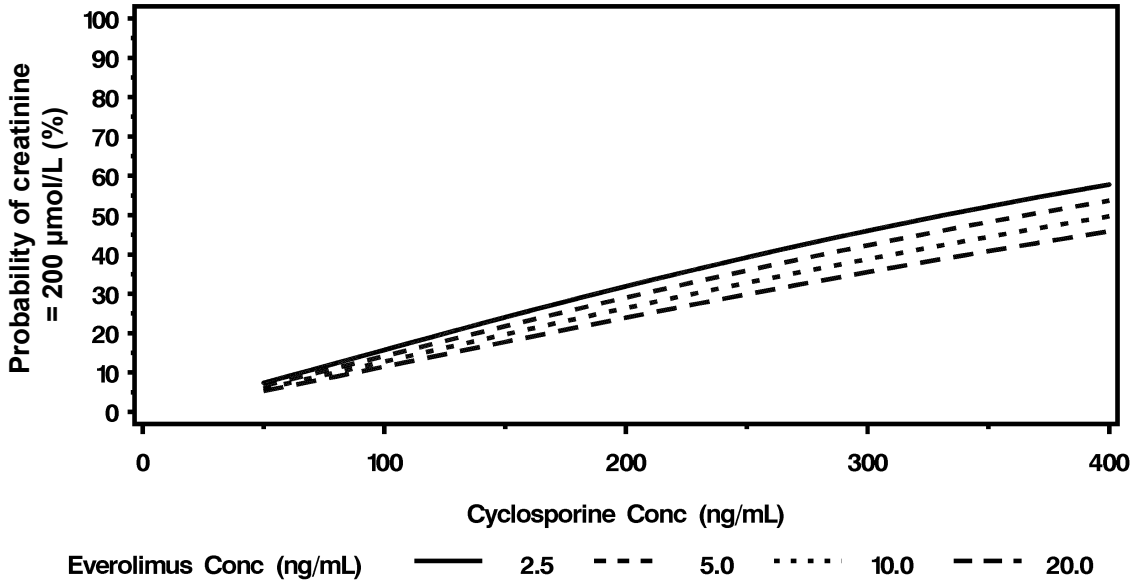
5.6.1 Renal function by simultaneous everolimus / CsA exposure

In order to explore the relationship between drug exposure and renal function, Cox regression was used to model "renal events" defined as the first occurrence of a creatinine value ≥ 200µmol/L (2.3mg/dL). The data of the first month (day 30) were discarded to reduce bias caused by perioperative circulatory issues; see Figure 5-2.

The two factors describing exposure (log-transformed) were included: the geometric means of everolimus and CsA C_{min} until the first occurrence of a creatinine value greater than 200µmol/L, or else censored on Day 225. The creatinine value at day 30 was used as a covariate.

The Cox regression analysis showed a strong effect of CsA exposure (p=0.0004) on renal events but no effect of everolimus exposure (p=0.275). The creatinine value at day 30 was a statistically significant covariate (p=0.0001).

Figure 5-2 Probability of Creatinine ≥ 200 µmol/L (Day 30-225) as a function of simultaneous everolimus and CsA trough levels – Heart study B253



The creatinine value at day 30 was used as a covariate (plot shown for the mean value of 130 µmol/L)

In everolimus treated patients PK/PD relationships demonstrated that CsA exposure strongly correlated to the renal dysfunction.

Supplemental analyses of exposure relationships for renal function

It is critical to define the appropriate period in which to examine drug effects on renal function. Defining a renal event as a change from baseline at randomization may be confounded due to factors unrelated to drug exposure that impact renal function during the peri-transplant period. Preoperatively, renal function may be impaired due to heart failure-related circulatory compromise. Further intraoperative hypoperfusion may lead to acute tubular necrosis, with renal compromise even to the point of requiring short-term dialysis (another confounder). Renal function after surgery is therefore highly variable and creatinine overall improves over time. Therefore, in order to properly evaluate renal impairment a later baseline, when renal function has stabilized, was used.

Cox regression did not show a statistically significant effect of everolimus or CsA on a 30% decrease in creatinine clearance from baseline. For a 30% decrease in creatinine clearance from month 1, Cox regression showed a statistically significant effect of CsA exposure (p=0.0051), while everolimus exposure had no statistically significant effect (p=0.79).

Having defined the population of patients with decreased creatinine clearance, the influence of CsA dosing on subsequent renal improvement, defined as a improvement in creatinine clearance was explored. Additional data from the subset of patients with a 30% decrease in creatinine clearance (patients identified in the analysis above),and subsequent data available for creatinine and CsA were analyzed. There was a notable association of a ≥10% improvement in creatinine clearance with larger decreases in CsA Cmin than occurred for

patients without such improvement (Table 5-20). Logistic regression analysis supported the association of CsA reduction and later renal improvement (p=0.0418).

Table 5-20 CsA Cmin changes among patients with and without improvement in creatinine clearance of 10% – Heart study B253

Increase in creatinine clearance	RAD 1.5 mg		RAD 3 mg		AZA	
	Decrease in CsA trough level – ng/mL (%)					
	n	ng/mL (%)	n	ng/mL (%)	n	ng/mL (%)
< 10%	13	-31.87 (-11.24%)	18	-20.52 (-5.53%)	7	-35.09 (-11.07%)
≥ 10%	57	-65.82 (-14.88%)	37	-120.66 (-36.23%)	24	-101.41 (-28.95%)

Longitudinal analysis for improvement in renal function after CsA reduction

Investigators managing renal function in cardiac transplant recipients were able to effect an improvement in the change in creatinine clearance in cardiac transplant recipients, and this change was in proportion to the percentage reduction in CsA. The analyses below in Figure 5-3 shows that after a ≥50% decrease in CsA (from initial exposure) the rate of change in renal function over time is not significantly different from AZA.

Mixed effects models were used to explore the extent to which reduction in CsA blood level is associated with renal function improvement. Reduction in CsA (relative to month1 dosing) to various degrees during the subsequent 11 months period were explored.

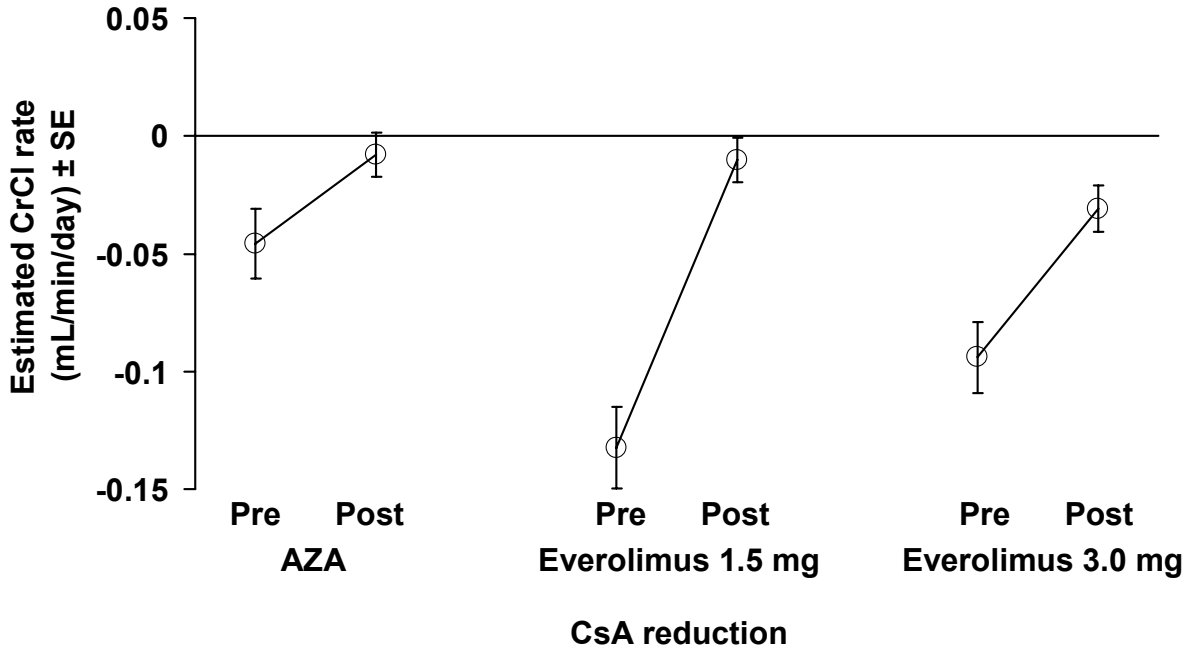
For patients meeting each cut-off of CsA reduction, a linear mixed-effects model is fitted with fixed-effects model: E(crcl) = b0 + b1 * csaday + b2*csaday*trt + b3*csaday_chg+ b4*csaday_chg*trt, where E(crcl) is the expected (average) creatinine clearance (CrCl) measured at the same time when CsA was measured; csaday is the number of days in the study when CsA is measured; csaday_chg is the number of days after the reduction in CsA ≥ the cut-off, with the convention csaday_chg = 0 before and at the date of reduction; trt represents the treatment arm. The model also uses random effects (which are patient-specific) for the intercept b0, the pre-reduction slope b1, and the change in slope b3. This model assumes a linear change in creatinine clearance measured up to csaday_chg, with slope given by b1. A change in slope for creatinine clearance is then allowed for measurements taken after the change. The parameter of interest is b3, the possible change in slope after the change in CsA (one would hope b3 > 0 reflecting improvement in Crcl after the reduction of CsA).

To evaluate the impact of treatment on pre-CsA reduction slope and on change in slope after CsA reduction, interaction terms csaday*trt (for pre-CsA reduction slope) and csaday_chg*trt (for slope after CsA reduction) were also included in the model.

The relationship between the pre-CsA reduction slope and the change in slope after CsA reduction was investigated using plots of the corresponding estimated random effects.

Rate of late BPAR ≥3A after CsA reduction was also assessed across treatment groups for patients who met each cut-off of CsA reduction.

Figure 5-3 Study B253- Reduction in Cyclosporine level of ≥50% and Change in Renal Function (change in creatinine clearance over time)[a]



[a] From repeated measures analysis, all slopes of estimated CrCl before CsA reduction were significantly different from 0 ($P \leq 0.002$).

The slope of estimated CrCl after CsA reduction in the everolimus 3.0 mg group is significantly different from 0 ($P = 0.002$).

All changes in slopes of estimated CrCl (before and after CsA reduction) were significantly different from 0 ($P \leq 0.045$).

Cohorts of patients defined by various decreases in CsA through 55% did not experience acute rejection at a higher rate than those cohorts without these decreases. In addition, the rates of acute rejection after CsA reduction for the everolimus 1.5 mg and 3.0 mg cohorts relative to AZA were numerically and statistically lower, respectively. Over the 24 month period the average number of BPARs were lower among those cohorts with CsA reduction (of any degree) relative to cohorts without CsA reduction. The average number of BPARs was always less in the everolimus arms relative to AZA.

The following were the main findings:

1. There was a statistically significant negative slope prior to CsA reduction, suggesting a decrease in creatinine clearance over time prior to CsA reduction for all treatment groups.
2. As shown in Figure 5-3, there is a statistically significant change in slope after the CsA reduction, indicating a reduced decline in renal function (creatinine clearance) for all treatment groups over time compared to renal function prior to reduction in CsA levels.
3. There was a statistically significant interaction between treatment and pre-CsA reduction slope:
    - There are statistically significant differences between the everolimus arms and the AZA arm in the pre-CsA reduction slope (AZA patients decrease creatinine clearance at a lower rate).

- There was no statistically significant difference between the 1.5mg and 3mg arms of everolimus on pre-CsA reduction slope.

4. For a given degree of CsA reduction, the change in slope in creatinine clearance was similar in each treatment arm.
5. There was no statistical difference in the slope of creatinine clearance after a 50% reduction in CsA level between everolimus 1.5 mg and AZA arms; and borderline statistical difference (P=0.06) in the slope of creatinine clearance after a 40% reduction in CsA levels between everolimus 1.5 mg and AZA arms
6. There was a strong negative association between the pre-CsA reduction slope and the change in slope after CsA reduction, suggesting that patients who decreased more rapidly prior to the CsA reduction had the greatest improvement after CSA reduction. This was true for all treatment groups.
7. Rate of BPAR ≥3A after CsA reduction was low in each treatment group. It was numerically lower for everolimus 1.5 mg and significantly lower for everolimus 3mg group (about 5-7%) compared with AZA group (about 21-23%). This difference is all the more notable in that the mean time to reduction in the AZA group was later than in the everolimus groups, which should provide an advantage to the AZA group in terms of a decreasing the likelihood of acute rejection.

Conclusion

These analyses demonstrated that investigators managing patients within the rigorous confines of a blinded study protocol were able to alter the deterioration in renal function via CsA exposure adjustment. These adjustments were made relatively early following transplant. Patients who had dose adjustments were at no greater risk for acute rejection than were patients who did not.

It is important to note PK/PD analyses support a relationship between CsA levels and renal dysfunction that could not be detected with simple comparisons of 1 year mean CsA level and renal function in everolimus-treated patients. These comparisons were confounded due to a two directional relationship between these 2 parameters. Specifically, creatinine values may change with adjustments in CsA levels (up or down depending on protocol dose adjustments, dose adjustments after a rejection or dose adjustments to palliate renal dysfunction), and CsA levels may be adjusted based on creatinine levels. For example, a patient with renal impairment with an elevated serum creatinine value is likely to receive lower levels of CsA. If this patient does not experience full recovery of renal function, the data from this patient would associate low CsA exposure with poor renal function. Similarly, if a patient had a low creatinine value, the clinician may not be concerned with a relatively high CsA level, leading to an association of high CsA exposure with good renal function.

5.6.2 Other Safety Relationships

The most commonly reported adverse events (AEs) suspected to be drug-related in both everolimus groups were leukopenia and thrombocytopenia, and those reported in the azathioprine group were leukopenia and cytomegalovirus infection. Examination of the frequency of events occurring with everolimus or azathioprine in the first year (days 1–450) demonstrated that hypertriglyceridemia and hypercholesterolemia numerically showed a weak

tendency, and thrombocytopenia a stronger overall tendency, to increase with higher everolimus trough concentrations.

5.6.3 Supportive data from kidney transplantation

During core kidney transplantation studies B201 and B251, everolimus-treated patients exhibited significantly elevated serum creatinine levels as compared to MMF-treated patients (Lorber 2005; Vitko 2004). Another parameters of renal dysfunction, the estimated creatinine clearance using Cockcroft–Gault formula (Cockcroft and Gault, 1975) showed a similar relationship, summarized in Table 5-21. However, everolimus has been shown to lack nephrotoxicity in preclinical and clinical safety studies (Schuler et al., 1997; Nashan 2002), though CsA-related nephrotoxicity is well known (Frampton and Faulds 1993; de Mattos et al., 2000). Consequently, the nephrotoxicity seen in the two kidney transplantation studies is likely to be attributable to co-administered CsA. Indeed, the results of another NDA study (Study B156) appear to support this hypothesis by showing that patients treated with reduced-dose CsA plus everolimus (3 mg/day) had higher GFR at 12 months than patients treated with full-dose CsA plus everolimus (3 mg/day) (Nashan 2004). Cox regression analyses in the pivotal studies B201/B251 (pooled data) used to model 'renal events' (creatinine values $\geq$ 200 μmol/L) showed that increased creatinine was strongly related to CsA exposure, and not to everolimus exposure. Results of two new randomized trials in de novo kidney transplant recipients (studies A2306 and A2307) have prospectively supported this hypothesis. In both studies reduced exposure CsA was used (target blood level at 2 hours after dosing 350 – 450 ng/mL in the maintenance phase after 3-4 months post-transplantation) with therapeutic drug monitoring for everolimus to keep all patient at a trough level of at least 3 ng/mL. As shown in Table 5-21 with that dosing regimen, improved serum creatinine and creatinine clearance were achieved, whilst maintaining good efficacy in preventing acute rejection (Vitko et al., 2004).

In core kidney transplantation studies B201 and B251 adverse events such as hypercholesterolemia and hypertriglyceridemia were found to be more frequent with higher everolimus Cmin values; however, no specific upper limit to the therapeutic range has been clearly identified from these data, as dose-limiting safety events were relatively infrequent. Furthermore, the hyperlipidemias in these studies responded to counter-measure therapies (Kovarik 2004); thus, everolimus-related adverse events appear to be manageable up to the highest trough levels observed in this population, and do not appear to be dose-limiting for the use of everolimus. See also section 9.3 for additional information on the kidney transplantation program.

Table 5-21 Creatinine Clearance in kidney transplantation studies at 12 months

	RAD 1.5 mg	RAD 3 mg	MMF
Mean (Median) Creatinine Clearance, Cockroft-Gault formula			
Study B201	52 (51)	47 (47)	54 (54)
Study B201	59 (58)	55 (52)	69 (67)
Study 2306	65 (65)	64 (63)	-
Study 2307	67 (64)	64 (64)	-

5.7 Summary of Safety Assessments – Heart transplant study B253

- Overall, adverse events were more frequent and the discontinuation rate was higher in the group receiving everolimus 3 mg/day than in the everolimus 1.5 mg/day group, suggesting that the 3 mg/day dose was less well tolerated than the 1.5 mg/day dose, despite the better efficacy seen.
- The overall infection rates were comparable between groups. However, there was a statistically significantly higher incidence rate of viral infections (in particular cytomegalovirus infections) in the AZA group than in each everolimus group. The incidence of bacterial infections (particularly pneumonia) was significantly higher in the everolimus 3 mg group compared to the AZA group.
- The rate of malignancies was low and similar across all treatment groups.
- Renal dysfunction was more frequent among everolimus-treated patients, and increasing CsA exposure, but not increasing everolimus exposure, was associated with an increased risk for renal dysfunction; improvement in renal function could be influenced by CsA reduction.
- Lipid abnormalities are characteristic of treatment with this class of agents, but with statin treatment the effect of everolimus exposure is manifest mostly in triglyceride increases, with HDL and LDL comparable to control groups. These changes were not associated with an increase in major adverse cardiovascular events.
- Graft and patient survival rate was excellent in all treatment groups, with no significant between-group differences.

6 Benefit and Risks Assessment

6.1 Integrated summary of benefit and risk

Current immunosuppressive regimens in transplantation have significantly improved graft loss due to rejection, contributing to long-term organ survival in transplant recipients. In heart transplantation improvement in the frequency of acute rejection and in allograft vasculopathy, both of which are associated with increased risk for graft loss and death, remain as significant unmet needs. The use of higher doses of immunosuppressants to overcome these problems is limited by the increased risk of side effects (e.g., infections, malignancies, in particular post-transplant lymphoproliferative disorder [PTLD], nephrotoxicity, and hypertension), so that no currently available immunosuppressive regimen is either fully safe nor able to fully prevent either acute rejection or allograft vasculopathy. Currently Cellcept® is the only adjunct agent

approved (as non-inferior to azathioprine) in the US for heart transplantation, and the available retrospective data support only modest effectiveness for allograft vasculopathy

The mechanism of action of everolimus provides 2 distinct avenues for benefit to prevent early and late graft loss. Firstly, the potent immunosuppressive (e.g. anti-rejection) effects were demonstrated convincingly in large well-controlled multi-center studies in two solid organ indications. In cardiac transplantation it provided the first secure evidence that improved acute rejection over AZA was possible. Furthermore, by inhibiting vascular smooth muscle cell proliferation, everolimus acts directly upon a key pathophysiologic feature of allograft vasculopathy. The ability to inhibit smooth muscle cell proliferation has been seen both *in vitro* and *in vivo* following rat aortic transplantation (Cole 1998) as well as clinically in drug-eluting stent and heart transplant studies. Thus, the ability of everolimus to inhibit smooth muscle proliferation, as well as the apparent synergy with CsA to reduce thee frequency of acute rejection, offers the potential to decrease morbidity, graft loss, and provide an second treatment option in an indication with significant unmet need.

The heart study B253 demonstrated a statistically significant reduction in average maximum intimal thickness of the coronary arteries from baseline to 12 and 24 months for both doses of everolimus compared with the AZA group. Further, both everolimus 1.5 and 3 mg/day were statistically superior with respect to allograft vasculopathy (change in MIT of 0.5 mm or greater at one year post-transplantation), which may directly lead, in the longer term, to outcomes such a major adverse cardiovascular events and death.

In this study the overall safety concerns included a higher drop-out rate and a higher incidence of serious AEs seen in the 3.0 mg/day everolimus, not in the recommended 1.5 mg/day group. As demonstrated, the elevation of serum creatinine levels is primarily due to CsA, and longitudinal analysis demonstrated clinicians were successful to modify depressed GFR with reduction of CsA. Lipid abnormalities are a well-documented and expected treatment emergent issue with the use of mTOR agents. The data demonstrated that with the use of statins, standard practice in heart transplantation, comparable LDL and HDL levels in the everolimus and AZA groups could be achieved, though triglycerides remained elevated with this regimen. In addition, everolimus imparted a key safety benefit in terms of the reduced incidence of cytomegalovirus infection, an important risk factor for chronic rejection. The expected outcome of long-term exposure to lipid abnormalities would be an increase in major adverse cardiovascular events, which, if anything, was decreased during follow-up of everolimus-treated patients.

Thus, the superior efficacy in preventing acute rejection, the predicted and significant effect demonstrated in preventing graft vasculopathy, combined with the predictable and manageable safety profile will provide a positive risk-benefit profile in heart transplantation. The effect on GFR demonstrated in this study will likely not occur in an open label setting with adequate attention to the levels of CsA used with RAD.

6.2 Role of therapeutic drug monitoring

The extensive efficacy and safety data presented lead to approval in most of the world of everolimus in renal and heart transplantation. The approved regimen is 0.75 mg orally (po) twice daily (bid) utilizing therapeutic drug monitoring (TDM) to achieve a trough blood concentration $\geq$ 3 ng/mL. This is based on the finding of excellent efficacy with both doses of

everolimus, but with better safety among patients on the 1.5 mg/day arms of the renal and heart studies. The addition of TDM offers the potential to improve both the efficacy and safety of everolimus. It is also recommended that CsA exposure be reduced during the maintenance phase (after the first month following the transplant).

6.3 TDM further enhances benefit-risk profile

A TDM-guided regimen is expected to enhance the efficacy and safety of an everolimus and CsA regimen in heart transplantation. There is a clear reduction in the incidence of acute rejection with average everolimus trough levels above 3 ng/mL, and only a modest increment in additional efficacy at exposures above 8 ng/ml. Beyond week 2, there is a distinct lack of effect of CsA exposure on efficacy failure within the concentration range tested. Overall, the data supports that once an exposure of everolimus of 3 ng/ml or more is assured, the CsA exposure may be liberally adjusted to reduce overall immunosuppression as appropriate, as well as palliate long term safety issues associated with chronic calcineurin inhibition.

The B253 heart study also demonstrated a statistically significant reduction in the change in average maximum intimal thickness of the coronary arteries from baseline at 12 and 24 months for both doses of everolimus compared with the AZA group. There appears to be an exposure effect of everolimus to reduce the incidence of vasculopathy, suggesting the management of vasculopathy might be further optimized if exposure were increased in those patients who are underexposed to everolimus.

6.4 Dosing Recommendations

Everolimus

Everolimus has proven efficacy to prevent acute rejection across a variety of CsA-exposures. PK/PD analysis suggests most patients will be well-served by a dose of everolimus of 1.5 mg/daily in divided dose. In order to assure flexible dosing of CsA can be pursued safely, the routine use everolimus whole blood therapeutic drug level monitoring is recommended, and can be used to support individualized patient dosing. Based on exposure-efficacy and exposure-safety analysis, patients achieving everolimus whole blood trough levels ≥3.0 ng/mL have been found to have a lower incidence of biopsy-proven acute rejection in both heart and kidney transplantation as compared to patients whose trough levels are below 3.0 ng/mL. There appears to be little incremental benefit to reduce rejection with higher everolimus exposures and an upper limit to the therapeutic range is recommended at 8 ng/mL. Exposure above 12 ng/mL has not been studied, nor has the use of everolimus as a monotherapy in transplantation.

It is especially important to monitor everolimus blood concentrations, in patients with hepatic impairment, during concomitant administration of strong CYP3A4 inducers and inhibitors, when switching formulation and/or if cyclosporine dosing is markedly reduced . Optimally, dose adjustments of everolimus should be based on trough levels obtained >4-5 days after the previous dosing change. There is an interaction of CsA on everolimus, and consequently, everolimus levels may decrease if CsA exposure is markedly reduced (i.e. trough concentration <50 ng/mL).

CsA dose recommendation in cardiac transplantation:

CsA dose recommendation in cardiac transplantation:

Heart transplant patients in the maintenance phase should have their exposure CsA reduced as tolerated in order to prevent kidney dysfunction. In heart transplant patients, the CsA exposure and dose may be based on CsA blood trough levels. Similar rates of acute rejection at all times following transplant across CsA quartile dosing suggests limited downside to reducing CsA exposure. Given this finding and other supportive analyses from PK/PD, CsA should be dosed at the median value for the lowest quartile of CsA exposure (rounded up for convenience). During the first month, when adequate CsA exposure is most critical CsA should be targeted to 250-400 ng/mL consistent with the B253 protocol. However, thereafter CsA should be reduced to a target of not below 175 ng/mL for months 2 and 3, 135 ng/mL for months 4-6 and 100 ng/mL beyond month 6. Prior to dose reduction of CsA, it should be ascertained that steady state everolimus whole blood trough concentrations are equal to or above 3 ng/mL.

7 Overall conclusions and recommendation for use

- Despite advances in immunosuppressive therapy, an unmet need remains for new compounds that are efficacious, safe, and possess unique benefits such as the potential to address both acute cellular rejection and the mechanisms of allograft vascular disease.
- Everolimus is an effective adjunctive immunosuppressive agent, with the additional benefit of inhibition of smooth muscle cell proliferation, which reduces the progression of allograft vasculopathy. As discussed above, there is a clear rationale for approving everolimus with CsA as a therapeutic option in heart transplantation. This rationale comes from a phase 3 program that include 48-month follow-up in heart transplantation an unprecedented efficacy and safety database in this indication, plus a large supportive safety experience in renal transplantation.
- The results of study B253 support a superior benefit/risk of everolimus 1.5 mg/day compared with AZA, provided that the CsA dose is reduced in patients with significant renal dysfunction. Therefore, RAD, now available to approximately 50% of heart transplant recipients worldwide, represents an important new treatment option for US heart transplant patients.
- Further refinements regarding the use of everolimus with CsA involves defining the optimal dose of CsA in combination with everolimus, as its use with some of the higher end of the range of exposures characterized in our dossier as “full-dose CsA” is associated with an increase in risk for renal dysfunction. Evidence from kidney transplantation suggests that with everolimus it is not necessary to dose CsA as high as is customary with MMF to achieve full antirejection benefit. With reduced CsA exposure excellent long term renal function has be demonstrated in kidney transplanation.
- The PK/PD modeling of everolimus and CsA in heart transplantation suggests similar exposure efficacy relationships to the larger renal transplant experience. With appropriate trough monitoring for everolimus, combined with the typical pattern of clinical

monitoring for acute cardiac rejection, there is an inherent ability to significantly reduce Neoral exposure to the degree necessary to achieve acceptable renal function.

- Novartis is committed to continue to evaluate whether the promise of TDM will be born out in the predicted maintained efficacy and improved safety, beyond that which has been demonstrated with fixed everolimus doses.
- Everolimus should be dosed initially as 1.5 mg/daily in divided dose (0.75 mg bid) then adjusted to achieve a predose trough level of at least 3 ng/ml. With everolimus administration there should be a reduction in CsA exposure. To manage both safety and maintain efficacy, clinicians should initially dose CsA at customary exposures for their individual centers, but then pursue CsA dose reduction to attain approximately 50% of the typical CsA exposure longer term, (see Dose recommendations, Section 6.4).
- US physicians that manage cardiac transplant recipients, and who have worked with everolimus in clinical trials have, by overwhelming majority, endorsed a position paper submitted to the FDA that called for the approval of everolimus (Everolimus in Heart Transplantation Position Paper 2005; see Appendix 2). The position paper recognizes the importance of everolimus' rejection benefit, the cytomegalovirus infection benefit and the IVUS findings. These finding led the group to conclude there was a need to have a mTOR agent with a well characterized toxicity profile in heart transplantation adequately described for prescribing information and available for their use. The transplant volume accounted by these 23 endorsers represented approximately 36% of the US total annual volume in 2003.

8 Bibliography

Cockcroft DW, Gault MH (1975) Creatinine clearance and age. Lancet; 2(7935): 612-613.

Cole OJ, Shehata M, Rigg KM (1998) Effect of SDZ RAD on transplant arteriosclerosis in the rat aortic model. Transplant Proc; 30(5):2200-3.

De Mattos AM, Olyaei AJ, Bennett WM (2000) Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis; 35(2): 333-46.

*Eisen HJ, Tuzcu EM, Dorent R, et al (2003) Everolimus for the Prevention of Allograft Rejection and Vasculopathy in Cardiac-Transplant Recipients. N Engl J Med; 349(9) 847-858.

Eisen HJ, Kobashigawa J, Starling RC, et al (2005) Improving Outcomes in Heart Transplantation – The Potential of Proliferation Signal Inhibitors. Transplantation Proceedings; 37(Suppl 4S): 4S-17S.

Frampton JE, Faulds D (1993) Cyclosporin: a pharmacoeconomic evaluation of its use in renal transplantation. Pharmacoeconomics; 4(5): 366-95.

*Halloran P (2004) Immuosuppressive Drugs for Kidney Transplantation. N Engl J Med; 351(26): 2715-2729.

Hochberg Y (1988) A Sharper Bonferroni procedure for multiple tests of significance. Biometrika; 75(4): 800-802.

Kapadia SR, Nissen SE, Tuzcu EM. (1999) Impact of intravascular ultrasound in understanding transplant coronary artery disease. Curr Opin Cardiol; 14(2): 140-50.

Kobashigawa JA (2003) First-year intravascular ultrasound results as a surrogate marker for outcomes after heart transplantation. J Heart Lung Transplan; 22(7): 711-4.

Kobashigawa JA, Tobis JM, Starling RC, et al (2005) Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. J Am Coll Cardiol; 45(9): 1532.

Kovarik JM, Hartmann S, Hubert M, et al (2004) Pharmacokinetic and Pharmacodynanamic Assessments of HMG-CoA Reductase Inhibitors When Coadministered with Everolimus. J Clin Pharmacol; 42(2):222-8.

Lorber MI, Mulgaonkar S, Butt KM, et al (2005) Everolimus versus mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: a 3-year randomized, multicenter, phase III study. Transplantation; 80(2): 244-52.

Mehra MR, Ventura HO, Stapleton DD, et al (1995) Presence of severe intimal thickening by intravascular ultrasonography predicts cardiac events in cardiac allograft vasculopathy. J Heart Lung Transplant; 14(4): 632-9.

Nankivell BJ, Gruenewald SM, Allen RD, et al (1995) Predicting glomerular filtration rate after kidney transplantation. Transplantation; 59(12): 1683-9.

Nashan B (2002) Review of the proliferation signal inhibitor everolimus. Expert Opin. Invest. Drugs; 11(12): 1845-1857.

Nashan B, Curtis J, Ponticelli C, et al (2004) Everolimus and Reduced-Exposure Cyclosporine in de novo Renal-Transplant Recipients: A Three-Year Phase II, Randomized, Multi-center, Open-Label Study. Transplantation; 78(9): 1332-1340.

Rickenbacher PR, Pinto FJ, Lewis NP, et al (1995) Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. Circulation; 92(12): 3445-52.

Schuler W, Sedrani R, Cottens S, et al (1997) SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. Transplantation; 64(1): 36-42.

Taylor DO (2003) Cardiac transplantation: drug regimens for the 21st century. Ann Thorac Surg; 75(6 Suppl): S72-8.

Vitko S, Margreiter R, Weimar W, et al (2004) Everolimus (Certican) 12-month safety and efficacy versus mycophenolate mofetil in de novo renal transplant recipients. Transplantation; 78(10): 1532-40.

Vitko S, Tedesco, Eris J, et al (2004) Everolimus with Optimized Cyclosporine Dosing in Renal Translpant Recipients: 6-Month Safety and Efficacy Results of Two Randomized Studies. Am J Transplant; 4(4): 626-35.

* see Appendix 3 References

9 Supplemental Information

9.1 Global Registration Status

List of Country Applications with Positive Recommendations

The Certican (everolimus) Tablet for use in kidney transplantation, and, the Certican (everolimus) Tablet for use in heart transplantation, have received a positive recommendation by Health Authorities in 48 countries. Commercial use (*) as of April 2005 is indicated.

Number	Country	Date of recommendation / authorization
1	Argentina *	30 July 2004
2	Australia	15 February 2005
3	Austria *	02 December 2003
4	Belgium	02 December 2003
5	Brazil	09 February 2004
6	Chile *	03 May 2004
7	Colombia *	20 August 2004
8	Costa Rica *	October 2004
9	Cuba	03 December 2004
10	Cyprus	01 December 2004
11	Czech Republic	01 December 2004
12	Denmark *	02 December 2003
13	Dominican Republic *	30 January 2004
14	Ecuador *	17 November 2004
15	El Salvador *	21 July 2004
16	Estonia	01 December 2004
17	Finland *	02 December 2003
18	France	02 December 2003
19	Germany *	02 December 2003
20	Greece *	02 December 2003
21	Guatemala *	25 February 2004
22	Honduras *	20 April 2004
23	Hungary	01 December 2004
24	Iceland	02 December 2003
25	India	02 September 2004
26	Israel	January 2005
27	Italy	02 December 2003
28	Jamaica *	21 May 2004
29	Latvia	01 December 2004

Number	Country	Date of recommendation / authorization
30	Lithuania	01 December 2004
31	Malta	01 December 2004
32	Mexico	08 July 2003
33	Netherlands *	02 December 2003
34	Nicaragua *	April 2004
35	Norway *	02 December 2003
36	Panama *	May 2004
37	Peru *	21 September 2004
38	Poland	01 December 2004
39	Portugal *	02 December 2003
40	Slovak Republic	01 December 2004
41	Slovenia	01 December 2004
42	South Africa	01 August 2005
43	Spain *	02 December 2003
44	Sweden *	18 July 2003
45	Switzerland	21 April 2005
46	Thailand	08 December 2004
47	Uruguay *	27 September 2004
48	Venezuela *	08 September 2004

* commercial product usage as of April 2005

9.2 Preclinical Pharmacology and Toxicology

The following sections provide a summary of preclinical pharmacology and toxicology effects of everolimus.

9.2.1 *In vitro* pharmacology- summary results

The *in vitro* experiments with everolimus demonstrated the immunosuppressive properties of the compound. They further showed that at the cellular and molecular level everolimus has the same mode of action as rapamycin, another macrocyclic lactone immunosuppressant.

Rapamycin has a mode of action which is different from that of both tacrolimus and CsA. The latter drugs prevent T cell proliferation by blocking transcriptional activation of early T cell-specific genes, thus inhibiting the production of T cell growth factors like interleukin-2 (IL-2). Rapamycin, in contrast, acts at a later stage, blocking not the production of growth factors but rather their effect. This compound inhibits T cell proliferation by indirectly blocking an intracellular proliferative signal, which is triggered by T cell growth factors, thus arresting the cells at the G1-phase of the cell cycle ("proliferation signal inhibitor, PSI"). It is of note that this effect is not restricted to IL2-driven proliferation of T cells; rapamycin inhibits in general growth factor-dependent proliferation of any hematopoietic as well as non-hematopoietic cell line tested so far. Like CsA and tacrolimus, rapamycin is not

immunosuppressive *per se*. It is a complex formed between the immunosuppressive compounds and certain abundant intracellular binding proteins which is the inhibitory principle. Rapamycin binds to the same binding protein as does tacrolimus, i.e., the 12kD cytosolic tacrolimus binding protein-12 (FKBP-12). Although the actual intracellular target protein, to which the FKBP-12/ rapamycin complex binds, could be identified in recent years, i.e., mTOR, it is still unclear how exactly rapamycin interference with mTOR leads to cell cycle arrest.

The *in vitro* T cell immunosuppressive activity of everolimus was determined by measuring its effect on mouse and human mixed lymphocyte reactions (MLR), as well as on proliferation of antigen-specific T cell clones. Its ability to suppress *in vitro* B cell immune responses was tested with T cell-dependent as well as independent antigens. To test for the ability of everolimus to inhibit growth factor-stimulated cell proliferation in general, the effect of the compound was tested in two *in vitro* systems, i.e., growth factor-induced proliferation of vascular smooth muscle cells, and IL-6-dependent proliferation of the B cell hybridoma B13-29-2. In all these *in vitro* assays everolimus inhibited cell proliferation with IC50 values in the low nanomolar range. The ability of everolimus to bind to FKBP-12 was shown in a competitive binding assay, and experiments with the IL-6-dependent hybridoma in the presence of excess molar concentrations of tacrolimus, in order to displace bound everolimus from FKBP-12, demonstrated that everolimus is not active *per se* but only so when complexed to FKBP-12. The mouse MLR also revealed synergistic immunosuppressive activity of everolimus and CsA; the different mode of action of CsA and tacrolimus provides a rationale for this synergistic activity of the two compounds.

9.2.2 *In vivo* pharmacology – summary results

The potential of everolimus as an immunosuppressant in the indication solid organ transplantation was demonstrated in preclinical allotransplantation models, using an experimental microemulsion formulation of everolimus for the oral application of the compound.

To test for its effects on acute rejection, orthotopic kidney allotransplantation was performed in rats, using three different donor-recipient strain combinations representing different degrees of MHC disparity. This animal model was also used to address the question of synergistic interaction of CsA and everolimus *in vivo*. The efficacy of everolimus was further tested in a non-human primate model of kidney allotransplantation, i.e., orthotopic kidney allotransplantation in cynomolgus monkeys. In all these models everolimus effectively prevented graft rejection at oral doses between 0.5 and 3.0 mg/kg per day. Finally, everolimus was tested in combination with CsA in the most difficult and stringent allotransplantation model, i.e., unilateral lung transplantation in cynomolgus monkeys. As a result, the combination of CsA and everolimus proved to be more efficacious than either compound alone, demonstrating the benefits of combining everolimus with CsA.

The potential of everolimus in chronic rejection was shown in aorta transplantation experiments in rats where the compound efficiently inhibited alloantigen-dependent as well as independent vascular changes.

Everolimus was tested for cardiovascular activity in normotensive rats; endocrine effects were studied in rats, while pulmonary effects were addressed in guinea pigs; neurological effects

were tested in a rat 'primary observation test'. In all cases the dose tested was 10- to 30-fold higher than the therapeutic dose in rats; no significant detrimental effect was noted.

9.2.3 Toxicology

Summary results

In acute toxicity studies, everolimus was well-tolerated by rats and mice without mortality or clinical signs indicating severe toxicity when administered as a single oral dose of up to 2000 mg/kg. In acute i.v. toxicity studies, there was no mortality in mice following a single dose of up to 96 mg/kg. In rats, however, mortality was observed 30 to 65 minutes following a single i.v. dose of 10 or 40 mg/kg. The estimated LD_{50} of everolimus was 6.3 mg/kg, but the vehicle might have contributed to the observed toxicity. Safety pharmacology evaluations of nervous system function in rats up to 50 mg/kg p.o., lung function in guinea pigs up to 30 mg/kg i.v., cardiovascular function in rats up to 100 mg/kg p.o. and in pigs up to 10 mg/kg i.v., and of endocrine parameters in rats up to 30 mg/kg p.o. did not reveal a potential of everolimus to cause serious toxicity after single application.

Repeated oral administration of everolimus to rats affected the thymus, spleen, lymph nodes and white blood cell counts. These effects were consistent with the pharmacological activity of the compound. Spontaneous heart lesions were exacerbated by the treatment with everolimus. Target organs of toxicity were lungs, eyes and male and female reproductive systems. Characteristic hematology findings in repeated oral toxicity studies in rats consisted of increases in red blood cell parameters such as packed cell volume, hemoglobin and erythrocyte counts and decrease in platelet count. Clinical chemistry revealed increases in cholesterol, triglycerides, amylase and decrease in albumin. The no-toxic-effect level (NTEL) in the rat after 4 weeks of oral treatment was 0.5 mg/kg/day corresponding to an approximate mean $AUC_{(0\text{-}24h)}$ value between 50 and 100 ng·h/mL. After a treatment period of 26 weeks, the NTEL was 0.15 mg/kg/day corresponding to a mean $AUC_{(0\text{-}24h)}$ value between 7 and 8 ng·h/mL, although histopathological findings at 0.5 mg/kg/day were rather of minor toxicological relevance.

After a 13-week oral administration to mice, the NTEL of 0.15 mg/kg/day was established for males and 0.5 mg/kg/day for females. The corresponding mean $AUC_{(0\text{-}24h)}$ values were 803 ng·h/mL for the males and 1258 ng·h/mL for the females. Target organs of toxicity were kidneys, lungs and male and female reproductive systems. Clinical chemistry findings included increases in cholesterol and creatinine, and decreases in albumin and albumin/globulin ratio.

Administration of everolimus over up to 2 years to rats and mice did not indicate any oncogenic potential up to doses of 0.9 mg/kg/day.

After repeated oral administration of everolimus to cynomolgus monkeys, treatment-related changes in thymus, spleen and lymph nodes were attributed to pharmacological activity of the compound. Except for the changes related to immunosuppression, there were no relevant histopathologic findings in the dose-escalating study up to 60 mg/kg/day or in the 4-week toxicity study up to 15 mg/kg/day. In contrast, heart lesions were observed at 5 mg/kg/day and higher in the 2-week dose range-finding study and in the 26-week toxicity study at 1.5 and 5 mg/kg/day. Poor general health condition of animals of the latter groups necessitated an

early sacrifice after 9/10 weeks of treatment. In addition to the heart findings, an increased incidence of pancreatic islet cell degeneration was seen at 5 mg/kg/day. Virological examinations of plasma from the 26-week toxicity study identified high titers of Coxsackievirus B4 in samples collected before and after the treatment period. Immunostaining with Coxsackievirus antibodies revealed positive results in heart tissue.

Hematology findings after repeated oral administration in monkeys included decreases in packed cell volume, hemoglobin and erythrocyte count, and increases in neutrophils, monocytes and fibrinogen. Clinical chemistry changes consisted of decreases in phosphorus and albumin, and increase in cholesterol. The NTEL after 4 weeks of treatment was set at 1.5 mg/kg/day corresponding to a mean $AUC_{(0\text{-}24h)}$ value of 1086 ng·h/mL and after 26 weeks at 0.5 mg/kg/day corresponding to a mean $AUC_{(0\text{-}24h)}$ value of 412 ng·h/mL (both sexes combined).

The oral administration of everolimus at 0.3 mg/kg/day for 52 weeks and at 0.9 mg/kg/day for 39 weeks to cynomolgus monkeys was associated with inflammatory changes in the gastrointestinal tract. These findings were considered to be the main cause of the poor health condition and early sacrifice of individual animals at 0.9 mg/kg/day and are probably secondary to the immunosuppression. Immaturity of male reproductive organs at 0.3 and 0.9 mg/kg/day may also be consequent to the impaired health condition of the animals. No relevant treatment-related changes were observed following administration of everolimus for 52 weeks at 0.1 mg/kg/day, corresponding to $AUC_{(0\text{-}24h)}$ values of 98 or 60 ng·h/mL for males and females, respectively.

The combination administration of everolimus and Neoral® for 4 weeks in rats and monkeys resulted in changes due to the pharmacological activity of the compounds and in findings reflecting toxicity, both notably exacerbated in animals receiving the combination compared to those receiving one or the other compound alone. There were no new target organs in the rat with the combination of both compounds, whereas monkeys showed unexpected findings of hemorrhage and arteritis with the combination in several organs such as gastrointestinal tract, heart, liver, kidneys, lymph nodes and pancreas. In view of the complexity of the possible mechanisms involved, the pathogenesis of arteritis remained uncertain. Since the monkeys were not specific pathogen-free and considering the changes in the gut, however, arteritis appears to be secondary to the high degree of immunosuppression. The low dose combination of CsA and everolimus at 50/0.25 mg/kg/day resulted in a higher degree of immunosuppression than when the compounds were administered alone, but was not associated with arteritis or poor health status as was seen for individual animals treated with the high dose combinations. For both animal species, the combination administration had no relevant effect on the toxicokinetic profile of Neoral, whereas exposure with everolimus was markedly increased. This might additionally have contributed to the more pronounced effects of the combination compared with those of the compounds alone.

A 4-week treatment with the combination of everolimus at 0.75 mg/kg and tacrolimus at 3 mg/kg in rats induced an increased severity of adverse effects when compared with the administration of the compounds alone. Cardiovascular and reproductive systems were particularly affected. With the combination of everolimus and tacrolimus, both at 0.75 mg/kg, a slightly increased severity of findings was restricted to few alterations compared with administration of everolimus or tacrolimus separately. As for the CsA, the combination

administration had no relevant effect on the toxicokinetic profile of tacrolimus, whereas exposure with everolimus was markedly increased.

Reproductive toxicity studies did not indicate any teratogenic effects of everolimus in rats or rabbits. There was a delay in embryo-fetal skeletal development in rats at 0.1 mg/kg/day ($AUC_{0\text{-}24h}$ of 20 ng/mL·h) and above. In rabbits the NTEL for embryotoxicity was 0.2 mg/kg/day ($AUC_{0\text{-}24h}$ of 61.2 ng/mL·h).

The oral fertility dose-range study in male rats revealed effects on spermatogenesis at 1.5 mg/kg, but there were no male-mentioned effects on female reproductive performance. In the 13-week oral male fertility study, none of the females mated with males treated at 5 mg/kg/day, became pregnant. Males at 5 mg/kg/day showed marked histopathological changes in the testes and epididymides, sperm motility and testicular sperm head count were markedly reduced, and testosterone levels were reduced. At these dose levels, effects were not completely reversible after a 13-week recovery period. At 0.5 mg/kg/day, a slight effect on testicular morphology was detected after 13 weeks of treatment, but this was not present any more in the recovery animals. There was no adverse effect on reproductive parameters at this dosage. No treatment-related effects were noted at the lowest dosage of 0.1 mg/kg/day. Effects on spermatogenesis in reproductive studies correlated with effects observed in other repeat toxicity studies.

Everolimus has been shown to be devoid of a clastogenic or mutagenic effect in *in vitro* tests. The compound has no potential to damage chromosomes or the spindle apparatus *in vivo*.

In conclusion, everolimus demonstrated a similar toxicity profile across animal species with the exception of additional target organs of lung and eyes in rats and kidneys in mice. The rat appears to be more sensitive than other species which could be related to the comparatively greater tissue distribution of the compound in the rat (V_D= 44-52 L/kg compared with V_D= 4 L/kg in the monkey). Some of the findings in the monkey may be due to an affected general health status of the animals, since these animals were not specific pathogen-free, and a relationship to the exacerbation of diseases as a consequence of severe immunosuppression has to be taken into consideration. Comparison of everolimus and rapamycin in 4-week toxicity studies indicates that the toxicity profiles are similar. Immunosuppression and toxicity, as well as systemic exposure to everolimus, were increased in combination with Neoral in rats and monkeys, and with tacrolimus in rats when compared with that of the compounds administered alone. everolimus has been shown to be devoid of mutagenic, teratogenic or oncogenic potential in animal studies.

9.2.4 Animal pharmacokinetics and drug metabolism

The PK behavior of everolimus was investigated in rat and monkey. Either ^{3}H-everolimus or ^{14}C-everolimus was used to measure absorption, distribution and elimination of everolimus. All samples were assayed for total radioactivity. Blood, bile and tissue samples were analyzed for parent drug by means of a liquid-chromatography-reverse isotope dilution (LC-RID) method. In addition, elaborated toxicokinetic investigations were part of most of the toxicology studies. Unchanged everolimus was measured in whole blood by HPLC and UV detection, by LC/MS, or by means of a specific ELISA. Organ distribution and disposition after single and multiple applications were studied in rat, as was the potential for milk excretion. The potential for drug-drug interaction was investigated in human liver

microsomal preparations. The metabolism of everolimus was looked at *in vitro* in microsomal preparations and liver slice preparations, respectively, of different species including man, and *in vivo* in rat, mouse and monkey. Metabolite profiles in blood, urine, bile and feces were determined by HPLC and radioactivity monitoring. Structure identification was attempted by means of MS. Special attention was given to rapamycin as a potential metabolite of everolimus. Blood distribution and protein binding studies, CNS penetration, intestinal metabolism and *in vitro* and *in vivo* absorption studies, and enzyme induction studies in rats were also part of the investigations.

Summary results

The oral absorption of everolimus given as a microemulsion is good in rat (40%) and lower in monkey (18%). The bioavailabilities observed in rat (14%-26%) and in monkey (6%) indicate the existence of a presystemic first pass metabolism. In both species, a concentration-dependent blood distribution is observed. everolimus is extensively distributed to tissues (V_d = 4-52 l/kg); the brain penetration of everolimus is dose-dependent. Metabolism of everolimus is extensive and complex in rat reflected by the metabolite profile of bile. However, in rat blood, five main metabolite peaks are found beside parent drug. Two of them could mainly result from chemical degradation rather than enzymatic biotransformation. Two peaks consisted of hydroxylated metabolites and one was identified as a direct phosphatidylcholine conjugate of everolimus. Similar first generation metabolites were detected in human liver microsomal incubations, as well as in a human ADME study. None of the metabolites, however, demonstrated significant biological activity in the mixed lymphocyte reaction (MLR) assay. They are not expected to contribute significantly to the immunosuppressive activity of everolimus. *In vitro* drug-drug interaction studies indicate that CYP3A inhibitors such as CsA and azoles would have the potential to inhibit everolimus metabolic clearance. Vice versa, everolimus inhibited competitively both CsA and dextromethorphan metabolism by CYP3A and CYP2D6, respectively. *In vivo* concentrations of everolimus, however, are most likely not sufficient to decrease the clearance of other coadministered drugs. In rat, everolimus related radioactivity is essentially eliminated by the bile. The systemic clearance consists essentially of metabolic clearance. No significant concentrations of everolimus are found in urine or bile. A lower (factor 10) systemic clearance is observed in cynomolgus monkeys. No indication of an increase in blood AUCs of everolimus after repeated drug administration was observed in rats and monkeys during the 4-week toxicology studies. However, when multiple daily doses of ^{3}H-everolimus were given to rats, AUC exposure of radioactivity increased by some 2.4-fold on Day 21 compared to Day 1. In this study, excretion of radioactivity was almost complete within 7 days after the last dose, with <0.3% of the total cumulated dose remaining in the carcass. Everolimus-related radioactivity was readily excreted into milk of treated suckling rats. In rats, an overproportional increase of AUCs is observed at higher doses whereas in monkeys dose normalized AUCs decrease with increasing doses. In mice, the increase in exposure is almost dose-proportional over a wide dose-range. When the administration of everolimus is combined with simultaneous application of a microemulsion formulation of CsA, a clear indication of a PK interaction is observed, resulting in higher exposures of everolimus, which is more pronounced in the monkey than in the rat. A similar drug interaction is seen when everolimus is combined with simultaneous applications of tacrolimus. Interaction between

everolimus and CsA at pre-systemic intestinal metabolism mechanisms has been demonstrated in rats which contributes to these drug-drug interaction effects. everolimus is also a substrate for Pgp-like mediated efflux systems. Intrinsic permeability is high, as shown in Caco-2 monolayer cultures and confirmed in a single-pass rat intestinal perfusion model, where everolimus demonstrated a permeability coefficient similar to propranolol. When rapamycin is given in similar microemulsion formulation as everolimus, the PK behavior is rather similar and exposure of rats and monkeys to the drug is comparable in rats and monkeys, after single and multiple applications

9.3 Kidney and Other Organ Transplantation

9.3.1 Core Supporting Studies - Kidney Transplant Program

Study B251 was a three-year, randomized, double-blind study comparing the efficacy and safety of everolimus 1.5 mg/day (0.75 mg b.i.d.), everolimus 3.0 mg/day (1.5 mg b.i.d.) and MMF 2 gm/day (1 gm b.i.d.) in combination with Neoral (standard doses) and steroids in adult *de novo* renal transplant recipients. The primary objective of this study was to compare the efficacy of two oral doses of everolimus with MMF as measured by the incidence of biopsy-proven acute allograft rejection, graft loss or death in the first six months of treatment. Biopsies were required at the time of any suspected rejection episode.

The primary efficacy results are shown in Table 9-1 . The proportion of everolimus patients experiencing 'efficacy failure' was similar between the treatment groups.

Table 9-1 Efficacy results in kidney transplant study B251 (ITT population)

Efficacy parameter	Everolimus 1.5 mg N=193 n (%)	Everolimus 3 mg N=194 n (%)	MMF 2 g N=196 n (%)	P-values, Everolimus vs MMF† vs. 1.5mg	P-values, Everolimus vs MMF† vs. 3mg
Efficacy failure within 6 months					
BPAR, graft loss, death or loss to follow-up	42 (21.8%)	46 (23.7%)	51 (26.0%)	0.389	0.706
BPAR	33 (17.1%)	39 (20.1%)	46 (23.5%)		
Graft loss/death	18 (9.3%)	12 (6.2%)	9 (4.6%)		
Graft loss	15 (7.8%)	7 (3.6%)	7 (3.6%)		
Death	5 (2.6%)	6 (3.1%)	2 (1.0%)		
Loss to follow-up	0	0	0		
Efficacy failure within 12 months					
BPAR, graft loss, death or loss to follow-up	48 (24.9%)	51 (26.3%)	54 (27.6%)	0.626	0.901
BPAR	37 (19.2%)	43 (22.2%)	47 (24.0%)		
Graft loss/death	21 (10.9%)	14 (7.2%)	12 (6.1%)		
Graft loss	17 (8.8%)	8 (4.1%)	10 (5.1%)		
Death	6 (3.1%)	7 (3.6%)	4 (2.0%)		
Loss to follow-up	1 (0.5%)	1 (0.5%)	1 (0.5%)		
Co-primary efficacy endpoint within 12 months					
Graft loss, death or loss to follow-up	22 (11.4%)	15 (7.7%)	13 (6.6%)	0.088	0.572

Cut-off date for 12-month data was day 381 for efficacy and day 450 for safety & disposition.
† CMH test.

The incidence rates of efficacy failure at Month 6 were slightly lower in both everolimus groups than in the MMF group, mainly driven by the incidence of biopsy-proven acute

rejection. At Month 12, the incidence of overall efficacy failure was similar across treatment groups. The incidence of the co-primary efficacy variable (graft loss, death or loss to follow-up at Month 12) was slightly higher for the everolimus 1.5 mg group. None of these differences was statistically significant.

For efficacy failure, both everolimus doses were equivalent to MMF with respect to confidence intervals using the same equivalence criteria (10%). For the co-primary endpoint at month 12, the everolimus 3 mg dose was equivalent, whereas the 1.5 mg group just misses equivalence.

Study B201 was a parallel study to B251 which is being conducted in Europe, Australia and South Africa. This study was also a three-year, randomized study comparing the efficacy and safety of everolimus 1.5 mg/day (0.75 mg b.i.d.), everolimus 3.0 mg/day (1.5 mg b.i.d.) and MMF 2 gm/day (1 gm b.i.d.) in adult *de novo* renal transplant recipients. As in B251, recipients of a single, primary, cadaveric, living unrelated or living related non-human leukocyte antigen identical renal transplant were randomized (1:1:1) into one of the three treatment groups, the composite endpoint of biopsy-proven rejection, graft loss or death within the first six months of treatment is used as the primary objective, and all patients receive background immunosuppression of Neoral and prednisone. See Table 9-2.

Table 9-2 Efficacy results in kidney transplant study B201 (ITT population)

Efficacy parameter	Everolimus 1.5 mg N=194 n (%)	Everolimus 3 mg N=198 n (%)	MMF 2 g N=196 n (%)	P-values, Everolimus vs MMF† vs. 1.5mg	vs. 3mg
Efficacy failure within 6 months					
BPAR, graft loss, death or loss to follow-up	52 (26.8%)	52 (26.3%)	58 (29.6%)	0.572	0.428
BPAR	42 (21.6%)	36 (18.2%)	46 (23.5%)		
Graft loss/death	15 (7.7%)	24 (12.1%)	18 (9.2%)		
Graft loss	7 (3.6%)	17 (8.6)	15 (7.7%)		
Death	9 (4.6%)	7 (3.5%)	3 (1.5%)		
Loss to follow-up	0	0	0		
Efficacy failure within 12 months					
BPAR, graft loss, death or loss to follow-up	58 (29.9%)	60 (30.3%)	61 (31.1%)	0.771	0.836
BPAR	45 (23.2%)	39 (19.7%)	47 (24.0%)		
Graft loss/death	18 (9.3%)	29 (14.6%)	21 (10.7%)		
Graft loss	9 (4.6%)	21 (10.6)	18 (9.2%)		
Death	10 (5.2%)	8 (4.0%)	5 (2.6%)		
Loss to follow-up	1 (0.5%)	3 (1.5%)	1 (0.5%)		
Co-primary efficacy endpoint within 12 months					
Graft loss, death or loss to follow-up	21 (10.8)	33 (16.7%)	23 (11.7%)	0.997	0.236

BPAR = Biopsy-proven acute rejection episode
Cut-off date for 12-month data was day 381 for efficacy and day 450 for safety & disposition.
† CMH test.

The incidence rates of efficacy failure at Month 6 were slightly lower in both everolimus groups than in the MMF group, mainly driven by the incidence of biopsy-proven acute rejection in both everolimus groups. At Month 12, the incidence of overall efficacy failure

was similar across treatment groups. A slightly lower incidence of biopsy-proven acute rejections was offset by slightly more graft loss/death in the everolimus 3 mg group.

The incidence of the co-primary efficacy variable (graft loss, death or loss to follow-up at Month 12) was slightly higher for the everolimus 3 mg group. None of these differences was statistically significant. The everolimus 1.5 mg treatment arm was equivalent to MMF with respect to confidence intervals using the same equivalence criteria (10%).

Summary of safety from core renal program

The experience in kidney transplantation provided a large comparative experience for the evaluation of safety in everolimus treated patients. By and large the safety in kidney patients approximated that in heart transplantation, with more adverse events reported in the higher dose everolimus arms, the issue of renal adverse events in patients receiving everolimus and full dose Neoral, and one study B201 also showing reduced CMV rates in everolimus relative to MMF (CMV prophylaxis was more widespread in the US, and so CMV rates were similar in everolimus and MMF in B251).

The most frequently reported AEs during the study are summarized in Table 9-3, showing the most frequently affected body system, and the most commonly reported AEs by preferred term (reported by ≥ 20% of patients in any treatment group in each case). While the data did not indicate any specific acute toxicity of everolimus to a major organ system, more everolimus patients experienced AEs of the metabolic and nutritional, urinary, red blood cell, and platelet, bleeding and clotting systems compared with MMF. There was an apparent dose-dependent effect of everolimus especially with red blood cell disorders.

Table 9-3 Number (%) of patients with frequently reported adverse events, including infections (≥ 20% in any group, by body system or preferred term) – 12-month analysis. Safety population – Renal studies B201 & B251

SMTT Body system or preferred term (common events)	RAD 1.5 mg (N= 387) n (%)	RAD 3 mg (N= 392) n (%)	MMF (N=392) n (%)
Total patients with an AE/infection	383 (99.0)	390 (99.5)	386 (98.5)
Body system[†]			
Metabolic & nutritional	308 (79.6)	324 (82.7)	281 (71.7)
Gastrointestinal	284 (73.4)	307 (78.3)	284 (72.4)
General	279 (72.1)	303 (77.3)	290 (74.0)
Urinary	251 (64.9)	274 (69.9)	221 (56.4)
Skin & appendages	188 (48.6)	197 (50.3)	181 (46.2)
Respiratory	183 (47.3)	193 (49.2)	187 (47.7)
Musculoskeletal	175 (45.2)	170 (43.4)	158 (40.3)
Red blood cell	134 (34.6)	165 (42.1)	120 (30.6)
Cardiovascular – general	154 (39.8)	161 (41.1)	172 (43.9)
C & P nervous system[‡]	133 (34.4)	143 (36.5)	145 (37.0)
Psychiatric	122 (31.5)	142 (36.2)	141 (36.0)
Vascular	93 (24.0)	107 (27.3)	81 (20.7)

SMTT Body system or preferred term (common events)	RAD 1.5 mg (N= 387) n (%)	RAD 3 mg (N= 392) n (%)	MMF (N=392) n (%)
Platelet, bleeding & clotting	82 (21.2)	90 (23.0)	60 (15.3)
Most common events, preferred term†			
Constipation	132 (34.1)	138 (35.2)	146 (37.2)
Anemia	112 (28.9)	137 (34.9)	101 (25.8)
Hypercholesterolemia	125 (32.3)	130 (33.2)	83 (21.2)
Hypertension	109 (28.2)	114 (29.1)	109 (27.8)
Urinary tract infection	115 (29.7)	112 (28.6)	100 (25.5)
Hyperlipidemia	97 (25.1)	104 (26.5)	65 (16.6)
Nausea	94 (24.3)	99 (25.3)	81 (20.7)
Diarrhea	90 (23.3)	98 (25.0)	74 (18.9)
Insomnia	77 (19.9)	88 (22.4)	84 (21.4)
Fever	69 (17.8)	83 (21.2)	59 (15.1)
Edema	77 (19.9)	82 (20.9)	74 (18.9)
Non-protein nitrogen increased	71 (18.3)	80 (20.4)	52 (13.3)
Edema legs	80 (20.7)	61 (15.6)	60 (15.3)

† Body systems and common AEs are ordered by highest frequency in either everolimus group
‡ C & P = Central and peripheral

The type and frequency of AEs with everolimus were broadly similar to those reported with MMF. at 12 months. AEs reported with ≥10% difference between the everolimus 1.5 or 3 mg groups and the MMF group included the following: viral infection (higher for MMF B201), hyperkalemia (higher for MMF B251) hypercholesterolemia, and hyperlipidemia. Everolimus potentially dose-related differences (≥ 5% between the 1.5 and 3 mg groups) were observed in B201 for the following AEs: diabetes mellitus (5% vs. 12%), lymphocele (11% vs. 17%), non-protein nitrogen (NPN) increased (9% vs. 15%), hypertrichosis (2% vs. 8%), and surgical wound complication (4% vs. 11%). Everolimus potentially dose-related differences (≥ 5% difference between the 1.5 and 3 mg) were observed for the following AEs: influenza-like symptoms (11% vs. 6%), edema-legs (31% vs. 23%), weight increase (21% vs. 14%), fluid overload (2% vs. 8%), moniliasis-GI (12% vs. 5%), nausea (34% vs. 41%), vomiting (16% vs. 24%), arthropathy (23% vs. 17.0%), insomnia (22% vs. 27%), anemia (29% vs. 36%), bronchospasm (7% vs. 2%), dyspnea (19% vs. 24%), epistaxis (3% vs. 8%), hypertrichosis (12% vs. 7%), and leukopenia (6% vs. 11%).

Infections

Infections and serious infections were comparable across treatments. In Study B251 the overall incidence of CMV infections was low and comparable between the everolimus 1.5 and 3 mg groups and the MMF group, respectively (5%, 4% and 6%) but was higher in the MMF group in study B201 (5%, 8% and 19%) and accounted for the significantly higher incidence of viral infection observed in this study. In both studies, the incidence of sepsis and opportunistic infections was similar between the recommended everolimus dose group (1.5 mg/day) and the MMF treatment group, and the incidence of sepsis and serious bacterial infections was not associated with average everolimus exposure.

Laboratory abnormalities

The most obvious laboratory effect seen that were attributable to everolimus was the effect on lipids, known to be a class effect for this type of drug. In this clinical program, total cholesterol and triglyceride concentrations ≥ 9.1 and ≥ 8.5 mmol/L respectively were regarded as clinically notable. Elevation of cholesterol and to a lesser extent, triglyceride levels are known to occur in transplant patients receiving concomitant immunosuppressive medications such as CsA and corticosteroids.

In general, mean total cholesterol and triglyceride concentrations tended to be higher during the 12 months post-transplant in the everolimus groups compared with MMF and the effect of everolimus was dose-dependent. Mean values and change from baseline at 12 months post-transplant as well as the incidence of clinically notable abnormalities and MACE (Major Adverse Cardiac Events) are provided in Table 9-4.

Table 9-4 Summary statistics for lipids and incidence of clinically notable abnormalities and MACE (Major Adverse Cardiac Events) – 12-month analysis – Renal studies B251 and B201

Parameter	RAD 1.5 mg		RAD 3 mg		MMF	
	B201	B251	B201	B251	B201	B251
Total Cholesterol						
Mean (change from baseline) TC (mmol/l)	6.4 (+1.7)	6.2 (+1.9)	6.7 (+2.2)	6.4 (+2.3)	5.9 (+1.4)	5.5 (+1.3)
TC ≥9.1mmol/L (% patients)	23.2%	20.8%	27.8%	32.3%	6.7%	6.2%
LDL/HDL ratio mean at month 12 (change from BL)	2.7 (-0.3)	2.6 (+0.1)	3.0 (-0.1)	2.6 (0.0)	2.9 (-0.3)	2.5 (0.0)
Triglycerides						
Mean (change from Baseline) TG (mmol/l)	2.7 (1.4)	2.9 (1.6)	3.0 (1.7)	3.1 (1.9)	2.1 (0.4)	2.3 (1.0)
TG ≥5.6mmol/L (% patients)	19.2%	17.1%	24.7%	26.0%	9.7%	11.9%
TG ≥8.5mmol/L (% patients)	4.7%	4.7%	5.6%	7.8%	1.5%	2.1%
Lipid lowering agents (% patients)	59.3%	62.7%	53.5%	66.5%	37.8%	50.0%
MACE (% patients)	3.1%	5.7%	2.5%	2.6%	4.1%	3.6%

MACE = Major adverse cardiac events (angina pectoris, myocardial infarction, & sudden death)

Creatinine and Creatinine Clearance

As in the heart study, in the renal studies B201 and B251 there were, on average, elevation of serum creatinine values with the everolimus/CsA combination. The differences between groups are demonstrated in the table below, and the elevations in the everolimus groups are not progressive. See Table 9-5

Table 9-5 Studies B201 and B251: 12 to 36-month summary statistics for serum creatinine (ITT population)

Study No.	Serum creatinine (µmol/L)					
	Month 12		Month 24		Month 36	
	N	Mean (median)	N	Mean (median)	N	Mean (median)
B201						

RAD 1.5 mg	168	175 (146) [a]	160	178 (145) [b]	164	166 (144) [a,b]
RAD 3.0 mg	167	216 (164) [b]	151	185 (159) [b]	151	196 (160) [b]
MMF 2 g	180	183 (146)	161	168 (133)	161	173 (134)
B251						
RAD 1.5 mg	173	190 (150) [b]	152	189 (145) [a,b]	143	194 (150) [b]
RAD 3.0 mg	166	189 (159) [b]	140	187 (167) [a,b]	129	212 (162) [a]
MMF 2 g	173	157 (133)	155	151 (131)	153	159 (124)

[a] p ≤0.05 RAD 1.5 vs 3.0 mg
[b] p ≤0.05 RAD vs MMF
Note: Analysis includes values at follow-up visits after discontinuation of study medication.

9.3.2 Optimized Dosing of Cyclosporine – Kidney Studies

Two (2) additional studies, open label, non-comparator trials (A2306, A2307), using therapeutic drug monitoring for everolimus were conducted with reduced doses of cyclosporine.

Study A2306 was a 1 year multicenter, randomized, open label, parallel group study of the safety, tolerability and efficacy of two doses (1.5 and 3mg/day with dose adjustment based upon everolimus trough level) of everolimus with steroids and reduced dose Neoral in de novo kidney transplant patients. The primary objectives were to compare renal function, as measured by serum creatinine, of 2 doses of everolimus and to assess whether acceptable renal function (improved creatinine vs historical data [studies B201/251] can be achieved at 6 months post-transplantation in de novo kidney transplant patients. The secondary objectives were to quantify the incidence of biopsy-proven acute rejection episodes, graft loss, death, loss to follow-up at 6 and 12 months in both groups; to assess the incidence of graft loss, death, biopsy-proven acute rejection, antibody-treated acute rejection, clinically-confirmed acute rejection, clinically confirmed chronic rejection, and biopsy-proven chronic allograft nephropathy at 6 and 12 months; to assess the incidence of graft loss, death or lost to follow-up at 12 months; to assess renal function, as measured by serum creatinine, calculated creatinine clearance (Cock-croft Gault) and calculated glomerular filtration rate (GFR) using the Nankivell formula at 6 and 12 months posttransplant; and to evaluate the safety of 2 oral doses of everolimus.

In A2306, 12 month results for the ITT population (N=237; 112 on everolimus 1.5 mg/day and 125 on 3.0 mg/day) demonstrated incidences of biopsy-proven rejection of 25.9 % for the 1.5 mg/day group and 19.2 % for the 3 mg/day group (results not shown). Across-study comparisons indicated that allograft function was better than seen with everolimus in the full-dose cyclosporine studies. On-treatment median serum creatinine values at Month 12 were 122 µmol/L in the 1.5 mg and 126 µmol/L in the 3 mg everolimus group (mean: 126 and 134 µmol/L). On-treatment median creatinine clearance values according to Cockcroft-Gault were 69 and 62 mL/min in the 2 everolimus groups (mean: 69 and 65 mL/min).

Study A2307 was a 1 year multicenter, randomized, open label, parallel group study of the safety, tolerability and efficacy of two doses (1.5 and 3mg/day with dose adjustment based upon everolimus trough level) of everolimus with Simulect, steroids and reduced dose Neoral in de novo kidney transplant patients. The primary objectives were to compare renal function,

as measured by serum creatinine, of 2 doses of everolimus and to assess whether acceptable renal function (improved creatinine vs historical data [study B156] can be achieved at 6 months post-transplantation in de novo kidney transplant patients. The secondary objectives were to quantify the incidence of biopsy-proven acute rejection episodes, graft loss, death, loss to follow-up at 6 and 12 months in both groups; to assess the incidence of graft loss, death, biopsy-proven acute rejection, antibody-treated acute rejection, clinically-confirmed acute rejection, clinically confirmed chronic rejection, and biopsy-proven chronic allograft nephropathy at 6 and 12 months; to assess the incidence of graft loss, death or lost to follow-up at 12 months; to assess renal function, as measured by serum creatinine, calculated creatinine clearance (Cockcroft Gault) and calculated glomerular filtration rate (GFR) using the Nankivell formula at 6 and 12 months posttransplant; and to evaluate the safety of 2 oral doses of everolimus.

The A2307 study had a similar design to A2306 with the addition of 2 doses of Simulect® (basiliximab) 20 mg at day 0 and day 4, and lower target C2 concentrations for cyclosporine through the first 8 weeks following transplantation. Analyses of the 12 month results (ITT, N=256; 117 on everolimus 1.5 mg/day and 139 on 3.0 mg/day) demonstrated incidences of biopsy-proven rejection of 13.7 % for the 1.5 mg/day group and 15.8 % for the 3 mg/day group (results not shown). On-treatment median calculated serum creatinine values at Month 12 were 128 μmol/L in the 1.5 mg and 126 μmol/L in the 3 mg everolimus group (mean: 132 and 132 μmol/L). On-treatment median creatinine clearance values (according to Cockcroft-Gault Formula) were 64 and 65 mL/min in the 2 everolimus groups (mean: 68 and 65 mL/min).

9.3.3 Tabular Summaries of Clinical Studies

The following tables provide summary details of the clinical studies in the NDA. All everolimus studies included in the tables below used the tablet formulation unless indicated otherwise.

Table 9-6 Single dose normal healthy volunteer and transplant studies

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Study duration	No. of patients randomized			RAD dose(s)
						Total no. of patients	RAD	Placebo	
W105	France (1)	Safety and tolerability, PK	Normal healthy male volunteers (NHV)	Randomized, double-blind, parallel group, placebo-controlled	13 days	24	16	8	0.5, 1, 2 and 4 mg
W107*	United Kingdom (1)	ADME	Male maintenance kidney transplant patients	Open-label	13 days	4	4	0	3 mg
W301	Switzerland (1)	PK comparison between adult tablet and fast-dispersible tablet formulations	NHV	Randomized, open-label, two-way crossover	22 days	16	16	0	1 mg
W302	Germany (1)	Pharmacokinetics of market form tablet under fed and fasted conditions	NHV	Randomized, open-label, two-period, two-treatment crossover	22 days	24	24	0	2 mg
W303	Europe (1)	Drug-drug interaction RAD/statins	NHV	Randomized, open-label, two-period, two-treatment crossover	30 days	24	24	0	2 mg
W101*	Germany (4)	Safety and tolerability, PK	Maintenance kidney transplant patients	Randomized, double-blind, placebo-controlled	11 days	54	36	18	0.25, 0.75, 2.5, 7.5, 15 and 25 mg

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Study duration	No. of patients randomized			RAD dose(s)
						Total no. of patients	RAD	Placebo	
A2408	USA (1)	Erythromycin (E)	NHV	Open-label, two-period, single-sequence, cross-over	19 days	17	16	0	2 mg / 500 mg E
A2409	USA (1)	Ketoconazole (K)	NHV	Open-label, two-period, single-sequence, cross-over	24 days	13	12	0	2 mg / 200 mg K
A2410	USA (1)	Verapamil (V)	NHV	Open-label, two-period, single-sequence, cross-over	16 days	16	16	0	2 mg / 80 mg V
B257#	Belgium, Germany, United Kingdom, US (8)	PK, safety and tolerability	Pediatric maintenance kidney transplant patients (stratified by <8 and ≥8 to 16 years old)	Open-label	14 days	20	20	0	1.2 mg/m^2
B202*#	Canada (2)	PK, safety and tolerability	*De novo* liver transplant patients	Open-label	Up to 42 days	26	26	0	7.5 mg
B258#	Canada, Europe, US (8)	PK, safety and tolerability	Pediatric maintenance liver transplant patients (stratified by <3 and ≥3 to 16 years old)	Open-label	14 days	16	16	0	1.2 mg/m^2

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Study duration	No. of patients randomized			RAD dose(s)
						Total no. of patients	RAD	Placebo	
B151*#	US (5)	PK, safety and tolerability	Maintenance lung and heart/lung transplant patients with or without pancreatic insufficient cystic fibrosis	Randomized, double-blind, treatment crossover	22 days	20	20	0	0.035 mg/kg (maximum of 2.5 mg) and 0.1 mg/kg (maximum of 7.5 mg)

*Capsule formulation #Conducted under a US IND
Note: Studies W105, W107, W301, W302 and W101 were not conducted under a US IND.

The following table provides details on the short term (4 weeks), multiple dose stable kidney transplant studies in the NDA.

Table 9-7 Short-term, multiple-dose maintenance kidney transplant studies

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Treatment/ study duration	No. of patients randomized			RAD dose(s)
						Total no. of patients	RAD	Placebo	
W102*	France Germany Norway (4)	Safety and tolerability, PK	Maintenance kidney	Randomized, double-blind, placebo-controlled	4 weeks	54	44	10	Capsule: 0.75 mg q.d. Tablet: 0.75, 2.5, 5 and 10 mg q.d.; 2.5 and 5 mg b.i.d.
B154**#	US (1)	Safety and tolerability, PK	Maintenance kidney	Randomized, double-blind, placebo-controlled	4 weeks	25	19	6	0.75, 2.5 and 7.5 mg q.d.

*Tablet and capsule formulation **Capsule formulation
#Conducted under a US IND Note: Study W102 was not conducted under a US IND.

The following table provides details of the core NDA multiple dose, *de novo* heart and kidney transplant studies with 12-month data in the NDA.

Table 9-8 Multiple-dose *de novo* heart *and* kidney transplant studies

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Primary endpoint (study treatment duration)	No. of patients randomized			Comparator	RAD dose(s)
						Total no. of patients	RAD	Compar-ator		
B253 #	Argentina, Canada, Europe[1], US (52)	Efficacy, safety and tolerability, PK	*De novo* heart	Randomized, double-blind, active-controlled	6 months (2 years)	630	420	234	AZA	0.75 and 1.5 mg b.i.d.
B251 #	Argentina, Brazil, Canada, US (44)	Efficacy, safety and tolerability, PK	*De novo* kidney	Randomized, double-blind, active-controlled	6 months (3 years)	583	387	196	MMF	0.75 and 1.5 mg b.i.d.
B201	Australia, Europe*, South Africa (54)	Efficacy, safety and tolerability, PK	*De novo* kidney	Randomized, 1 year double-blind, 2 year open-label, active-controlled	6 months (3 years)	588	392	196	MMF	0.75 and 1.5 mg b.i.d.
A2306 #	US (8), Italy, Brazil, CDN, Spain, Poland, Venezuela Belgium	Safety, efficacy tolerability with reduce Neoral	*De novo* kidney	OL, parallel, non-comparator,	12 months	237	237	0	-	0.75 and 1.5 mg b.i.d.
A2307 #	US (7), Italy, Australia, France, Argentina, Germany, Czeh R. Columbia, Norway, Switzerland	Safety, efficacy and tolerability with reduced Neoral and SImulect	*De novo* kidney	OL, parallel, non-comparator,	12 months	256	256	0	-	0.75 and 1.5 mg b.i.d.

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Primary endpoint (study treatment duration)	No. of patients randomized			Comparator	RAD dose(s)
						Total no. of patients	RAD	Compar-ator		
B157 #	Canada, Germany, United Kingdom, US (8)	Safety and tolerability, PK	*De novo* kidney	Randomized, double-blind, dose-finding	6 months (1 year + 2 year extension)	103	103	N/A	None	0.5, 1 and 2 mg b.i.d.
B156 #	France, Germany, Italy, US (12)	Efficacy, safety and tolerability	*De novo* kidney	Randomized, open-label with Simulect®	6 months (3 years)	111	111	N/A	High vs. low cyclo-sporine trough level	1.5 mg b.i.d.
B351#	Europe, US (12)	Efficacy, safety and tolerability, PK	Pediatric *de novo* kidney	Open-label, single arm	12 months (3 years)	19	19			0.5, 1 and 2 mg b.i.d.

#Conducted under a US IND (Note: Study B201 was not conducted under a US IND)

[1]Austria, Belgium, Denmark, France, Germany, Italy, Norway, Poland, Spain, Switzerland, United Kingdom

*Austria, Belgium, Czech Republic, France, Germany, Italy, Netherlands, Norway, Russia, Spain, Switzerland, UK

Table 9-9 Multiple dose liver and lung transplant studies

Study no.	Countries (total number of centers)	Objective(s)	Patient population	Design	Primary endpoint (study treatment duration)	No. of patients randomized			Comparator	RAD dose(s)
						Total no. of patients	RAD	Compar-ator		
B158 #	Canada, France, Germany, United Kingdom, Nether-lands, US (16)	Safety and tolerability, PK	*De novo* liver	Randomized, double-blind, placebo-controlled	12 months (3 years)	119	90	30	Placebo	
B159 #	Australia, Canada, Europe[1], US (40)	Efficacy, safety and tolerability, PK	Maintenance lung and heart/lung at high risk for bronchiolitis obliterans syndrome (BOS)	Randomized, double-blind, active-controlled	12 months (3 years)	230	120	110	Azathioprine	1.5 mg b.i.d. (0.75 mg b.i.d. arm discont.'d)
B152 #	Australia, Denmark, Germany, US	Efficacy, safety and tolerability	Lung and heart/lung patients with BOS1 or BOS2	Randomized, open-label, active-controlled	12 months (1 year+ 2 year extension)	31	15	16	ATGAM and Azathioprine	2 mg b.i.d.

#Conducted under a US IND; [1]France, Germany, Italy, Netherlands, Spain, Sweden, United Kingdom