OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA	**21-560 and 21-628**
Submission Date(s)	**10/4/02 (chemistry only), 12/20/02 (original), 2/14/03, 5/2/03 (safety update), 6/10/03, 7/9/03, 7/18/03, 10/13/03**
Brand Name	**Certican**
Generic Name	**Everolimus (code name; RAD001, SDZ RAD)**
Primary Reviewer	**Jang-Ik Lee, Pharm.D., Ph.D.**
Supporting Reviewers	**Jenny J. Zheng, Ph.D. (Pharmacometrics)** **Seong Jang, Ph.D. (Dissolution)**
Team Leader	**Philip Colangelo, Pharm.D., Ph.D.**
OCPB Division	**DPE III (HFD-880)**
OND Division	**ODE IV DSPIDP (HFD-590)**
Sponsor	**Novartis Pharmaceuticals Corp.**
Relevant IND(s)	**52,003**
Submission Type; Code	**Original, 1S (NME)**
Formulation; Strength(s):	**Immediate release tablets;** **0.25 mg, 0.5 mg, 0.75 mg, and 1.0 mg**

Proposed Indication:

> *N21-560***: Prophylaxis of organ rejection in allogeneic kidney transplant patients**
> *N21-628***: Prophylaxis of organ rejection in allogeneic heart transplant patients**

Proposed Dosage and Administration: **Oral doses of 0.75 mg b.i.d. or larger to maintain whole blood concentrations of everolimus ≥ 3.0 ng/mL in combination with cyclosporine and corticosteroids**

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1. EXECUTIVE SUMMARY

Everolimus, 40-O-(2-hydroxyethyl)-rapamycin, is a macrolide immunosuppressant which is derived by chemical modification of the natural product rapamycin that certain strains of *Streptomyces hygroscopicus* produce. Everolimus acts as a proliferation signal inhibitor that blocks growth factor-driven transduction signals in the cellular response to alloantigens. Everolimus has been developed as adjunctive therapy to cyclosporine and steroids in the prophylaxis of acute rejection in patients receiving allogeneic kidney and heart transplants. Two dosage forms are intended for marketing: immediate release (IR) tablets and dispersible tablets. This review is for the original applications for IR tablets and additional applications (N21-561 and N21-631) for dispersible tablets are separately reviewed. The sponsor is not pursuing pediatric indications because of a potential risk to endocrine function. The Agency denied the pediatric exclusivity request because the sponsor prematurely discontinued the pivotal pediatric study.

The Human Pharmacokinetics and Bioavailability section of the original application contains 24 Clinical Pharmacology and Biopharmaceutics (CPB) study reports (see 6.2. Individual Studies). Among the study reports, this reviewer reviewed 16 essential reports completely and 3 pediatric study reports partially. The sponsor also provided the synopses and partial raw data of two ongoing studies (A2306, A2307) and a labeling revision based on them in the 120-day safety update. The synopses and labeling revision were excluded in this review because their format was not a final reviewable one from a CPB standpoint. Dr. Jenny J. Zheng reviewed the pharmacometrics data, while Dr. Seong Jang reviewed the dissolution part of this submission. Labeling recommendations are deferred because the clinical Division's action for these NDAs will be approvable due to insufficient dosing and safety information.

1.1. Recommendation

The CPB information in this application is not sufficient to support the approval of everolimus. Deficiencies and recommendations are listed below.

(1) From a CPB standpoint, neither the everolimus dosage regimen proposed by the sponsor (see *What is the proposed dosage and route of administration?*) nor the regimens studied in the pivotal clinical studies (B201, B251, and B253) supports a safe and efficacious administration of everolimus for the proposed indications. Particularly, the starting dose appears to be too low and the upper limit of everolimus concentration or dose was not determined. The sponsor needs to adequately determine a starting dose and a target trough concentration range (upper as well as lower limits) by conducting concentration-controlled studies with adequate therapeutic drug monitoring (TDM). If everolimus is to be administered in combination with a full dose of cyclosporine in *de novo* heart transplant patients, we recommend 1.5 mg b.i.d. as a starting dose and a steady state trough concentration range of 4 – 9 ng/mL as a target concentration for subsequent dosage adjustment with TDM. This recommendation is based on our retrospective/exploratory analysis of the exposure-response relationship data obtained from Study B253.

(2) Basic everolimus pharmacokinetic parameters were not adequately determined in target patients of interest (see remarks in Basic Pharmacokinetics section in 6.2. Individual Studies). The sponsor needs to provide adequately estimated values for the clearance (CLb/F), volume of distribution (Vz,b/F), and elimination half-life ($t_{1/2}$) of everolimus at the range of possible clinical doses following single and multiple (steady state) oral doses to target patients of interest using to-be-marketed Certican tablets or formulations that were tested for bioequivalence compared to the tablets.

(3) The CPB information on *in vivo* drug-drug interactions with everolimus is not sufficient. In addition to the *in vivo* drug-drug interaction studies provided in this submission, the sponsor needs to conduct additional *in vivo* interaction studies with other drugs/substrates that are known to affect CYP3A and/or P-glycoprotein and would be potentially coadministered with everolimus to transplant patients. Such drugs/substrates could include but are not limited to digoxin, erythromycin, glyburide, ketoconazole, nifedipine, phenytoin, ritonavir, and oral contraceptives.

(4) In order to more consistently control the quality of the final tablet product, we recommend that the sponsor change the dissolution specification for Certican immediate release tablets to Q = _____ in 30 min (i.e., change from proposed Q = _____ .

1.2. Phase IV Commitments

Not applicable

_____   Date: _____

Jang-Ik Lee, Pharm.D., Ph.D.
Clinical Pharmacology and Biopharmaceutics Reviewer
Division of Pharmaceutical Evaluation III

_____   Date: _____

Jenny J. Zheng, Ph.D.
Pharmacometrics Reviewer
Division of Pharmaceutical Evaluation III

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RD/FT Initialed by Philip Colangelo, Pharm.D., Ph.D. _____ Date: _____

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3. SUMMARY OF CPB FINDINGS

Basic Everolimus Pharmacokinetic Parameters: Following a single oral dose from 1 mg to 4 mg of everolimus to healthy volunteers, time to maximum concentration (Tmax), dose normalized maximum blood concentration (Cmax,b/Dose), and dose normalized area under the blood concentration-time curve (AUCb/Dose) of everolimus were in the range of 0.5 – 1 hr, 4.9 – 11.1 (ng/mL)/mg, and 55 – 66 (ng-hr/mL)/mg, respectively. The respective apparent blood clearance (CLb/F), apparent volume of distribution at elimination phase (Vz,b/F), and elimination half-life ($t_{1/2}$) of everolimus were in the range of 16.5 – 19.7 L/hr, 842 – 1328 L, and 31.5 – 55.8 hr.

When determined in *de novo* kidney transplant patients (Study B157) receiving concomitant Neoral, the respective mean ± SD Cmax,b and AUCb within a 12-hr dosing interval (AUCτ,b) of everolimus were 5.6 ± 3.7 ng/mL and 28 ± 23 ng-hr/mL following the first oral dose of 1 mg. Median Tmax was 3 hr (range, 2- 9 hr). Following twice daily doses of 1 mg for 6 days, the respective Cmax,b and AUCτ,b were 11.6 ± 4.4 ng/mL and 81 ± 34 ng-hr/mL. Median Tmax was 2 hr (range, 1 – 5 hr). The median accumulation ratio calculated from the AUCτ,b determined on Day 1 and Day 7 was 3.0 (range, 1.1 – 51). The CLb/F, Vz,b/F, and $t_{1/2}$ were not adequately determined. Everolimus pharmacokinetic parameters were comparable over time (2, 3, and 6 months).

Absorption, Distribution, Metabolism, and Elimination: Following a single oral dose of ^{14}C-everolimus 3 mg to three stable renal transplant patients receiving concomitant Neoral (Study W107), the Cmax and AUC of radioactivity was higher in blood than in plasma (ratio ~ 2.4). The respective $t_{1/2}$ was 81 hrs and 33 hrs for the radioactivity and parent compound. Approximately 11% of the radioactive dose was circulating in blood. Parent compound, mono-hydroxylated metabolites, and hydrolytic metabolites accounted for 40%, 25.0%, and 10.6% of the AUCb of the total radioactivity. The metabolites were much less active (by two orders) than everolimus *in vitro*. Rapamycin, the active metabolite, accounted for only 1.2 % of the total radioactivity. The 80% and 5% of the administered radioactive dose were recovered in feces and urine, respectively, during the collection interval of 10 days. No parent drug was detected in excreta. This data indicates that everolimus undergoes extensive metabolism and is primarily excreted by non-renal routes.

Protein Binding: The everolimus uptake to human erythrocytes was approximately 85% at the blood concentration range of 5 - 100 ng/mL. The fraction of everolimus associated to neutrophils and lymphocytes was approximately 1%. The percentage in plasma was around 14%. The free fraction in human plasma was 0.25 and considered to be concentration independent.

Dose Proportionality: Everolimus dose-concentration relationship was modestly under-proportional when assessed in *de novo* kidney (Study B201) and heart transplant patients (Study B253). Particularly, the mean dose-normalized trough blood concentration (Cmin,b,ss/Dose) following the dose of 1.5 mg b.i.d. were lower up to 22% compared with the mean Cmin,b,ss/Dose following 0.75 mg b.i.d (Study B251).

Exposure-Efficacy Relationship: In general, the proportion of patients free from the primary composite events (%FPCE; biopsy-confirmed acute rejection, graft loss, patient death, lost to follow-up) censored up to 7.5 months showed increasing trend up to the everolimus $C_{min,b,ss}$ of 5 - 7 ng/mL followed by gradual decrease without recognizable pattern. At doses studied (0.75 mg b.i.d. and 1.5 mg b.i.d.), the minimum and maximum %FPCE determined in *de novo* kidney and heart transplant patients were 62% and 96%, and 60% and 80%, respectively. The %FPCE determined in the mycophenolate mofetil (1 g b.i.d., Studies B201 and B251) and azathioprine (1 – 3 mg/kg/day, Study B253) control groups was 80% and 57%, respectively.

Exposure-Safety Relationship: The incidence of hypertriglyceridemia was correlated with everolimus $C_{min,b,ss}$ (r = 0.94, p = 0.02) in Studies B201 and B251. The incidence of thrombocytopenia was correlated with the everolimus $C_{min,b,ss}$ (r = 0.92, p = 0.03) in Study B253. The proportion of patients with decrease in creatinine clearance (CrCL) by 30% or greater censored up to 7.5 months (Study 253) was lowest (approx. 36%) at the everolimus $C_{min,b,ss}$ of 6 ng/mL compared to the proportions (approx. 53% and 64%) at lower (<4 ng/mL) and higher (>13 ng/mL) extreme everolimus $C_{min,b,ss}$. The proportion in the azathioprine control group was approximately 38%.

Everolimus Dose: The sponsor inadequately determined everolimus dosage regimen; particularly, the starting dose appears to be too low and there is no upper limit of dose or concentration. Furthermore, everolimus dose appears to be an unreliable predictor for everolimus AUC or efficacy/safety responses. Following fixed doses of 0.75 mg b.i.d. and 1.5 mg b.i.d., the inter-individual variability in everolimus exposure was up to 59% (Study B251) and, therefore, there was a marked overlap in the frequency distribution of $C_{min,b,ss}$ despite the 2-fold dose difference. Based on the retrospective/exploratory analyses of exposure-efficacy and exposure-safety response relationships, if everolimus is administered in combination of full dose cyclosporine to *de novo* heart transplant patients, this reviewer recommends 1.5 mg b.i.d. as a starting dose and the $C_{min,b,ss}$ range of 4 – 9 ng/mL as a target concentration for subsequent dosage adjustments with TDM.

Special Populations: Compared to healthy controls, patients with moderate hepatic impairment (Child-Pugh scores between 7 and 9, Study A2303) had higher AUCb by 115%, lower mean CLb/F by 47%, and longer mean $t_{1/2}$ of everolimus by 36 hr (43 *versus* 79 hours). Therefore, everolimus dose needs to be reduced by approximately one-half in patients with moderate hepatic impairment. Renal impairment is not likely to significantly impact on everolimus pharmacokinetics because everolimus is extensively metabolized and the metabolites are mainly excreted in feces. A negligible portion of variability (3.9%) in everolimus CLb/F could be explained by CrCL at 14 days after *de novo* renal transplant (Study B157). Black renal transplant patients have 20% higher CLb/F compared with non-black patients. Age and weight (both over the adult ranges) and gender did not affect everolimus pharmacokinetics to a clinically relevant extent.

Food Effect: Following a 2-mg dose of IR tablets under fed conditions (Study W302), everolimus Tmax was prolonged by 1.3 hr, and mean everolimus Cmax,b and AUCb were decreased by 60% and 16%, respectively. It would be prudent to administer Certican tablets on a consistent basis either with food or without food to avoid variations in everolimus exposure between doses.

Drug-Drug Interaction Potential Assessed *In Vitro*: Everolimus is a substrate of CYP3A (Km, $1.8 - 3.0$ µg/mL) and P-glycoprotein. Compounds known to inhibit CYP3A also inhibited everolimus metabolism. Everolimus competitively inhibited cyclosporine metabolism with a Ki value of 2.2 ± 0.5 µg/mL. Considering that everolimus Cmax,b,ss following 1.5-mg dose was around 20 ng/mL, the Cmax,b/Ki ratio was 0.009 and, therefore, a significant effect of everolimus on the metabolism of the representative CYP3A substrate is not expected. The permeability coefficient (P_{eff}) of ^{3}H-everolimus in the apical-to-basolateral transport through a Caco-2 cell monolayer was higher than the P_{eff} of mannitol but much lower than the P_{eff} of propranolol. The basolateral-to-apical P_{eff} was much greater than the apical-to-basolateral P_{eff} at both concentrations. The addition of verapamil, a strong P-glycoprotein inhibitor, virtually completely eliminated the difference. Cyclosporine addition partially reduced the difference.

Drug-Drug Interaction *In Vivo*: A concomitant single oral dose of Neoral 175 mg increased the respective mean Cmax,b and AUCb of everolimus determined after a single oral dose of 2 mg by 82% and 168% (Study A2304). The Neoral coadministration numerically increased the mean $t_{1/2}$ from 25 hr to 29 hr without affecting the median Tmax. Individual increases in the AUCb were highly variable at the range of 46% - 365%. A concomitant single oral dose of Sandimmune 300 mg increased the respective mean Cmax,b and AUCb by 6% and 74%. Individual increases in the AUCb were at the range of 0% - 254%.

The effect of everolimus on atorvastatin or pravastatin exposure and *vice versa* were moderate (up to 16% change) at their clinical doses (Study W 303). No dose adjustment for everolimus or either of these two statins would be necessary for coadministration.

A CYP3A enzyme induction by rifampin 600 mg daily for 8 days increased the mean everolimus CLb/F determined following a single oral dose of 4 mg by 172%. The respective mean decreases in Cmax,b and AUCb were 58% and 63%. The mean $t_{1/2}$ was significantly shortened from 32 hr to 24 hr. Everolimus dose needs to be adjusted when CYP3A inducers are started or discontinued.

A population pharmacokinetic analysis indicated that the coadministration of macrolide antibiotics (erythromycin or azithromycin) and itraconazole may decrease everolimus CLb/F by 20% and 74%, respectively.

Everolimus in Biopharmaceutics Classification System (BCS): Everolimus is a Class 3 drug (high solubility, low permeability) with respect to BCS.

Bioequivalence between Clinical and To-Be-Marketed Tablets: The clinical and to-be-marketed IR tablets were bioequivalent.

Dissolution: Sodium dodecylsulfate (SDS) was used to enhance dissolution of drug substance. To enable complete dissolution, the addition of 0.2% SDS (critical micelle concentration) was necessary. The drug substance is not stable in strong acidic media, up to pH 3. In weak acidic media (aqueous buffer pH 4.5 and 6.8), the drug substance is stable. However, the increase in pH with 0.4% SDS has no advantage over water with 0.4% SDS. Unexpectedly greater solubility and dissolution were achieved with 0.2% SDS compared to 0.4% SDS, resulting in less

discriminating dissolution profiles with 0.2% SDS compared to 0.4% SDS. The dissolution method for Certican tablets, USP apparatus 2 (paddle) at 50 rpm in 500   f water with 0.4% SDS, is acceptable. At this condition, IR tablets dissolved greater than   in 30 min. Thus, we recommend dissolution specifications of Q =   min for IR tablets.

4. QUESTION-BASED REVIEW

4.1. General Attributes

What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product?

Everolimus, 40-O-(2-hydroxyethyl)-rapamycin, is a macrocyclic lactone derived by chemical modification of the natural product rapamycin (sirolimus) that certain strains of *Streptomyces hygroscopicus* produce.

Chemical Name: (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R),-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.0^{4,9}]-hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone

Structure:
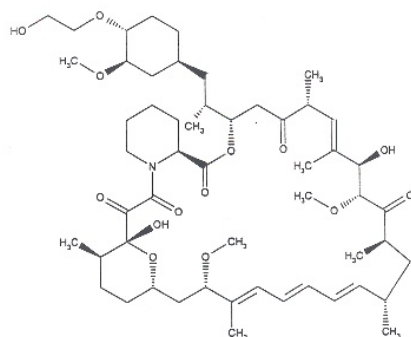
Molecular Weight: 958.25 ($C_{53}H_{83}NO_{14}$)

Physicochemical Properties: Everolimus is a white to faintly yellow powder. Everolimus is more lipophilic than sirolimus. The reported everolimus solubility is < 0.01% (0.1 mg/mL) in water, 0.1 N HCl, and citrate buffer (pH 2.0 - 10.0).

Formulation and Composition: The sponsor developed two Certican immediate release (IR) tablets using a solid dispersion principle for the dissolution of fine colloidal particles of everolimus. Table 1 lists the composition in each strength of IR and dispersible tablets.

Table 1. Composition (mg / tablet) of Certican immediate release tablets.

Ingredients	Strength (mg)				Function	Reference to Standards
	0.25	0.5	0.75	1.0		
					active substance	Novartis
					antioxydant	Ph.Eur., NF
					filling agent	Ph.Eur., NF
					carrier	Ph.Eur., USP
					solvent	Ph.Eur., USP
					solvent	Ph.Eur., NF
					protective gas	Ph.Eur., NF
					lubricant	Ph.Eur., NF
					disintegrant	Ph.Eur., NF
					filling agent	Ph.Eur., NF
Total	80.00	125.00	187.50	250.00		

* corresponds to the drug substance stabilized with butylhydroxytoluene, ** removed during processing, *** processing aid

What is the proposed mechanism of drug action and therapeutic indications?

Mechanism of Action: On a molecular level, everolimus forms a complex with the cytoplasmic protein FKBP-12 and inhibits the growth factor-stimulated phosphorylation of p70 S6 kinase that involves in the initiation of protein synthesis. Because the phosphorylation is under the control of FRAP (FKBP12-Rapamycin-Associated-Protein, also known as mTOR, mammalian target of rapamycin), a key regulatory protein that governs cell metabolism, growth, and proliferation, it is suggested that the everolimus-FKBP-12 complex binds to FRAP and interferes with its function.

On a cellular level, the immunosuppressive activity of everolimus is explained by its ability to inhibit IL-2/IL-15-stimulated T cell proliferation. The antigen-induced activation of antigen-specific T cells by cytokine production such as IL-2 and subsequent proliferation of the activated T cells (clonal-expansion) are the hallmark features of a T cell immune-response. While cyclosporine and tacrolimus prevent the activation (inhibition of G0 to G1 phase transition), everolimus and rapamycin inhibit the proliferation of T cells (cell cycle arrest at the late G1 stage). The difference in the mechanisms of action for everolimus and cyclosporine provides a rationale for their combination.

Indication: The proposed indication is the prophylaxis of organ rejection in allogeneic kidney or heart transplant patients. Everolimus is proposed to be administered in combination with cyclosporine and corticosteroids, and may be used in a regimen including monoclonal antibody (e.g., Simulect).

What is the proposed dosage and route of administration?

General Recommendation: The sponsor proposed 0.75 mg b.i.d. as a starting everolimus dose for kidney and heart transplant populations without clear scientific rationale. Based on retrospective/exploratory analyses of exposure-efficacy and exposure-safety response, however, this reviewer recommends a starting dose of 1.5 mg b.i.d. The sponsor recommends administering everolimus dose as soon as possible after surgical transplant procedure in combination with cyclosporine. In order to minimize variability, everolimus needs to be administered on a consistent schedule either with or without food.

Dosage Adjustments and Therapeutic Drug Monitoring (TDM): The sponsor proposed an increase in everolimus dose at $1 - 2$ week intervals when everolimus trough blood concentrations (Cmin,b,ss) remain below 3 ng/mL. Based on retrospective/exploratory analyses of exposure-efficacy and exposure-safety response relationships, however, this reviewer recommends $4 - 9$ ng/mL as target Cmin,b,ss. This reviewer also recommends routine TDM starting as early as 3 days after the first dose and dose adjustments based on not only Cmin,b,ss but also tolerability, individual response, and the clinical situation. Monitor everolimus Cmin,b,ss especially for blacks, patients with hepatic impairment, during concomitant administration of potent CYP3A4 and/or P-glycoprotein inhibitors or inducers, and in cases of marked cyclosporine dose change.

4.2. General Clinical Pharmacology

What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology and clinical studies?

Everolimus exposure-response relationships were explored based on the combined pharmacokinetic, safety, and efficacy data obtained from *de novo* kidney (Studies B201 and B251) and heart transplant patients (Study B253). The exposure parameter was mean Cmin,b,ss determined from 7 days after transplant to the time of an efficacy or safety event, or a censored time point (7.5 months post transplant), whichever came first. It is noted that the frequency of Cmin,b,ss monitoring was not the same over time (e.g.; 0.25, 0.5, 1, 2, 3, 6 months). The respective efficacy and safety parameters were freedom from primary composite endpoints (PCE) and decrease in creatinine clearance (CrCL) by 30% or greater (DCrCL30). Practically, the proportion of transplant patients free from PCE (%FPCE) and with DCrCL30 (%DCrCL30) were calculated and compared between everolimus treatment and active control groups. The PCE includes biopsy-confirmed acute rejection, graft loss, patient death, and lost to follow-up. Although the sponsor assessed the incidence of hypercholesterolemia (> 250 mg/dL), hypertriglyceridemia (> 250 mg/dL), leukocytopenia (< 4 x 10^9/L), thrombocytopenia (< 100 x 10^9/L), and nephrotoxicity (serum creatinine > 200 μmol/L, Study B253 only) as safety parameters, this reviewer excluded the detailed results because those parameters contained inseparable confounding factors (hypercholesterolemia and hypertriglyceridemia), were unserious and reversible in clinical nature (thrombocytopenia), had no relationship with everolimus exposure (leukocytopenia), or were less reliable (serum creatinine).

This reviewer chose PCE as an efficacy parameter because PCE is more relevant than acute rejection episode alone to determine the success or failure of an immunosuppressive therapy in transplant patients under the intent-to-treat concept. This reviewer chose DCrCL30 as a safety

parameter because one of the most serious adverse effects that can be reliably quantified following a cyclosporine-based immunosuppressive therapy is DCrCL and everolimus is additive to the cyclosporine-induced nephrotoxicity. In Study B253, the mean baseline CrCL estimated using Cockroft-Gault calculation formula was around 68 mL/min and the 30% decrease resulted in CrCL of 47 mL/min.

Are the active moieties appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes. Refer to 4.6 Analytical.

What are the characteristics of the exposure-response relationships for efficacy and safety?

Degree of Linearity in Dose-Concentration Relationship: The dose-concentration relationship is not quite linear at the potential clinical doses at steady state. The relationship was modestly under-proportional when assessed in *de novo* renal transplant patients (Study B201). The respective mean dose-normalized values at steady state for $C_{min,b,ss}$/Dose, maximum blood concentrations ($C_{max,b,ss}$/Dose), and areas under the blood concentration-time curves at the 12-hr dosing interval ($AUC_{\tau,b,ss}$/Dose) were lower by 18% (90% confidence interval of geometric mean ratio [90% CI of GMR], 75% - 90%), 15% (76% - 96%), and 20% (72% - 90%) following the dose of 1.5 mg b.i.d. as compared with 0.75 mg b.i.d. (Table 2).

Table 2. Everolimus pharmacokinetic parameters (mean ± SD) determined at steady state in *de novo* renal transplant patients receiving concomitant cyclosporine doses (Study B201).

Everolimus Pharmacokinetic Parameter	0.75 mg b.i.d. (reference)			1.5 mg b.i.d. (test)		
	Month 2	Month 3	Month 6	Month 2	Month 3	Month 6
	n = 34	n = 40	n = 33	n = 40	n = 38	n = 30
Tmax* (hr)	1.1 (1-5)	1.0 (1-6)	1.0 (1-5)	1.0 (1-5)	1.1 (1-5)	1.0 (0-5)
$C_{min,b,ss}$ (ng/mL)[#]	5.0 ± 2.3	5.2 ± 2.6	5.5 ± 2.2	7.5 ± 2.4	9.2 ± 4.7	8.7 ± 3.4
$C_{min,b,ss}$/Dose(ng/mL/mg)[#]	6.6 ± 3.1	6.9 ± 3.4	7.3 ± 2.9	5.0 ± 1.6	6.1 ± 3.1	5.8 ± 2.3
Geometric Mean Ratio (90% CI)[#]	**0.82 (0.75 – 0.90)**					
$C_{max,b,ss}$ (ng/mL)	11.5 ± 3.9	12.8 ± 4.9	12.1 ± 3.5	19.7 ± 6.8	22.3 ± 9.3	22.5 ± 9.7
$C_{max,b,ss}$/Dose (ng/mL/mg)	15.3 ± 5.2	17.1 ± 6.5	16.2 ± 4.7	13.1 ± 4.6	14.9 ± 6.2	15.0 ± 6.4
Geometric Mean Ratio (90% CI)	**0.85 (0.76 – 0.96)**					
$AUC_{\tau,b,ss}$ (ng-hr/mL)	83 ± 28	91 ± 38	90 ± 29	133 ± 38	153 ± 75	149 ± 56
$AUC_{\tau,b,ss}$/Dose (ng-hr/mL/mg)	111 ± 38	121 ± 51	120 ± 39	89 ± 25	102 ± 50	100 ± 38
Geometric Mean Ratio (90% CI)	**0.80 (0.72 – 0.90)**					

* median (range), [#] based on 1989 predose troughs from 361 patients

In similar *de novo* kidney (B251, data not shown) and heart transplant studies (B253, Table 3), the mean $C_{min,b,ss}$/Dose were lower by 22% at the higher dose, respectively, while the mean $C_{max,b,ss}$/Dose and $AUC_{\tau,b,ss}$/Dose were comparable (the 90% CI of GMR within 80 – 125%).

Table 3. Everolimus pharmacokinetic parameters (mean ± SD) determined at steady state in *de novo* heart transplant patients receiving concomitant cyclosporine doses (Study B253).

Everolimus Pharmacokinetic Parameter	0.75 mg b.i.d. (reference)			1.5 mg b.i.d. (test)		
	Month 2	Month 3	Month 6	Month 2	Month 3	Month 6
	n = 22	n = 23	n = 20	n = 20	n = 20	n = 14
Tmax* (hr)	2 (1 - 5)	2 (1 - 5)	2 (1 - 5)	2 (1 - 5)	2 (0 - 5)	2 (1 - 5)
$C_{min,b,ss}$ (ng/mL)#	4.1 ± 3.5	5.1 ± 3.8	4.8 ± 3.3	8.7 ± 5.1	9.1 ± 6.3	8.5 ± 5.6
$C_{min,b,ss}$/Dose(ng/mL/mg)#	5.5 ± 4.7	6.8 ± 5.1	6.0 ± 4.4	5.8 ± 3.4	6.1 ± 4.2	5.7 ± 3.7
Geometric Mean Ratio (90% CI)#	**0.83 (0.77 – 0.89)**					
$C_{max,b,ss}$ (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
$C_{max,b,ss}$/Dose (ng/mL/mg)	13.5 ± 5.1	13.1 ± 5.8	13.6 ± 6.4	13.3 ± 5.8	12.4 ± 4.5	13.8 ± 8.3
Geometric Mean Ratio (90% CI)	1.03 (0.86 – 1.23)					
$AUC_{\tau,b,ss}$ (ng-hr/mL)	79 ± 30	82 ± 43	80 ± 39	159 ± 63	158 ± 60	164 ± 87
$AUC_{\tau,b,ss}$/Dose (ng-hr/mL/mg)	106 ± 40	110 ± 57	104 ± 52	106 ± 42	105 ± 40	104 ± 58
Geometric Mean Ratio (90% CI)	1.07 (0.88 – 1.29)					

* median (range), # based on 2328 predose troughs from 410 patients,

Concomitant cyclosporine administration does not seem to affect the non-linearity in the everolimus pharmacokinetics: cyclosporine trough concentrations were comparable between everolimus dose groups in both kidney (Table 4) and heart transplant study (Table 5).

Table 4. Comparison of cyclosporine trough concentrations (mean ± SD $C_{min,b,ss}$, ng/mL) between kidney transplant patient groups stratified by everolimus doses (Study B201).

	Control (MMF)		Everolimus 0.75 mg bid		Everolimus 1.5 mg bid	
	n	$C_{min,b,ss}$	n	$C_{min,b,ss}$	n	$C_{min,b,ss}$
Week 1	163	248 ± 170	160	252 ± 154	164	263 ± 182
Week 2	158	252 ± 157	154	242 ± 162	155	248 ± 115
Month 1	156	226 ± 120	149	231 ± 137	150	232 ±135
Month 2	144	190 ± 75	136	210 ± 100	137	205 ± 114
Month 3	138	175 ± 72	132	193 ± 105	127	218 ± 176
Month 6	111	170 ± 61	110	172 ± 86	108	165 ± 95

Table 5. Comparison of cyclosporine trough concentrations (mean ± SD $C_{min,b,ss}$, ng/mL) between heart transplant patient groups stratified by everolimus doses (Study B253).

	Control (Azathioprine)		Everolimus 0.75 mg bid		Everolimus 1.5 mg bid	
	n	$C_{min,b,ss}$	n	$C_{min,b,ss}$	n	$C_{min,b,ss}$
Week 1	167	223 ± 128	167	220 ± 120	167	226 ± 132
Week 2	176	259 ± 122	171	254 ± 104	176	253 ± 111
Week 3	172	259 ± 100	165	262 ± 122	164	264 ± 113
Month 1	180	271 ± 106	163	270 ± 119	159	255 ± 111
Month 2	163	250 ± 96	154	253 ± 109	150	246 ± 95
Month 3	144	233 ± 93	140	231 ± 94	130	215 ± 89
Month 6	123	205 ± 97	112	201 ± 109	115	185 ± 87

Degree of Linearity in Trough Concentration-AUC Relationship: The relationship between everolimus Cmin,b,ss (predose trough concentration) and AUCτ,b,ss (postdose AUC) was linear (n = 417, r = 0.86, p < 0.001) when determined in *de novo* kidney transplant patients (Figure 1, Study 251). Another *de novo* renal transplant study (B201; n = 242, r = 0.94, p < 0.001) and a *de novo* heart transplant study (B253; n = 129, r = 0.90, p < 0.001) showed slightly better results (figures not shown).

Exposure-Efficacy Relationship: The sponsor initially determined everolimus exposure-efficacy relationship using a median-effect model for the proportion of patients free from biopsy-confirmed acute rejection observed up to six months after *de novo* kidney and heart transplants at each quintile of everolimus Cmin,b,ss. Although the sponsor's analysis showed a positive relationship between the Cmin,b,ss and the proportion (r >= 0.93, p <= 0.02), the analysis ignored other important efficacy variables such as graft loss and patient death, and was not useful to determine a clinically relevant target concentration range (both lower and higher limit) for everolimus dosage adjustment using TDM. A histogram and Kaplan-Maier survival analysis for the proportion of transplant patients free from the primary composite endpoints (%FPCE) with various ways of patient stratification based on Cmin,b,ss were not successful. Therefore, to determine the target concentration range, this reviewer applied an approach that calculates the %FPCE from the PCE of 78 adjacent patients (39 patients below and above) at the Cmin,b,ss of the patient of interest.

Figure 1. Relationship between everolimus Cmin,b,ss (predose troughs) and AUCτ,b,ss (postdose AUC) determined in *de novo* kidney transplant patients (Study B251; n = 417, r = 0.86, p < 0.001)

According to the reviewer's approach, the %FPCE censored up to 7.5 months were increasing from 62% to 96%, decreasing from 96% to 82%, and then increasing again from 82% to 94% at the everolimus Cmin,b,ss range of <5 ng/mL, 5 – 6.5 ng/mL, and >=6.5 ng/mL, respectively, in *de novo* kidney transplant studies (B201 and B251, Figure 2). In contrast, the mycophenolate mofetil (MMF 1 g b.i.d.) control showed the %FPCE of 79%. In *de novo* heart transplant study (B253), the %FPCE were increasing from 61% to 77%, staying at the plateau of 77%, and then slowly declining from 77% to 72% at the everolimus Cmin,b,ss range of <5 ng/mL, 5 – 10 ng/mL, and >=10 ng/mL, respectively (circles in Figure 3). In contrast, the azathioprine control (1 – 3 mg/kg/day)

Figure 2. Proportion (%) of *de novo* kidney transplant patients free from primary composite events (Study B201 + B251, N = 617, censored at 7.5 months).
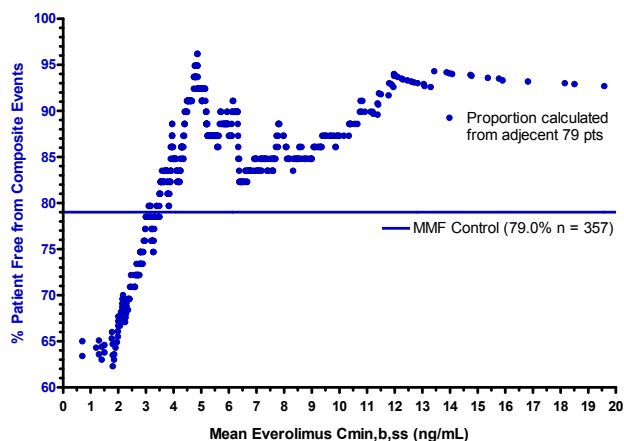
showed the %FPCE of 57.4%. Note that the exposure-efficacy relationships were determined under the cyclosporine trough concentrations shown in Tables 4 and 5, and that the mean proportions of noncompliant patients to biopsy for acute rejection confirmation were 7.7% (range, 5.9% – 10.7%), 10.3% (5.7% - 17.5%), and 8.6% (5.2% - 15.0%) in groups of 0.75 mg, 1.5 mg, and azathioprine control, respectively, when assessed up to 7.5 months.

Exposure-Safety Relationship: Similar to the exposure-efficacy relationship analysis, the sponsor initially analyzed everolimus exposure-safety relationship using a median-effect model for the incidences of hypercholesterolemia, hypertriglyceridemia, leukocytopenia, thrombocytopenia, and

Figure 3. Proportion (%) of *de novo* heart transplant patients (Study B253,) free from primary composite events (efficacy) and with decrease in creatinine clearance by 30% greater from baseline (safety).

nephrotoxicity. In Study B201 and Study B251, while the incidence of hypertriglyceridemia correlated with everolimus $C_{min,b,ss}$ (increase from 59% to 77%; $r = 0.94$, $p = 0.02$), the incidences of hypercholesterolemia, leukocytopenia, and thrombocytopenia were not related to the $C_{min,b,ss}$ ($r < 0.54$, $p > 0.05$). While the incidence of leukocytopenia was numerically lower in everolimus-treated than MMF-control patients (26% *versus* 36%), the incidences of hypercholesterolemia (82% *versus* 60%), hypertriglyceridemia (68% *versus* 47%), and thrombocytopenia (11% *versus* 7%) were greater. In Study B253, while the incidence of thrombocytopenia was correlated with the $C_{min,b,ss}$ (increase from 5% to 9%; $r = 0.92$, $p = 0.03$), the incidences of hypercholesterolemia, hypertriglyceridemia, leukocytopenia, and nephrotoxicity were not related to the $C_{min,b,ss}$. While the incidence of leukocytopenia was numerically lower in everolimus-treated than azathioprine-control patients (26% *versus* 36%), the incidences of hypercholesterolemia (53% *versus* 29%), hypertriglyceridemia (53% *versus* 35%), thrombocytopenia (7% *versus* 1%), and nephrotoxicity (26% *versus* 36%) were greater. Note that the exposure-efficacy relationships were determined under the cyclosporine trough concentrations shown in Table 5.

This reviewer reanalyzed the exposure-safety relationship observed in Study B253 using the approach mentioned in **Exposure-Efficacy Relationship**. Instead of serum creatinine levels, this reviewer used %DCrCL30 as an index of nephrotoxicity. The %DCrCL30 censored up to 7.5 months were stable around 53%, decreasing from 53% to 36%, increasing from 36% to 64%, and then slowly decreasing from 64% to 55% at the everolimus $C_{min,b,ss}$ range of <4 ng/mL, 4 – 6 ng/mL, 6 – 13 ng/mL, and >=13 ng/mL, respectively (diamonds in Figure 3). In contrast, the azathioprine control showed the %DCrCL30 of 37.6%. This approach could not be applied for *de novo* kidney transplant studies because baseline CrCL values could not be accurately estimated.

Dose with Respect to Relationship in Dose-Concentration-Response: Everolimus dose appears to be an unreliable predictor for everolimus concentration, AUC, or efficacy/safety responses. The inter-individual variability in everolimus pharmacokinetic parameters was very
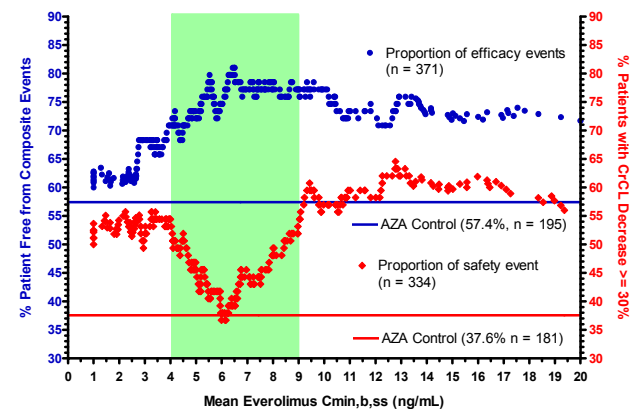
large following a fixed dose (see the question on inter-individual variability). Even with a two fold increase in everolimus dose from 0.75 mg b.i.d. to 1.5 mg b.i.d., there was a marked overlap in the frequency distribution of everolimus Cmin,b,ss (Study B253, Figure 4). On combining the everolimus exposure-efficacy and exposure-safety relationships shown above, the Cmin,b,ss of 4 – 9 ng/mL appears to be relatively efficacious and safe concentration range for *de novo* heart transplant patients (shaded area in Figure 3). This range may be similar for *de novo* kidney transplant patients under the assumption that the safety response is comparable.

Figure 4. Frequency distribution of everolimus Cmin,b,ss determined from *de novo* heart transplant patients (n = 371, Study B253)
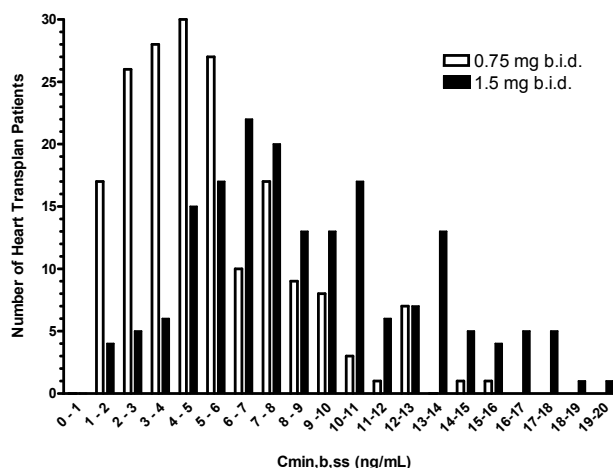
On stratifying the *de novo* kidney transplant patients in Study B201 and Study B251 based on the recommended concentration range, the Kaplan-Maier survival curve of %FPCE for the group of everolimus Cmin,b,ss 4 – 9 ng/mL was superior to that for the groups of MMF control (log rank test, p = 0.0018) or Cmin,b,ss < 4 ng/mL (p = 0.0007) but not inferior to that for the group of Cmin,b,ss >= 9 ng/mL (p = 0.63, Figure 5A). Similarly, the survival curve for Cmin,b,ss 4 – 9 ng/mL was superior to that for azathioprine control (p < 0.0001) or Cmin,b,ss < 4 ng/mL (p = 0.013) but not inferior to that for Cmin,b,ss >= 9 ng/mL in *de novo* heart transplant study (B253, Figure 5B). In terms of the %DCrCL30 determined in Study B253 (Figure 6), the survival curve for Cmin,b,ss 4 – 9 ng/mL was not significantly inferior to that of azathioprine control (p = 0.26) but superior to that of Cmin,b,ss < 4 ng/mL (p = 0.013) or >= 9 ng/mL (p = 0.0029).

Figure 5. Kaplan-Maier survival curves for primary composite events stratified by everolimus Cmin,b,ss (p values for comparison with the group of Cmin,b,ss 4 – 9 ng/mL)

A. *De novo* kidney transplant (Study B201 + B251)   B. *De novo* heart transplant (Study B253)
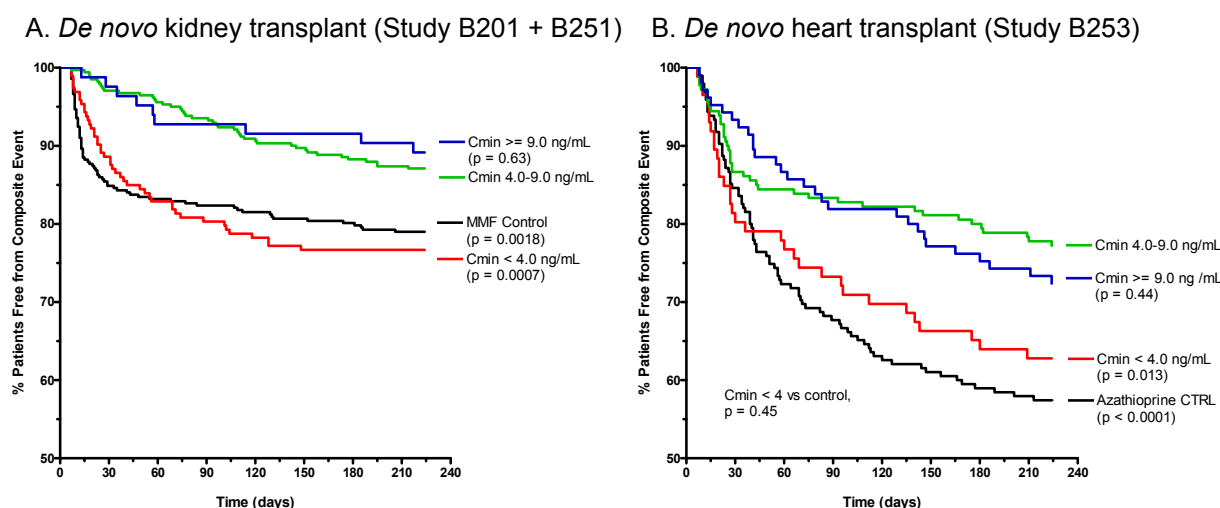
In retrospect, when assessed based on the target Cmin,b,ss range determined by this reviewer, neither fixed everolimus dose regimens studied in Study B253 were clinically efficacious and safe: at 0.75 mg b.i.d. regimen, respective 38% and 11% of enrolled patients were under- and over-dosed; at 1.5 mg b.i.d. regimen, respective 8% and 45% were under- and over-dosed (Table 4).

Overall, if everolimus is to be administered in combination with a full dose of cyclosporine to *de novo* heart transplant patients, this reviewer recommends 1.5 mg b.i.d. as a starting dose and the Cmin,b,ss range of 4 – 9 ng/mL as an target concentration for subsequent dosage adjustments. In conjunction, this reviewer recommends conducting adequate TDM at least weekly until the trend in everolimus trough concentrations is stable. This reviewer also recommends conducting concentration-controlled phase III studies to assess whether the starting dose of 1.5 mg b.i.d. and the target Cmin,b,ss of 4 – 9 ng/mL are clinically feasible for the *de novo* kidney and heart transplant patients concomitantly receiving a full dose of cyclosporine and steroids.

Figure 6. Kaplan-Maier survival curves for decrease in CrCL by >= 30% stratified by everolimus Cmin,b,ss (*de novo* heart transplant study B253, p values for comparison with the group of Cmin,b,ss 4 – 9 ng/mL).
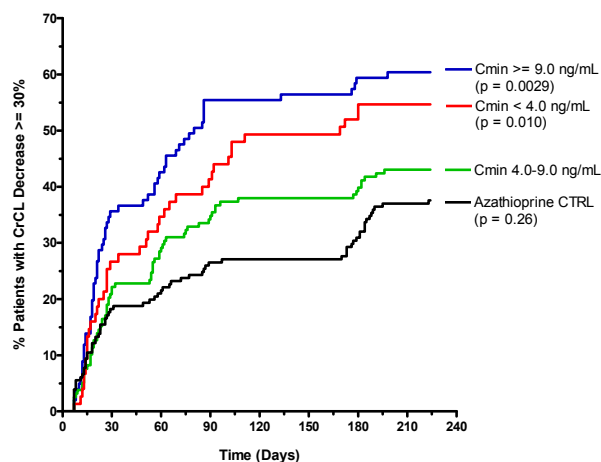
Table 6. Distribution of transplant patients in each range of mean everolimus Cmin,b,ss.

Study	Everolimus Dose	Mean Everolimus Cmin,b,ss (ng/mL)			Total
		< 4	4 - 9	>= 9	
De Novo Kidney Transplant (B201 + B251)	0.75 mg b.i.d.	155 (51%)	142 (47%)	4 (1%)	301
	1.5 mg b.i.d.	38 (12%)	225 (71%)	53 (17%)	316
	Total	193 (23%)	367 (49%)	57 (28%)	617
De Novo Heart Transplant (B253)	0.75 mg b.i.d.	71 (38%)	93 (50%)	21 (11%)	185
	1.5 mg b.i.d.	15 (8%)	87 (47%)	84 (45%)	186
	Total	86 (23%)	180 (49%)	105 (28%)	371

Time Dependency in Pharmacokinetics: Everolimus pharmacokinetic parameters were comparable between the time points compared (2, 3, and 6 months) in Studies B201 (Table 2), B251 (data not shown), and B253 (Table 3): the visit-effect was not statistically significant ($p > 0.05$) and each 90% CI of GMR for paired visits were within the range of 80 – 125%. The time dependency in everolimus pharmacokinetics was assessed at 2, 3, and 6 months because everolimus had a significant drug-drug interaction with cyclosporine and targeted cyclosporine concentration ranges (100 – 300 ng/mL) were not different within the time periods.

Onset or Offset of Pharmacological Response: Not applicable

How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

Basic Pharmacokinetic Parameters

No single study in this application compared basic everolimus pharmacokinetic parameters determined in healthy subjects and transplant patients. The pharmacokinetic parameters determined in healthy subjects following a single oral dose in the range from 1 to 4 mg are shown below in Table 7.

Table 7. Basic everolimus pharmacokinetic parameters (mean ± SD) determined in healthy volunteers following a single oral dose.

Everolimus Dose	1 mg	1.5 mg	2 mg	2 mg	4 mg
Study	W301	A2407	W302	A2303	A2302
Subjects (n)	16	24	24	8	12
Tmax (hr)*	0.75 (0.5 - 1.0)	1.0 (0.5 - 2.0)	0.5 (0.5 – 2.0)	0.5 (0.5-2.0)	0.5 (0.5-1.0)
Cmax,b (ng/mL)	4.9 ± 0.9	12.3 ± 4.8	17.9 ± 5.9	15.4 ± 8.6	44.2 ± 13.3
Cmax,b/Dose# (ng/mL)/mg	4.9 ± 0.9	8.2 ± 3.2	9.0 ± 3.0	7.7 ± 4.3	11.1 ± 3.3
AUC,b (ng·hr/mL)	62 ± 26	99 ± 28	122 ± 52	114 ± 45	219 ± 69
AUC,b/Dose# (ng·hr/mL)/mg	62 ± 26	66 ± 19	61 ± 26	57 ± 23	55 ± 17
CLb/F (L/hr)	18.9 ± 8.4	16.5 ± 5.5	19.1 ± 7.4	19.4 ± 5.8	19.7 ± 5.4
Vz,b/F (L)	1328 ± 359	986 ± 494	842 ± 315	1219 ± 593	ND
$t_{1/2}$ (hr)	55.8 ± 22.9	41.0 ± 11.2	31.5 ± 6.4	43 ± 18	32.2 ± 6.1

* median (range); # calculated from mean Cmax or AUC divided by dose; ND, not determined

When determined in *de novo* renal transplant patients receiving concomitant cyclosporine doses from Day 1 post transplant (Study B157), the respective mean ± SD Cmax,b and AUCτ,b of everolimus were 5.6 ± 3.7 ng/mL and 28 ± 23 ng-hr/mL following the first oral dose of 1 mg (Table 8 in the next page). Median Tmax was 3 hr (range, 2 – 9 hr). Following twice daily doses of 1 mg for 6 days, the respective Cmax,b and AUCτ,b were 11.6 ± 4.4 ng/mL and 81 ± 34 ng-hr/mL under the cyclosporine concentration of 199 ± 79 ng/mL. Median Tmax was 2 hr (range, 1 – 5 hr). The median accumulation ratio calculated based on the AUCτ,b determined on Days 1 and 7 was 3.0 (range, 1.1 – 51). The dose-normalized parameters were comparable following 2-mg dose.

Other basic everolimus pharmacokinetic parameters than those shown in Table 8 were not adequately determined in target patients of interest. Specifically, CLb/F, Vz,b/F, and $t_{1/2}$ determined in transplant patients following single or multiple everolimus dosing at the full range of potential clinical doses using to-be-marketed tablets or formulations that were tested for bioequivalence to the to-be-marketed tablets were undetermined or unreliable (see remarks in Basic Pharmacokinetics section in 6.2. Individual Studies). The mean CLb/F value calculated from the everolimus dose divided by mean AUCτ,b using data from Study B157 was 12.3 L/hr following 1-mg dose (Table 8). Based on the analysis of population pharmacokinetic data obtained from Studies B201 and B251 (see Dr. Zheng's pharmacometrics review), the respective mean values for CLb/F and apparent central volume of distribution (Vc,b/F) was 8.8 L/hr and 110 L. Based on the sponsor's calculation (not reviewed in detail), the mean $t_{1/2}$ value

determined administering a single dose of everolimus capsules (2.5 mg) to six kidney transplant patients receiving cyclosporine coadministration were unexpectedly shorter (25 ± 6 hrs) than the values determined in healthy subjects not receiving cyclosporine.

Table 8. Basic everolimus pharmacokinetic parameters (mean ± SD) determined in *de novo* renal transplant patients following first and multiple oral doses in combination with oral cyclosporine administrations (Study B157).

Everolimus Dose	1 mg		2 mg	
Time of Measurement	Day 1	Day 7	Day 1	Day 7
Subjects (n)	32	32	30	32
Cyclosporine $C_{min,b,ss}$ (ng/mL)		199 ± 79		227 ± 93
T_{max} (hr)*	3 (2-9)	2 (1-5)	3 (2-12)	2 (1-8)
$C_{max,b}$ (ng/mL)	5.6 ± 3.7	11.6 ± 4.4	9.8 ± 7.0	21.9 ± 10.5
$C_{max,b}$/Dose[#] (ng/mL)/mg	5.6 ± 3.7	11.6 ± 4.4	4.9 ± 3.5	11.0 ± 5.3
$C_{min,b}$ (ng/mL)		4.7 ± 2.6		9.5 ± 5.2
$C_{min,b}$/Dose[#] (ng/mL)/mg		4.7 ± 2.6		4.8 ± 2.6
$AUC_{\tau,b}$ (ng·hr/mL)	28 ± 23	81 ± 34	56 ± 37	164 ± 78
$AUC_{\tau,b}$/Dose[#] (ng·hr/mL)/mg	28 ± 23	81 ± 34	28 ± 18	82 ± 39
Accumulation Ratio on AUC*		3.0 (1.1 – 51)		2.7 (1.2 – 79)
CL_b/F (L/hr)^		12.3		12.2

* median (range); [#] calculated from mean parameter value divided by dose; ^ calculated from dose divided by mean $AUC_{\tau,b}$

Absorption, Distribution, Metabolism and Elimination (Mass Balance Study)

Absorption and Bioavailability of Everolimus: In a mass balance study (W107), everolimus-derived radioactivity and everolimus concentrations in blood reached Cmax 1 - 2 hours after a single oral dose of ^{14}C-everolimus 3 mg (72.72 µCi) to three stable renal transplant patients receiving Neoral at the target $C_{min,b,ss}$ of 80 - 200 ng/mL (Figure 7). The respective Cmax values were 71 ± 11 pmol/mL and 36.7 ± 2.1 pmol/mL (Table 9 in the next page). The Cmax and AUC of radioactivity was higher in blood than in plasma (ratio, approx. 2.4) which reflects the uptake of everolimus into blood cells. The AUC of parent compound accounts for 36.7%

Figure 7. Concentration-time profiles (mean ± SD) of everolimus-derived radioactivity, everolimus, and rapamycin in blood (Study W107)

of the AUC of everolimus-derived radioactivity. The respective $t_{1/2}$ determined with last three measurable blood concentrations were 81 hrs and 33 hrs for the everolimus-derived radioactivity and the parent compound.
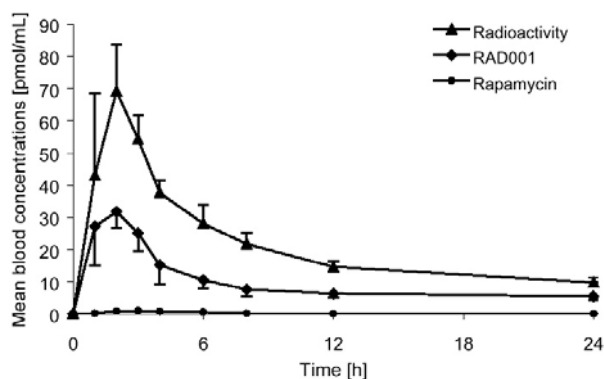
Approximately 11% of the radioactive dose (parent drug and metabolites) was circulating in blood at Tmax when calculated with the mean Cmax of radioactivity and an assumed blood

volume of 5L. Because no human study was conducted following an intravenous administration of everolimus, bioavailability could not be calculated by the ratio of AUCpo/AUCiv of radioactivity or parent drug.

Table 9. Pharmacokinetic parameters (mean ± SD) for parent compound and ^{14}C-everolimus-derived radioactivity in blood and plasma estimated following a single dose of ^{14}C-everolimus 3 mg (72.72 µCi) to three stable renal transplant patients (Study W107)

Parameter	Unit	Radioactivity in Blood	Radioactivity in Plasma	Everolimus in Blood
Cmax	pmol/mL	71 ±11	29 ± 9	36.7 ± 2.1
	ng/mL			35.1 ± 2.0
AUC_{0-t}*	pmol-hr/mL	1120 ± 101	442 ± 103	413 ± 69
	ng-hr/mL			396 ± 66
$AUC_{0-\infty}$	pmol-hr/mL	1211 ± 96	523 ± 80	445 ± 86
	ng-hr/mL			426 ± 82
Median Tmax	hr	2	2	1.5
Terminal $t_{1/2}$	hr	81 ± 16	100 ± 45	33 ± 6

* t = last measurable time point

Metabolites in Blood: Parent compound was the major component in blood accounting for 39.9% of the AUC(0-24hr),b of total radioactivity (Table 10). Mono-hydroxylated metabolites (p42 and p 50) account for 25.0% of the AUC, while hydrolytic metabolites (p36 and p40) account for 10.6%. These metabolites were found to be at least two orders of magnitude less active than everolimus in a mixed lymphocyte reaction assay (see Pharmacology and Toxicology review). Rapamycin, the active metabolite, was present as a minor species accounting 1.2 % only. The parent compound and listed metabolites accounted for a total of 82.3% of the AUC. The concentrations of most everolimus metabolites declined roughly in parallel with the parent compound.

Table 10. Pharmacokinetic parameters (mean ± SD) and relative amounts for everolimus and its metabolites in blood determined in 3 stable kidney transplant patients (Study W107)

Everolimus and Metabolites	Tmax (hr)	Cmax (pmol/mL)	AUC_{0-24} (pmol-hr/mL)	% of AUC_{0-24} of Radioactivity
PKF229-255 (p36, hydrolyzed)	2	4.5 ± 4.7	21.5 ± 12.6	4.1
PKF226-320 (p40, hydrolyzed)	2	6.3 ± 4.9	34.1 ± 8.7	6.5
46-OH-RAD (p42)	3	4.9 ± 0.6	65.7 ± 8.2	12.6
24-OH-RAD / 25-OH-RAD (p50)	3	6.8 ± 3.6	64.5 ± 13.3	12.4
Unknown (p57)	2	6.4 ± 1.7	29.3 ± 6.1	5.6
Rapamycin	2	0.8 ± 0.3	6.3 ± 0.8	1.2
Everolimus	2	33.1 ± 13.5	207.5 ± 26.3	39.9
others				17.7
Total radioactivity	2	69.3 ± 14.3	520.7 ± 54.1	100.0

Recovery in Urine and Feces: The radioactivity was mainly excreted in feces during the collection interval of 0 - 240 hours: 79.5 ± 6.0 %, 5.1 ± 1.7 %, and 84.6 ± 7.3% of the administered radioactive dose were recovered in feces, urine, and total, respectively. Everolimus excretion was relatively slow: only about 30% of the radioactivity was excreted for three days

after dosing. The excretion was still ongoing 10 days after dosing. No parent drug was detected in excreta, which suggests virtually complete metabolism of ^{14}C-everolimus administered. Metabolites excreted in feces and urine appeared to be polar compounds when determined using chromatographic patterns. Everolimus metabolites in feces were not separable in the radiochromatogram used in this study. The metabolites in urine were hardly detectable.

Extraction Ratio

Accurate extraction ratio for everolimus can not be calculated in the absence of absolute bioavailability (F) data. When the respective F and hepatic blood flow are assumed to be 0.11 (see *Mass Balance Study* above) and 1.35 L/min, the extraction ratio is 0.025 and 0.016 in healthy volunteers and kidney transplant patients, respectively.

What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The intraindividual and interindividual variability in everolimus pharmacokinetic parameters were in the moderate to high range of 24 – 59% when determined in *de novo* kidney (Study B251) and heart (Study B253) transplant patients for the first 6 months (Table 11). The interindividual variability determined in healthy subjects (Table 7) was comparable to that in transplant patients. The intraindividual variability was not adequately determined in healthy volunteers.

Table 11. Intraindividual and interindividual variability (coefficient of variation, %) in everolimus pharmacokinetic parameters at steady state observed for the first 6 months after transplant.

Everolimus PK Parameter	*De Novo* Kidney Transplant (Study B251)		*De Novo* Heart Transplant (Study B253)	
	Intraindividual	Interindividual	Intraindividual	Interindividual
Cmin,b,ss/Dose	44*	59*	38**	40**
Cmax,b,ss/Dose	26[#]	33[#]	30[##]	33[##]
AUCτ,b,ss/Dose	24[#]	35[#]	30[##]	37[##]

*   based on 2025 Cmin,b,ss from 370 patients
** based on 2328 Cmin,b,ss from 410 patients
[#]   based on 417 steady state concentration-time profiles from 170 patients
[##] based on 129 steady state concentration-time profiles from 55 patients

4.3. Intrinsic Factors

What intrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?

Hepatic Impairment: The patients with moderate hepatic impairment (Child-Pugh score between 7 and 9) had significantly lower everolimus elimination compared with the healthy subjects that were matched for sex, age (± 5 years), weight (± 10%), and height (± 5 cm, Study A2303). This is probably because everolimus is extensively metabolized and metabolites are predominantly excreted in bile. The patients had higher mean AUCb, lower mean CLb/F, and longer mean $t_{1/2}$ by 115% (245 ± 91 *versus* 114 ± 45 ng-hr/mL), 47% (9.1 ± 3.1 *versus* 19.4 ± 5.8

L/hr), and 36 hr (43 *versus* 79 hours, Table 12). The differences in mean Cmax (11.7 ± 4.3 *versus* 15.4 ± 8.6, p = 0.32) and Vz,b/F (936 ± 301 *versus* 1219 ± 593, p = 0.19) were not statistically significant. The median Tmax was not different.

Table 12. Effect of moderate hepatic impairment on everolimus pharmacokinetics (mean ± SD) determined following a single oral dose of 2 mg (Study A2303).

Everolimus PK Parameter	Matched Healthy Controls (n = 8)	Patients with Hepatic Impairment (n = 8)	Difference	p-value
Tmax (hr)*	0.5 (0.5-2.0)	0.5 (0.5-1.1)		
Cmax,b (ng/mL)	15.4 ± 8.6	11.7 ± 4.3	-24%	0.32
AUCb (ng-hr/mL)	114 ± 45	245 ± 91	+115%	0.01
CLb/F (L/hr)	19.4 ± 5.8	9.1 ± 3.1	-47%	0.01
Vz,b/F (L)	1219 ± 593	936 ± 301	-23%	0.19
$t_{1/2}$ (hr)	43 ± 18	79 ± 42	+36 hr	0.04

* mean (median)

Everolimus AUCb was positively correlated with total bilirubin levels (r = 0.857, p = 0.0001), negatively correlated with albumin levels (r = 0.717, p = 0.002), and positively correlated with prothrombin time with borderline significance (r = 0.492, p = 0.053). The fractions of ^{3}H-everolimus bound to plasma proteins were comparable (73.8 ± 3.6 % *versus* 73.5 ± 2.4 %) between the hepatic patients and matched controls.

Renal Impairment: Renal impairment is not likely to produce a significant impact on everolimus exposure because everolimus is extensively metabolized and the metabolites are predominantly excreted in feces. The sponsor did not conduct a clinical pharmacology study in patients with renal impairment but, instead, determined the relationship between CrCL and everolimus CLb/F using the data obtained from *de novo* renal transplant patients at 14 days after transplant procedure (n = 81, Study B157). According to the pharmacometrics review (see 6.3. Pharmacometrics Review), a negligible extent of variability in everolimus CL/F could be explained by CrCL (3.9%) and the relationship was not statistically significant (p = 0.08).

Race: According to the pharmacometrics review (see 6.3. Pharmacometrics Review), blacks (n = 65) had an average 20% higher everolimus CLb/F than non-blacks. However, no significant difference in CLb/F was detected for Asians (n = 17).

Age (Pediatrics): The sponsor provided three pharmacokinetic study reports conducted in pediatric transplant patients using dispersible tablets (B257, B258, and B351; see 6.2. Individual Studies). The pivotal multiple dose study (B351) was prematurely discontinued because of the safety concerns evidenced by depressed testosterone levels and renal function. The sponsor is not pursuing pediatric indications for everolimus at this time. Furthermore, the Agency declined the pediatric exclusivity request based on the three studies. Therefore, this reviewer did not completely review the studies. Based on the sponsor's conclusion, everolimus CLb/F increased linearly with body surface area (m^2) and mean CLb/F per body surface area was approximately two-fold higher in pediatric patients compared with adult patients.

Age (Adults), Body Weight, and Gender: Age (ranged from 17 to 69 years old) and body weight (49 to 106 kg) have no effect on everolimus exposure. Similarly, the exposure between

males (n=60) and females (n=31) were not different. According to the pharmacometrics review (see 6.3. Pharmacometrics Review), although there was a statistically significant influence of age and weight on the CLb/F, the relationships were very shallow and data showed considerable scatter. A one-year increase in age in adults resulted in a 0.34% decrease in the CLb/F. Hence, the CLb/F would be 9.5 and 8.2 L/hr at the extremes of age of 20 and 65 years old, respectively. With respect to weight, a one-kg increase in body weight would result in a 0.44% increase in the CLb/F. Hence, the CLb/F would be 7.6 and 10.0 L/hr at the extremes of weight of 40 kg and 100 kg, respectively.

Based upon what is known about exposure-response relationships and their variability, and the groups studied; what dosage regimen adjustments, are recommended for each of these subgroups?

Hepatic Impairment: Based on approximate two-fold increase in AUCb shown in the previous question (Study A2303), everolimus dose needs to be reduced by approximately one-half in patients with moderate hepatic impairment (Child-Pugh B). Further dose reduction may be needed for patients with severe hepatic impairment (Child-Pugh C); however, these patients were not studied. In the hepatic impairment study, Child-Pugh scores did not exclusively identify subjects with elevated exposure in need of a potential dose reduction: three hepatically impaired subjects based on the scores had everolimus AUCs within the range of healthy subjects. As proposed by the sponsor, bilirubin levels alone (e.g., 1.5 mg/dL) may be a better indicator for everolimus dosage adjustment.

Pregnancy and Lactation: The sponsor provided no CPB information on everolimus obtained from pregnant women or nursing mothers. In animal studies (refer to Pharmacology and Toxicology Review), everolimus and/or its metabolites crossed placenta and transferred into milk. The potential risk for pregnant or lactating women or fetus is not known: the sponsor proposed everolimus as a Pregnancy Category C drug. There is no dosing recommendation for pregnant women or nursing mothers. Women of childbearing potential are advised to use effective contraception while they are on and after everolimus treatment (for up to 8 weeks).

Race: Blacks may need a slightly higher everolimus dose (approx. 20%) to achieve similar systemic exposure as non-blacks. Adjust everolimus dose for blacks with adequate TDM

Pediatric Patients: See previous question.

Age (Adults), Body Weight, Gender, and Renal Impairment: No dosage adjustment is recommended based on age (elderly), gender, and renal impairment.

4.4. Extrinsic Factors

What extrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?

Drugs, foods, and dietary supplements that inhibit CYP3A and/or P-glycoprotein activity are likely to increase everolimus exposure through pharmacokinetic interaction when they are given concomitantly and, as a result, to produce overimmunosuppresion that increases the chance to

infection, lymphoproliferative disorder, and other adverse reactions such as thrombocytopenia. Drugs that have immunosuppressive effects are also likely to produce overimmunosuppression by pharmacodynamic interaction. In contrast, drugs, foods, and dietary supplements that induce CYP3A and/or P-glycoprotein activity are likely to decrease everolimus exposure and to produce efficacy failure. Smoking and alcohol use are not likely to modify everolimus exposure.

Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments do you recommend for each of these factors?

It would be inadequate to adjust everolimus dose simply based on the existence of the extrinsic factors mentioned in the previous question because everolimus dose is not a good predictor for exposure (see **Dose with Respect to Relationship in Dose-Concentration-Response**). This reviewer recommends implementing everolimus dosage adjustment with adequate TDM to achieve everolimus trough concentrations within an clinically appropriate range.

Drug-Drug Interactions

In Vitro Basis to Suspect in Vivo Drug Interactions

In an *in vitro* metabolism study using human liver microsomes, the majority of the metabolites showed a mass increase of 16, corresponding to a single hydroxylation, or a loss of 14 mass units, corresponding to demethylation. In addition to metabolites formed via enzymatic reactions, a ring-opened derivative of everolimus (PKF 229-255) and its dehydrated analog (PKF 226-320) were also formed in the absence of NADPH or in incubation buffer alone (approx. 10%/hr at 37ºC). The metabolism of everolimus was qualitatively similar to that of its structural analog rapamycin reported in the literature. When determined over the concentration range of $0.35 – 20$ µM ($0.34 – 19.2$ µg/mL) in two human liver microsomal preparations, the metabolic pathways followed Michaelis-Menten kinetics with the Km-values of 1.9 and 3.1 µM and Vmax values of 46 and 100 nmol/hr/mg microsomal protein. The intrinsic clearance was 24 and 32 mL/hr/mg microsomal protein.

Inhibition of Everolimus Metabolism: Compounds known to inhibit CYP3A metabolism also inhibited everolimus metabolism. TAO, a mechanism based inhibitor for CYP3A, effectively inhibited everolimus metabolism, while furafylline, a mechanism based inhibitor for CYP1A2, had no effect (Table 13 in the next page). All other CYP3A substrates studied inhibited everolimus metabolism at concentrations at which they are known to inhibit competitively. Most relevant were cyclosporine, tacrolimus, rapamycin, ketoconazole, and lovastatin. In another *in vitro* study using human liver microsomes, itraconazole strongly inhibited everolimus metabolism with IC_{50} of 0.18 ± 0.11 µM. However, fluconazole up to 2 µM did not significantly inhibit everolimus metabolism. Therefore, comedication of everolimus with fluconazole rather than ketoconazole or itraconazole may be considered appropriate.

Table 13: Effect of characteristic CYP inhibitors / substrates and potentially coadministered compounds on everolimus (1 µM) metabolism by human liver microsomes.

Inhibitor / Substrate	IC$_{50}$ (µM)	Inhibitor / Substrate	IC$_{50}$ (µM)
Immunosuppressant		*Antidiabetic*	
Cyclosporine	2.2	Tolbutamide	> 500
Tacrolimus	0.47	Glyburide	66
Rapamycin	0.8	*Antihypertensive*	
Azathioprine	> 200	Nifedipine	9.7
Antifungal		Diltiazem	85
Ketoconazole	0.03	*Antiarrhythmic*	
Corticosteroids		Quinidine	181
Prednisone	> 200	*Anti Parkinson*	
Prednisolone	40	Bromocriptine	0.43
Steroids		*Antihypercholesterolemic*	
Progesterone	46	Fluvastatin	> 200
Ethynylestradiol	14	Lovastatin	8.3
Dexamethasone	16	*Oxytocic*	
Antiulcer		Sparteine	> 500
Cimetidine	> 500	*Skeletal Muscle Relaxant*	
Antibiotic		Chlorzoxazone	> 500
Erythromycin	41	*Other*	
Analgesic / antipyretic		Dextromethorphan	> 200
Diclofenac	447	Furafylline*	> 200
Phenacetin	> 500	TAO*	10 (IC$_{75}$)

* mechanism based inhibitors

Role of CYP Enzymes in Everolimus Metabolism

In the metabolism study mentioned above, metabolic profiles were comparable when everolimus was incubated with microsomes from cells expressing specifically CYP3A4. Everolimus metabolism by CYP3A was consistent with its selective inhibition in human liver microsomes by a series of CYP3A inhibitors including the mechanism-based inhibitor, TAO. The IC$_{50}$ values for cyclosporine, rapamycin, and ketoconazole were 2.2 µM, 0.8 µM, and 0.03 µM, respectively. In contrast, everolimus metabolism was not detectable when everolimus was incubated with microsomes from cells expressing CYPs other than CYP3A4 including 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A5.

CYP Enzyme Inhibition/Induction by Everolimus

Everolimus competitively inhibited cyclosporine metabolism with a Ki value of 2.3 ± 0.5 µM (2.2 ± 0.5 µg/mL), which was similar to the Km value of everolimus metabolism. Everolimus was a mixed inhibitor of dextromethorphan O-demethylation with a Ki value of 1.7 ± 0.3 µM (1.6 ± 0.5 µg/mL). The everolimus Cmax,b,ss measured following an oral dose of 1.5 mg was approximately 20 ng/mL, approximately one hundredth of the Ki values. Therefore, a significant effect on the metabolism of the representative CYP3A or 2D6 substrate is not expected. At concentrations up to 200 µM (192 µg/mL), everolimus had no effect on CYP1A2 and CYP2E1 as indicated by the lack of effect on phenacetin and chlorzoxazone metabolism, respectively

(Table 14). Using paclitaxel, tolbutamide and S-mephenytoin as probes, everolimus had little or no effect on CYP2C8, CYP2C9, and CYP2C19, respectively.

Table 14. Effect of everolimus on the metabolism of characteristic CYP substrates.

CYP Isozyme	Substrate	IC_{50} / Ki	Pathway / Metabolite
1A2	Phenacetin	> 200 µM	O-deethylation
2C8	Paclitaxel	~ 23 µM	6-α-hydroxypaclitaxel
2C9	Tolbutamide	~ 33 µM	4-hydroxylation
2C19	S-Mephenytoin	~ 117 µM	4-hydroxylation
2D6	Bufuralol	~ 5 µM	1-hydroxylation
2D6	Dextromethorphan	~ 7 µM / 1.7 µM	O-demethylation
2E1	Chlorzoxazone	> 200 µM	6-hydroxylation
3A	Cyclosporine	~ 6 µM / 2.3 µM	hydroxylation
			N-demethylation
3A	Terfenadine	~ 31 µM	azacyclonol
		> 200 µM	alcohol metabolite
		~ 9 µM	acid metabolite

Effect of P-glycoprotein

Caco-2 Permeability: The permeability coefficient (P_{eff}) of ^{3}H-everolimus at 0.2 and 1.0 µM in the apical-to-basolateral transport through a Caco-2 cell monolayer was around 1.6 - 2.0 x 10^{-6} cm/sec, which was 3 - 4 times higher than the P_{eff} value for mannitol (0.5 x 10^{-6} cm/sec) but was much lower that the P_{eff} for propranolol, a 90% absorbed transcellular compound (31.2 x 10^{-6} cm/sec). The basolateral-to-apical P_{eff} was much greater than the apical-to-basolateral P_{eff} at the concentrations (33 – 45 x 10^{-6} cm/sec). The addition of verapamil, a strong P-glycoprotein inhibitor, virtually completely eliminated the difference in P_{eff}: verapamil 100 µM increased the apical-to-basolateral transport to 23 x 10^{-6} cm/sec but decreased the basolateral-to-apical transport to 18 x 10^{-6} cm/sec. The addition of cyclosporine, another P-glycoprotein substrate/inhibitor, partially reduced the difference: cyclosporine 10 µM increased the apical-to-basolateral to 15 x 10^{-6} cm/sec but decreased the basolateral-to-apical transport to13 x 10^{-6} cm/sec. These results suggest that a drug transporter such as P-glycoprotein is involved in everolimus efflux.

Co-Administration of Another Drug in Everolimus Labeling, Co-Medications Likely to Be Administered,

Everolimus is proposed to be administered as a part of triple or quadruple immunosuppressive therapy (e.g., cyclosporine + prednisone + everolimus) for kidney and heart transplant patients. To kidney and heart transplant patients, everolimus is likely to be coadministered with anti-infective (erythromycin, ketoconazole, acyclovir, rifampin, sulfamethoxazole/trimethoprim, retonavir), anti-hypertensive (nifedipine, diltiazem, verapamil), anti-hyperglycemic (glyburide), and other drugs (digoxin, phenytoin, norgestrel/ethinyl estradiol). In addition, there seems to be a long list of drugs that are potentially administered with everolimus in transplant patients. The sponsor conducted drug interaction studies in humans only with cyclosporine, rifampin, atorvastatin, and pravastatin.

A population pharmacokinetic analysis (see 6.3. Pharmacometrics Review),) indicated that the co-administration of macrolide antibiotics erythromycin or azithromycin may result in a decrease in everolimus clearance by approximately 20% and the coadministration of itraconazole may decrease everolimus clearance by 74%. The analysis did not detect an influence on everolimus concentrations from concomitant use of atorvastatin, pravastatin, simvastatin, gemfibrozil, quinolone antibiotics (ciprofloxacin, levofloxacin, norfloxacin, or ofloxacin), fluconazole, trimethoprim-sulfamethoxazole, dihydropyridines (amlodipine, isradipine, or nifedipine), diltiazem and verapamil.

Compared with the labeling for sirolimus, a structural analog, or cyclosporine and tacrolimus, drugs in similar pharmacological class, the information on drug-drug interactions in the proposed labeling for Certican is very limited. The sponsor needs to conduct additional drug-drug interaction studies for drugs that may be co-administered with everolimus and, in the labeling, to construct a list of drugs stratified by the potential effect on everolimus exposure (e.g.; drugs that may increase everolimus concentration, drugs that may decrease everolimus concentration, drugs that may not affect everolimus concentration). Metabolism-based drug-drug interaction studies in patients can be waived for drugs whose Cmax / Ki ratios are adequately determined *in vitro* studies and demonstrated as < 0.1. Findings regarding drug-drug interactions in population pharmacokinetic analysis are considered to be preliminary data and, therefore, cannot be used to prove absence or to determine the extent of drug-drug interaction in patients.

Effect of Co-Medication on the Exposure-Response Relationship

Other immunosuppressive drugs, if co-administered, may potentially influence both efficacy and safety responses to everolimus exposure. Cyclosporine increases everolimus exposure through the inhibition of CYP3A and P-glycoprotein (pharmacokinetic) and efficacy response through immunosuppressive mechanism (pharmacodynamic). Although everolimus alone may not be nephrotoxic, everolimus can aggravate the nephrotoxicity caused primarily by cyclosporine when co-administered. Everolimus and cyclosporine can also be potentially additive in causing hypercholesterolemia and hypertriglyceridemia. Combined use of the two drugs potentially increases the chance of adverse effects due to overimmunosuppression such as infection and malignancy. Everolimus and steroids can also have similar pharmacodynamic interaction and exaggerate efficacy and safety responses to everolimus.

Effect of Cyclosporine on Everolimus Exposure: A concomitant single oral dose of Neoral 175 mg increased the respective mean $C_{max,b}$ and AUC_b of everolimus determined after a single oral dose of 2 mg by 82% and 168% (Study A2304, Table 14 in the next page). The Neoral coadministration numerically increased the mean $t_{1/2}$ from 25 hr to 29 hr without affecting the median Tmax. Individual increases in everolimus AUC_b were highly variable at the range of 46% - 365%. In the same study, a concomitant single oral dose of Sandimmune 300 mg increased the respective mean $C_{max,b}$ and AUC_b of everolimus determined after a single oral dose of 2 mg by 6% and 74% (Table 16 in the next page). The Sandimmune coadministration did not affect the mean $t_{1/2}$ and median Tmax. Individual increase in the AUC_b was highly variable at the range of 0% - 254%.

Table 15. Effect of a concomitant single oral dose of Neoral 175 mg on everolimus pharmacokinetics (mean ± SD) determined following a single oral dose of 2 mg to 12 healthy subjects (Study A2304).

Everolimus PK Parameter	Baseline	With Neoral Co-Administration	Geometric Mean Ratio	90% CI
Tmax (hr)*	1.0 (0.5 – 1.0)	1.0 (0.6 – 2.5)		
Cmax,b (ng/mL)	11.6 ± 3.3	20.5 ± 3.5	**1.82**	**1.63 – 2.04**
AUCb (ng-hr/mL)	74 ± 26	193 ± 47	**2.68**	**2.22 – 3.24**
$t_{1/2}$ (hr)	25.2 ± 8.2	29.0 ± 4.6		

* median (range)

Table 16. Effect of a concomitant single oral dose of Sandimmune 300 mg on everolimus pharmacokinetics (mean ± SD) determined following a single oral dose of 2 mg to 12 healthy subjects (Study A2304).

Everolimus PK Parameter	Baseline	With Neoral Co-Administration	Geometric Mean Ratio	90% CI
Tmax (hr)*	0.5 (0.5 - 1.0)	1.0 (0.5 - 1.0)		
Cmax,b (ng/mL)	15.3 ± 5.2	17.2 ± 9.9	1.06	0.88 - **1.27**
AUCb (ng-hr/mL)	92 ± 30	167 ± 82	**1.74**	**1.49 - 2.04**
$t_{1/2}$ (hr)	26.4 ± 9.1	27.7 ± 5.4		

* median (range)

In addition, the sponsor compared the everolimus pharmacokinetics following twice daily doses of everolimus 1.5 mg at steady state when co-administered with full-dose (Cmin,b,ss = 190 ± 115 ng/mL, n = 33) and reduced-dose (92 ± 61 ng/mL, n = 35) cyclosporine at 4 weeks after renal transplant and concluded that everolimus pharmacokinetics were not differentially influenced (Study B156). The study was not reviewed because the sponsor did not propose reduced cyclosporine dose regimens in this application.

Based on the result of the cyclosporine-everolimus interaction studies, a clinically significant decrease in everolimus exposure (2 to 3 fold) is expected if either Neoral or Sandimmune that has been co-administered is removed. Therefore, everolimus dose needs to be adjusted accordingly in these cases.

Everolimus-Statin Interaction: A concomitant single oral dose of atorvastatin 20 mg or pravastatin 20 mg slightly decreased everolimus exposure following a single oral dose of 2 mg (Table 17 in the next page, Study W303). The respective mean Cmax,b of everolimus was reduced by 9% (90% CI of GMR, 0.75 - 1.10) or 10% (0.75 - 1.06) following atorvastatin or pravastatin coadministration. For everolimus AUCb in both cases, the lower 90% confidence bounds were slightly outside the bioequivalence interval (90% CI of GMR, 0.76 - 1.06 or 0.79 - 1.12, respectively). There was no apparent change in the mean $t_{1/2}$ or median Tmax.

In the same study, the concomitant everolimus dose increased the mean Cmax of atorvastatin by 11% (90% CI of GMR, 0.89 - 1.37) but decreased the AUC(0-tz) of total HMG-CoA reductase inhibitor by 7% (90% CI of GMR, 0.78 - 1.11, Table 18 in the next page). The everolimus co-administration did not significantly influence on the AUC, $t_{1/2}$, and Tmax of atorvastatin and the Cmax of total HMG-CoA reductase inhibitor. The concomitant everolimus dose decreased the

mean Cmax and AUC of pravastatin, and the mean Cmax and AUC of total HMG-CoA reductase inhibitor by 10% (90% CI of GMR, 0.64 - 1.27), 5% (0.74 - 1.23), 16% (0.65 - 1.10), and 2% (0.76 - 1.27), respectively (Table 18). The $t_{1/2}$ and Tmax of pravastatin were comparable.

Table 17. Effect of a concomitant single oral dose of atorvastatin 20 mg or pravastatin 20 mg on everolimus pharmacokinetics (mean ± SD) determined following a single oral dose of 2 mg to 12 healthy subjects (Study W303).

	Everolimus PK Parameter	Baseline	With Statin	Geometric Mean Ratio	90% CI
Atorvastatin	Tmax (hr)	0.5 (0.5 - 1.5)	0.5 (0.5 - 1.0)*		
	Cmax,b (ng/mL)	17.1 ± 4.0	16.4 ± 6.6	0.91	**0.75** - 1.10
	AUCb (ng-hr/mL)	120 ± 37	118 ± 46	0.95	**0.77** - 1.18
	$t_{1/2}$ (hr)	34 ± 13	34 ± 11		
Pravastatin	Tmax (hr)	0.5 (0.5 - 1.5)*	0.5 (0.5 - 1.0)*		
	Cmax,b (ng/mL)	16.7 ± 4.4	15.3 ± 4.4	0.90	**0.76** - 1.06
	AUCb (ng-hr/mL)	109 ± 43	98 ± 28	0.94	**0.79** - 1.12
	$t_{1/2}$ (hr)	34 ± 11	36 ± 17		

* median (range)

Table 18. Effect of a concomitant single oral dose of everolimus 2 mg on atorvastatin or pravastatin pharmacokinetic parameters (mean ± SD) determined following a single oral dose of 20 mg to 12 healthy subjects (Study W303).

	Statin PK Parameter	Baseline	With Everolimus	Geometric Mean Ratio	90% CI
Atorvastatin	Tmax (hr)*	0.5 (0.5 - 1.0)	0.5 (0.5 - 8.0)		
	Cmax (ng/mL)	11.1 ± 4.9	12.0 ± 5.4	1.11	**0.89 - 1.37**
	AUC (ng-hr/mL)	208 ± 62	209 ± 67	1.02	0.94 - 1.11
	$t_{1/2}$ (hr)	26 ± 5	26 ± 5		
	HMG Cmax (ng/ml)	11.9 ± 2.5	12.5 ± 3.5	1.06	0.93 - 1.21
	HMG AUC(0-tz) (ng-hr/mL)	212 ± 73	191 ± 71	0.93	**0.78** - 1.11
Pravastatin	Tmax (hr)*	1.0 (0.5 - 2.1)	1.0 (1.0 - 1.5)		
	Cmax (ng/mL)	24.4 ± 19.4	21.4 ± 11.6	0.90	**0.64 - 1.27**
	AUC (ng-hr/mL)	72 ± 40	68 ± 26	0.95	**0.74** - 1.23
	$t_{1/2}$ (hr)	3.7 ± 2.1	3.4 ± 1.5		
	HMG Cmax (ng/ml)	21.5 ± 13.9	17.9 ± 5.7	0.84	**0.65** - 1.10
	HMG AUC(0-tz) (ng-hr/mL)	54 ± 31	51 ± 17	0.98	**0.76 - 1.27**

* median (range)

Given the minimal effect (up to 16% decrease in Cmax) of everolimus on statin exposure and *vice versa*, no dose adjustments for everolimus or the two stains appear to be necessary for their coadministration.

Effect of Rifampin on Everolimus Exposure: A CYP3A enzyme induction by rifampin 600 mg daily for 8 days increased the mean everolimus CLb/F determined following a single oral

dose of 4 mg by 172% with an individual range of 0 - 451% (Study A2302, Table 19). The respective mean decreases in Cmax,b and AUCb were 58% (p = 0.0001) and 63% (p = 0.0001). The mean $t_{1/2}$ was significantly shortened from 32 hr to 24 hr (p = 0.0001); however, median Tmax was not different. The interindividual variability in the magnitude of the interaction as estimated from the CV of AUC values was 52 %.

Table 19. Effect of CYP3A induction by rifampin 600 mg daily for 8 days on everolimus pharmacokinetic parameters (mean ± SD) determined following a sing oral dose everolimus 4 mg to 12 healthy subjects (Study A2302).

Everolimus PK Parameter	Baseline	After Rifampin Induction	Geometric Mean Ratio	90% CI
Tmax (hr)*	0.5 (0.5-1.0)	0.5 (0.5-1.0)		
Cmax,b (ng/mL)	44.2 ± 13.3	18.3 ± 3.9	**0.42**	**0.36 – 0.50**
AUCb (ng-hr/mL)	219 ± 69	83 ± 37	**0.37**	**0.30 – 0.46**
CLb/F (L/hr)	19.7 ± 5.4	55.1 ± 19.0	**2.72**	**2.19 – 3.38**
$t_{1/2}$ (hr)	32.2 ± 6.1	23.9 ± 5.2		

* median (range)

Mechanistic Basis for Pharmacodynamic Drug-Drug Interactions

As mentioned above, everolimus and cyclosporine, and everolimus and steroids can have pharmacodynamic drug-drug interactions by different immunosuppressive mechanisms. Everolimus may affect response to vaccination and vaccination during everolimus treatment may be less effective. The use of live vaccines should be avoided due to a risk of actual infection by the pathogens in the vaccines.

Unresolved Issues on Active Metabolites and Protein Binding

Protein Binding: Everolimus is strongly bound to human erythrocytes. The erythrocyte uptake was approximately 85% at the blood concentration range of 5 - 100 ng/mL. The fraction of everolimus associated to human neutrophils and lymphocytes is extremely low (approx. 1%), while the fraction in human plasma was around 14 %. At higher blood concentrations than 100 ng/mL, the blood cell uptake was concentration-dependent and saturable, and the ratios change rapidly with an increase in plasma concentration. The free fraction (fu) in the plasma was 0.25 and considered to be concentration independent. Overall, the plasma protein binding is not an important factor in the disposition of everolimus: a change in the concentration of plasma proteins will not dramatically alter the free fraction.

What issues related to dose, dosing regimens or administration are unresolved, and represent significant omissions?

As stated above, a starting dose, and efficacious and safe concentration range of everolimus need to be adequately determined.

4.5. General Biopharmaceutics

Based on BCS principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

Everolimus is a low permeability drug based on the in vitro permeability study using a Caco-2 cell monolayer (see **Caco-2 Permeability**). The reported everolimus solubility is < 0.01% (1 mg / 10 mL) in aqueous media (see **Physicochemical Properties**) and, therefore, the highest dose strength of everolimus tablet (1 mg) would be soluble in 250 mL of aqueous media. Based on the permeability and solubility data, everolimus is a Class 3 drug with respect to BCS.

What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

The Certican IR tablets to be marketed (final market image, FMI) were bioequivalent to the tablets used in clinical studies (market formulation, MF).

Bioequivalence between Everolimus MF and FMI tablets in Different Strengths: When a single oral dose of everolimus 1 mg was administered to healthy subjects as 1 x 1-mg FMI (reference), 4 x 0.25-mg MF (test), 2 x 0.5-mg MF (test), and 4 x 0.25-mg FMI (test) tablets in a four-way crossover study (A2301), individual difference in everolimus Tmax between the test and reference treatments ranged from -0.5 hr to +1.0 hr. The median difference was 0 hr for all three comparisons. Everolimus Cmax,b satisfied equivalence criteria for all three test treatments relative to the reference (Table 20). All comparisons in everolimus AUCb between test and reference treatments satisfied the bioequivalence criteria of 0.8 – 1.25. The residual area for extrapolation of the truncated AUC(0-tz),b to the full AUCb generally ranged from 7% to 35%. The mean $t_{1/2}$ was comparable between treatments.

Table 20. Bioequivalence between everolimus MF and FMI tablets in different strengths administered as a single dose of 1 mg (n = 19, Study A2301).

Everolimus PK Parameter	Reference	Test					
	1 x 1-mg FMI tablets	4 x 0.25 mg MF tablets	Geometric Mean Ratio (90% CI)	2 x 0.5 mg MF tablets	Geometric Mean Ratio (90% CI)	4 x 0.25 mg FMI tablets	Geometric Mean Ratio (90% CI)
Tmax (hr)*	1.0 (0.5-1.0)	0.5 (0.5-1.5)		0.5 (0.5-1.5)		1.0 (0.5-1.0)	
Cmax,b (ng/mL)	7.3 ± 2.9	6.7 ± 2.0	0.93 (0.83-1.05)	7.0 ± 1.9	1.01 (0.89-1.14)	6.9 ± 1.6	1.01 (0.90-1.14)
AUC(0-tz),b (ng-hr/mL)	43 ± 18	40 ± 14	0.98 (0.85-1.13)	45 ± 19	1.10 (0.95-1.27)	46 ± 15	1.15 (0.99-1.34)
AUCb (ng-hr/mL)	55 ± 18	51 ± 15	0.94 (0.84-1.05)	57 ± 19	1.06 (0.95-1.18)	58 ± 15	1.10 (0.98-1.22)
t1/2 (hr)	37 ± 9	34 ± 10		39 ± 8		38 ± 8	

* median (range); FMI, final market image; MF, market formulation; CI, confidence interval

If different-strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to-be-marketed product?

Not applicable.

What is the effect of food on the bioavailability of the drug from the dosage form? What dosing recommendation should be made regarding administration of the product in relation to meals or meal types?

Following a single oral administration of two 1 mg IR tablets to 24 healthy male subjects under fasted conditions (Study W302), everolimus was rapidly absorbed (Figure 8): all subjects had quantifiable concentrations at 0.5 hr postdose; this constituted Tmax for 17 subjects. When everolimus was administered after a high-fat meal defined in the Agency's guidance for food effect bioavailability studies, everolimus Tmax was delayed in 21 subjects; the median delay was 1.5 hr (range, 0 to 4 hr; Table 21). Mean Cmax was notably decreased in all 24 subjects by 60% under the fed condition (Figure 8, Table 21). The GMR of fed/fasted AUCb remained in the equivalence range of 80 – 125% for 10 subjects. The other

Figure 8. Influence of high-fat meal on everolimus concentration-time profile following a single oral dose of two 1 mg immediate release tablets (○ fasted, ● high-fat meal, n = 24, Study W302)

14 subjects had changes outside the range: 12 subjects showed reductions in the ratio by 21% - 69% and 2 subjects showed increases in the ratio by 73 - 152%. The overall food effect on the extent of absorption was a reduction of 16%. The mean CLb/F, Vz,b/F, and $t_{1/2}$ was comparable between fed and fasted conditions.
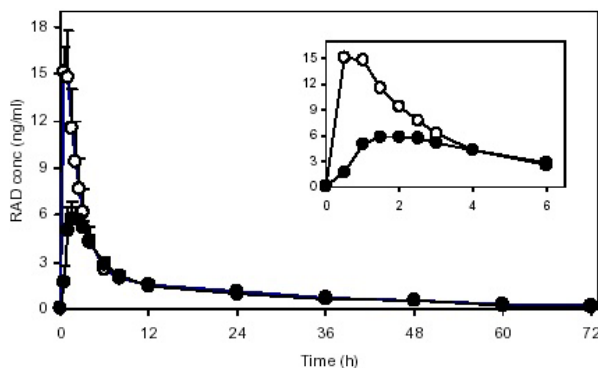
Table 21. Influence of high-fat meal on everolimus pharmacokinetics (mean ± SD) determined following a single oral dose of 2 mg as two 1 mg immediate release tablets to 24 healthy male subjects (Study W302).

Everolimus PK Parameter	Fasted Condition (Reference)	Fed Condition (Test)	Geometric Mean Ratio	90% Confidence Interval
Tmax (hr)*	**0.5 (0.5 – 2.0)**	**2.0 (0.5 – 6)**		
Cmax,b (ng/mL)	17.9 ± 5.9	7.1 ± 2.0	**0.40**	**0.35 – 0.46**
AUC(0-tz),b (ng·hr/mL)	99 ± 50	75 ± 19		
AUC∞,b (ng·hr/mL)	122 ± 52	97 ± 19	0.84	**0.74** – 0.95
CLb/F (L/hr)	19.1 ± 7.4	21.3 ± 3.7		
Vz,b/F (L)	842 ± 315	928 ± 179		
$t_{1/2}$ (hr)	31.5 ± 6.4	30.5 ± 4.9		

* median (range)

In Study W102, six clinically stable renal transplant patients received daily everolimus doses of 2.5 mg for 28 days in addition to cyclosporine and corticosteroids. On day 15, they were

randomized to take the everolimus dose either after an overnight fast or after a high-fat meal. On day 21 the alternative administration condition was assigned. Everolimus administration after a high-fat meal delayed median Tmax by 1.75 hr, and reduced respective mean Cmax,b and AUCb by 51% and 21%.

Clinical Implications: Given the marked effect on the rate and moderate effect of food on the extent of everolimus absorption, it would be prudent to administer Certican tablets to the individual patient on a consistent basis either with food or without food to avoid unnecessary fluctuations in everolimus exposure over time.

When would a fed BE study be appropriate and was one conducted?

A fed bioequivalence study was not conducted and seems to be unnecessary as long as everolimus is administered consistently with respect to food and adequately with TDM.

How do the dissolution conditions and specifications assure in vivo performance and quality of the product?

Proposed dissolution method and specification for IR tablets

Apparatus: Paddle method (USP: apparatus 2)
Media: water + 0.4% sodium dodecylsulfate
Volume: 500 mL
Speed of rotation: 50 ± 2 rpm
Analytical method: HPLC with UV detection
Dissolution specification: Q=   in 30 min

Note that 0.4% SDS concentration is higher than its critical micelle concentration (i.e., 0.2%). Thus, in order to apply this dissolution method to evaluate the *in vivo* performance (e.g., in vivo-in vitro correlation) of the product further validation is needed. Please refer to the dissolution review for details (see 6.4. Dissolution Review).

What other significant, unresolved issues related to in vitro dissolution or in vivo bioavailability and bioequivalence need to be addressed?

No issues in this regard for IR tablets.

If the NDA is for a modified release formulation of an approved immediate product without supportive safety/efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?

Not applicable.

If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?

Not applicable.

If replicate design studies were conducted and individual BE was analyzed, what were the outcomes with respect to variability and subject-by-formulation interactions?

Not applicable.

4.6. Analytical

How are the active moieties identified and measured in human specimens in CPB studies?

Everolimus blood concentrations were measured by reverse-phase high performance liquid chromatographic (HPLC) methods using mass spectrometric (LC-MS) or tandem mass spectrometric detection (LC-MS/MS), or enzyme-linked immunosorbent assay (ELISA). The total radioactivity in blood and urine samples were measured using a direct liquid scintillation counting method. Everolimus metabolites in blood, urine and feces were detected by a HPLC-Radiometric method.

In the LC-MS method, the active moieties were detected at a selected ion monitoring mode with an atmospheric pressure chemical ionization (APCI). In order to enhance sensitivity, a negative ion mode was used for everolimus ($[M]^-$, m/z = 956.7) and 40-O-(3'-hydroxy)propyl-rapamycin (internal standard, ($[M]^-$, m/z = 971.6). In the LC-MS/MS method, the moieties were detected at a multiple reaction monitoring mode with an electrospray ionization (ESI, positive ion detection). Selected masses (m/z) of parent and daughter compounds were 975.5 and 908.5, and 989.5 and 922.5 for everolimus and SDZ 223-756 (internal standard for everolimus), respectively. In the ELISA, the moieties were detected using a monoclonal anti-rapamycin antibody. In the HPLC-Radiometric method, retention time markers were added to the samples prior to analysis. Supplementary HPLC methods were applied for the assignment and identification of metabolite peaks by extensive chromatographic comparison of *in vivo* and *in vitro* samples. The HPLC fractions containing the isolated metabolite peaks in low concentrations were analyzed on a mass spectrometric method with nanospray technique.

Which metabolites have been selected for analysis and why?

Only everolimus concentrations were measured in all CPB studies except the mass balance study (W107) because the parent drug accounted for the majority (approx. 40%) of the AUCb following the administration of ^{13}C-everolimus, most identifiable metabolites accounting for the 35% of the AUCb were known to be much less active by two orders than the parent drug, and rapamycin accounting for only 1.2% was the only active metabolite identified (see *Absorption, Distribution, Metabolism and Elimination* section of this review).

For all moieties measured, is free, bound or total measured? What is the basis for that decision and is it appropriate?

In CPB studies for everolimus, whole blood concentrations of everolimus were measured. The whole blood concentration assay appears to be advantageous for the development of commercial assay using current technology in the commercial blood concentration assays of sirolimus, tacrolimus, and cyclosporine.

What bioanalytical methods are used to assess concentrations?

<u>Standard Curve, Limit of Quantitation, Accuracy, Precision, Selectivity, Sample Stability, Quality Control</u>:

Measurement of Everolimus Blood Concentration: The limit of quantification (LOQ) for the chromatographic methods and the ELISA was around 0.4 ng/mL and 2 ng/mL, respectively. In the chromatographic methods, a liquid-liquid extraction or semi-automated solid phase extraction technique was used for analyte preparation. The assay methods were validated at the method development stage and their in-process performance was included in most CPB study reports. The assay methods produced equivalent results over the everolimus concentration range of 3 - 32 ng/mL which covers most of the concentration range achieved in CPB studies. Single-dose studies in which lower concentrations in the terminal phase of the profile were important preferentially used the chromatographic method due to its lower LOQ. Everolimus blood samples were stable after 20 freezing/thawing cycles and while stored at -20°C for up to 10 months. The stability of cyclosporine was not determined. Table 22 listed the in-process assay performance of the assays used in each CPB study.

LC-MS Method: Calibration curves, represented by the plots of the peak area ratio (y) of everolimus to SDZ 223-756 versus the concentration (x) of the calibration standards, were generated using weighted $(1/x^2)$ linear least-squares regression $(y = mx + b)$. The calibration curves consisted of 6 calibration standards in duplicate over the calibration range. For an analytical run to be accepted, at least one of the duplicate standards at the lowest and at the highest concentrations needs to have an acceptable accuracy (relative error, RE > 20%). The calibration curves were linear over the concentration range of 0.375 – 253 ng/mL (correlation coefficient, r > 0.99) and, therefore, the LOQ was 0.375 ng/mL. The intra-day accuracy determined using 6 replicates of 5 quality control concentrations (QC; 0.629, 2.52, 10.1, 50.7, and 253 ng/mL) ranged from 89.7% to 103.0%. The intra-day precision (CV) was from 2.4% to 10.3%. The inter-day accuracy and precision were at the range of 93.6% – 102% and 4.5% – 8.6%, respectively. Retention times were approximately 2.3 min and 2.4 min for everolimus and the internal standard, respectively.

Table 22. Summary of in-process performance of the analytical methods used for the measurement of everolimus blood concentrations.

Study	Site	Report No.	Analyte	Method	Calibration Range (ng/mL)	LOQ (ng/mL)	Precision* (%) RSE (US) or CV	Accuracy* (%) Recovery (US) or Bias
2301	CH	00-1020	everolimus	LC-MS	0.2 - 100	0.2	5.1 to 7.2	-1.5 to 4.0
2302	CH	00-1021	everolimus	LC-MS	0.3 - 100	0.3	4.9 to 11.5	-7.6 to -1.0
2303	CH	00-1022	everolimus	LC-MS	0.3 - 202.3	0.3	5.1 to 9.9	-4.7 to 3.0
2304	CH	00-1023	everolimus	LC-MS	0.45 - 400	0.45	7.5 to 14.2	**5.1 to 16.7**
		00-1023-01	cyclosporine	RIA	15.6 - 2000	20 - 60	**5.5 to 56.11**	**-21.7 to 1.3**
2407	CH	01-0507	everolimus	LC-MS	0.257 - 51.4	0.257	7 to 10.9	-0.8 to 0.4
B157	CH	98-3048	everolimus	ELISA	1.56 - 100	**2.0 or 3.0**	**13.3 to 16.1**	-7.7 to -1.8
	Fr	98-3048-01	cyclosporine	RIA	25.0 - 1600	30	5.1 to 10.6	-12.0 to 0.4
B201	Fr	00-1904 (6 mo)	everolimus	LC-MS	0.2 - 50	0.2	7.5 to 8.7	-1.0 to 1.2
	CH	00-1904B (6 mo)	cyclosporine	EMIT	25 - 500	33.2	4.5 to 13.7	-8.0 to 2.4
				RIA	15.63 - 500	20.0	9.7 to 15.1	1.9 to 5.5
B251	US	98-1880	everolimus	LC-MS I	0.372 - 300	0.372	5.8 to 9.2	-0.1 to 4.5
				LC-MS II	0.386 -409	0.368	4.6 to 10.5	0.6 to 8.5
			cyclosporine	LC-MS I	7.03 - 1760	7.03	7.2 to 9.2	-7.1 to -5.6
				LC-MS II	5.24 - 1748	5.24	5.3 to 7.6	-2.1 to 4.8
B253	CH	98-3077 (6 mo)	everolimus	ELISA	1.56- 100	2.0	11.1 to 34.1	**-6.8 to 19.8**
			cyclosporine	EMIT	25 - 500	33.2	3.1 to 13.9	-10.2 to 3.6
				RIA	15.63 - 1000	20 or 50	**9.1 to 21.0**	-4.9 to 8.6
B257	US	99-2464	everolimus	LC-MS	0.372 - 300	0.372	4.51 to 10.7	-6.0 to 8
			cyclosporine	LC-MS	7.03 - 1760	7.03	0.92 to 11.8	-6.3 to 3.0
B258	CH	99-2033	everolimus	LC-MS	0.4 - 100	0.4	6.1 to 9.3	-6.0 to 6.2
			cyclosporine	RIA	15.63 - 2000	50	3.4 to 9.2	1.0 to 11.0
B351	US	00-311	everolimus	LC-MS	0.373 - 80.0	0.373 or 0.380	**0.1 to 17.1**	-10.0 to 4.2
			cyclosporine	LC-MS	9.29 - 2000	9.29 or 9.32	2.0 to 6.0	-12.3 to 6.3
W101	CH	1997/180	everolimus	LC-MS	0.04 - 252.81	0.75	**9 to 17**	-12 to -7
	Fr	RADW 101	cyclosporine	RIA	14.9 – 2537.9	15	**5.7 to 17.7**	-1.7 to 3.5
W105	Fr	99-021	everolimus	LC-MS	0.3 - 100	0.3	6 to 8	-11 to 10
W107	Fr	98-075	everolimus	LC-MS	0.3 - 100	0.3	3 to 13	-14 to 13
			rapamycin**	LC-MS	0.2 - 50	0.3	4 to 6	-13 to 4
	CH	98-417B	cyclosporine	ELISA	3.125 - 400	10	**5.2 to 24**	**5.4 to 32**
W301	Fr	99-037	everolimus	LC-MS	0.2 - 100	0.2	3.6 to 11	-5.9 to 8.0
W302	CH	99-1904	everolimus	LC-MS	0.45 -400	0.45	7.2 to 12.8	0.9 to 5.4
W303	CH	99-1905	everolimus	LC-MS	0.253 - 202.3	0.253	7.1 to 8.7	-4.9 to 2.7
	US	99-1905-01	atorvastatin	enzyme inhibition assay	0.36 - 16	0.36	6.8 to 13.4	-8.6 to 6.2
			pravastatin		0.36 - 40	0.36	8.4 to 12.7	-6.7 to 9.6
	Fr	99-1905-03	atorvastatin	LC-MS	0.5 - 50	0.5	5.1 to 12.4	-1.6 to 18.0
		99-1905-02	pravastatin	LC-MS	0.5 - 50	0.5	11.0 to 14.3	-19.5 to -2.0

* within-study values; ** simultaneously determined with everolimus; CH, Switzerland; ELISA, enzyme-linked immunosorbent assay; EMIT, enzyme-multiplied immunoassay technique; Fr, France; LC-MS, liquid chromatographic methods with mass spectrometric or tandem mass spectrometric detection; LOQ, limit of quantitation; RIA, radioimmunoassay; US, United States.

LC-MS/MS Method: Calibration curves were generated using weighted (l/x^2) polynomial regression ($y = ax^2 + bx + c$). Calibration curves were accepted when r is > 0.95 and the 2/3 of the calibration samples had back-calculated values of 85% - 115% (80% - 120% for LOQ). The calibration curves were linear over the range of 0.368 – 409 ng/mL (r > 0.99) and , therefore, the LOQ was 0.368 ng/mL. The mean absolute recoveries for everolimus were 52.4% (range, 40.1% - 59.0%), respectively. The intra-day accuracy determined using 6 replicates of 5 QCs (0.368, 1.23, 40.9, 205, and 358 ng/mL) ranged from 98.3% to 112%. The intra-day precision was from 1.9% to 10.7%. The inter-day accuracy and precision were at the range of 99.4% – 109% and 4.5% – 8.9%, respectively. Retention times were approximately 2.3 min and 2.5 min for everolimus and internal standard, respectively.

ELISA Method: The standard curve working range was 2 – 100 ng/mL. Quality control samples (2, 3, 9, and 30 ng/mL) were included in duplicate in 10 separate assay runs over a period of 2 weeks. The within assay variation (CV) was from 6.7 % to 11.8 %. The between assay variation was from 8.6 to 23.0%. The accuracy calculated as the deviation (%) between the observed and nominal concentrations was 0.5%, -4%, 4%, and 8.3 % for the quality controls, respectively. The LOQ was set to the concentration of the lowest QC sample giving accuracy and variations better than 30 %. In most of the individual assay the LOQ was 2 ng/mL. In one assay out of 10, the LOQ was 3 ng/mL. The relationship between the ELISA and LC-MS methods were linear (r > 0.95) when 116 samples were measured together.

Measurement of ^{14}C-Everolimus-Derived Radioactivity in Plasma, Blood, Urine and Feces: The total radioactivity in plasma or urine was directly measured in duplicate using a scintillation counter at a counting time of 120 sec with picofluor 40 scintillant. The total radioactivity in whole blood and fecal samples were dried in air and combusted to CO_2 using a sample oxidizer (combustion time, 0.5 min) prior to scintillation counting. Fecal samples were homogenized with water prior to combustion. The quench curve was standardized with a Packard extended range quenched standard set for ^{14}C (No. 6018595, assay value 128,700 ± 1.3%).

Determination of Everolimus Metabolites by HPLC-Radiometric Method: Prior to HPLC analysis for everolimus metabolites, blood samples were extracted with diethylether and methanol, urine samples were freeze-dried, but feces samples were homogenated and extracted with methanol. Retention time markers were added to the samples prior to analysis. Quantities of metabolite (peaks) were calculated from the total sample radioactivity, the specific radioactivity of the parent drug administered and the area-percentages of the corresponding peaks.

Measurement of Cyclosporine Concentrations: Cyclosporine concentrations was measured from whole blood samples by radioimmunoassay assay (RIA), enzyme-multiplied immunoassay technique (EMIT), LC-MS, and LC-MS/MS methods. Table 22 listed the in-process assay performance of the methods. The RIA used the commercial reagents (INCSTAR Cyclo-Trac) and assay manual (DIASORIN Corp., Stillwater, MN). The EMIT used the Emit Cyclosporine A Specific Assay reagents and instruction manual (Dade Behring Inc., Cupertino, CA).

In the LC-MS method, cyclosporine ([M+H]$^+$, m/z = 1203) and internal standard D$_{12}$-cyclosporine ([M+H]$^+$, m/z = 1215) were detected in positive ion mode. Retention times were approximately 4.1, and 4.2 min for D$_{12}$-cyclosporine and cyclosporine, respectively. The

calibration curves were linear over the concentration range of 6.95 - 1530 ng/mL (r > 0.99) and, therefore, the LOQ was 6.95 ng/mL. The intra-day accuracy determined using 6 replicates of 4 quality control concentrations (15.2, 60.1, 301, and 1510 ng/mL) ranged from 90.4% to 114%. The intra-day precision was from 2.6% to 10.9%. The inter-day accuracy and precision were at the range of 100% to 108% and 5.3% to 11.8%, respectively.

In the LC-MS/MS method, selected m/z of parent and daughter compounds were 1219.8 and 1202.8, and 1232.9 and 1214.9 for cyclosporine and D_{12}-cyclosporine, respectively. Retention times were around 3.05, and 3.1 min for D_{12}-cyclosporine and cyclosporine, respectively. The calibration curves were linear over the concentration range of 5.23 - 1748 ng/mL (r > 0.99) and, therefore, the LOQ was 5.23 ng/mL. The mean absolute recovery was 51.3% (range, 47.5% - 53.9%). The intra-day mean accuracy determined using 6 replicates of 4 quality control concentrations (5.23, 174, 871, and 1524 ng/mL) ranged from 88.2% to 110%. The intra-day precision was from 0.9% to 4.2%. The inter-day accuracy and precision were at the range of 91.3% – 102% and 5.1% – 7.3%, respectively.

Measurement of Statin Concentrations: The plasma concentrations of atorvastatin and pravastatin were measured using an LC-MS/MS method in selected reaction monitoring mode with ESI interface following a liquid-liquid extraction procedure. The HMG-CoA reductase inhibition activity of atorvastatin and pravastatin was measured by an enzyme inhibition bioassay. Plasma extracts containing HMG-CoA reductase inhibitors were incubated with buffer solution containing ^{14}C-HMG-CoA, cofactors, and HMG-CoA reductase from human liver microsomes. The ^{14}C-mevalonate was separated from the substrate after lactonization to ^{14}C-mevalonolactone by HC1, on a small ion exchange column. The effluent from the column was directly collected into scintillation vials and counted. The ^{14}C-mevalonolactone, measured in cpm, was used to construct a standard curve. The in-process assay performance of the two analytical methods is listed in Table 22.

5. DETAILED LABELING RECOMMENDATIONS

Labeling recommendations are deferred because the clinical Division's action for these NDAs in this review cycle will be 'approvable' due to insufficient dosing and safety information.

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6. APPENDICES

6.1. Proposed Labeling

Please refer to \\CDSESUB1\N21560\N_000\2002-12-19\labeling\proposed.pdf

6.2. List of CPB Studies

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
Basic Pharmacokinetics (See also Studies B201, B251, and B253)					
A1101	to assess the PK parameters and dose proportionality of single ascending oral doses of everolimus in Japanese subjects	randomized, parallel group, time-lagged, ascending dose	24 healthy subjects 24/0 24 Japanese	0.5 mg as 2 x 0.25 mg MF tablets, 1 mg as 1 x 1 mg MF tablet, 2 mg as 2 x 1 mg MF tablets, or 4 mg as 4 x 1 mg MF tablets PO, single dose	**Not useful**: PK parameters not calculable at 0.5 and 1 mg doses
B154	to evaluate the safety, tolerability and PK of everolimus	randomized, double-blind, matching placebo, time-lagged	18 stable renal transplant recipients	0.75 – 7.5 mg as 0.25, 1, or 10 mg capsules PO, QD	**Not relevant to proposed regimen (QD with capsules) -> not reviewed**
W101	to determine the single ascending dose PK of everolimus during steady state dose of Neoral to determine the effect of single dose everolimus on the steady state PK of Neoral	multi-center, randomized, placebo-controlled, parallel groups, ascending dose	54 stable renal transplant recipients 44M / 10F 54W 27 – 65 yearr	0.25, 0.75,2.5, 7.5, 15, 25 mg as 0.25, 1, 10 mg capsules + cyclosporine PO, single dose	**Used capsules without assessing bioequivalence to FMI tablets** also for dose proportionality no PK parameters at 0.25 mg dose
W102	to characterize the single- and multiple-dose PK of everolimus to explore relationships between systemic exposure and changes in pertinent laboratory parameters to assess the influence of steady-state everolimus on steady-state cyclosporine PK	multi-center, randomized, double-blind, placebo-controlled, sequential	54 stable renal transplant recipients	0.75, 2.5, and 10 mg as capsules or tablets with various strengths PO, single or 2 divided doses, for 28 days	**Not associated with labeling claim -> not reviewed** also for relative bioavailability, proportionality, food effect, everolimus -> CsA interaction

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
W105	to assess the PK parameters and dose proportionality of single ascending oral doses of everolimus	randomized, parallel group, time-lagged, ascending dose	16 healthy subjects 16/0 13W, 0B, 1A, 2O 21 – 35 yr	0.5 mg as 2 x 0.25 mg MF tablets, 1 mg as 1 x 1 mg MF tablet, 2 mg as 2 x 1 mg MF tablets, 4 mg as 4 x 1 mg MF tablets, or placebo tablets PO, single dose	**Not useful**: PK parameters not calculable at 0.5 and 1 mg doses, sample size too small (n = 4 each)

Dose Proportionality (See Study W101)

Mass Balance and Metabolism

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
W107	to assess the absorption, disposition, kinetics, and biotransformation of ^{14}C-everolimus and metabolites	open-label	3 (3/0) stable renal transplant recipients 3W 25 – 41 yr	3 mg as 1 mg capsules of solid dispersion formulation, simultaneously administered with Neoral (target, 80 – 200 ng/mL) PO, single dose	also for ADME

Protein Binding (see 2303)

Bioavailability and Bioequivalence (See also Study A2407)

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
A2301	to determine the bioequivalence of a single 1-mg dose of everolimus administered as various MF and FMI tablets	randomized, open-label, 4-way crossover	19 (12/7) healthy subjects 12W, 6B, 1O 18 – 45 yr	1 mg as 4 x 0.25 mg MF, 4 x 0.25 mg FMI, 2 x 0.5 mg MF, or 1 x 1 mg FMI tablets PO, 4 single doses	
W301	to evaluate the bioequivalence of single 1-mg doses of everolimus administered as dispersible and immediate release tablets	randomized, open-label, 2-way crossover	16 (16/0) healthy subjects 14W, 1A, 1O 18 – 50 yr	1 mg as 4 x 0.25 mg dispersible or 1 x 1 mg MF tablets PO, 2 single doses	

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
Food Effect					
A2407	to evaluate the PK of the everolimus dispersible tablet under fed and fasted conditions to determine the relative bioavailability of everolimus dispersible tablet to the FMI tablet	randomized, open-label, 3-way crossover	24 (22/2) healthy subjects 23W, 1O 19 – 42 yr	1.5 mg as 6 x 0.25 mg dispersible tablets or 2 x 0.75 mg tablets (FMI) PO, 3 x single doses	also for relative BA/BE
W302	To determine the food effect (high-fat meal) on everolimus pharmacokinetics	randomized, open-label, 2-way crossover	24 (24/0) healthy subjects 23W, 1B 18 – 55 yr	2 mg as two 1 mg tablet (MF) under fed and fasted conditions PO, 2 x single doses	Also for basic PK parameters
Drug-Drug Interaction Studies (See also Studies B201, B251, and B253)					
A2302	to investigate the effect of the CYP3A4 inducer rifampin on the PK of everolimus	open-label, 2-period, sequential	12 (6/6) healthy subjects 12W 30 – 61 yr	4 mg as 4 x 1 mg MF tablets ± 8-day rifampin 600 mg PO, two single doses	everolimus-rifampin interaction
A2304	to determine the effect of a single dose of Neoral and Sandimmune on the PK of everolimus following single doses	randomized, 2-period, crossover	24 (21/3) healthy subjects 14W, 9B, 1A 21 – 44 yr	2 mg as 2x 1 mg tablets (FMI) PO, two single doses ± Neoral 175 mg or Sandimmune 300 mg	everolimus-cyclosporine interaction
B156	To characterize the steady-state PK of everolimus and assess whether full- *vs* reduced-dose CsA had a differential influence on everolimus PK	multicenter, randomized, open-label, parallel group	109 *de novo* renal transplant recipients	1.5 mg as 3 x 0.5 mg tablets PO, BID + full or reduced CsA dose	CsA -> everolimus interaction **Not associated with labeling claim -> not reviewed**
W303	to evaluate the single-dose PK of everolimus and HMG-CoA reductase inhibitors (pravastatin and atorvastatin) when coadministered	randomized, open-label, 3-way crossover	24 (24/0) healthy subjects 24W 24 – 49 yr	2 mg as 2 x 1 mg MF tablets, ± 20 mg pravastatin or 20 mg atorvastatin PO, two single doses	everolimus-statin interaction

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
Special Populations (See also Studies B201 and B251 for population pharmacokinetics)					
A2303	to compare the PK of everolimus in subjects with moderate hepatic impairment to matched healthy control subjects	open-label, single-dose, case-control	8 pts (7/1) with hepatic impairment 3W, 5O 43 – 60 yr	2 mg as 2 x 1 mg FMI tablets PO, single dose	with 8 (7/1) healthy controls also for protein binding
B151	to compare the PK of 2 different single dose everolimus	randomized, double-blind, stratified, crossover	24 stable lung ± heart transplant recipients ± cystic fibrosis	0.035 and 0.1 mg/kg as 0.25 and 1 mg capsules PO, 2 single doses	**not indicated -> not reviewed**
B202	To compare the PK of everolimus at one, two, or three identical doses.	open-label	26 *de novo* liver transplant recipients ± T-tube	7.5 mg as 0.25 or 1 mg capsules nasogastric or nasoduodenal, 1 - 3 single dose(s)	**not indicated -> not reviewed**
B257	to evaluate the safety and single dose PK of everolimus in pediatric patients	multi-center (3 US, 3 Europe), open-label	19 (14/5) pediatric patients with stable renal transplant 16W, 2B, 1A 3 - 16 yr	~ 1.2 mg/m^2 BSA as 0.1 or 0.25 mg dispersible tablets PO, single dose + Neoral + corticosteroids ± azathioprine	**not reviewed** planned 20 13 in 3-11 yo, 6 in 12-16 yo
B258	to evaluate the safety and single dose PK of everolimus in pediatric patients	multi-center (5 US, 2 Europe, 1 Canada), open-label	24 (15/9) pediatric patients with stable liver transplant 14W, 7B, 3O 1 - 16 yr	~ 1.2 mg/m^2 BSA as 0.1 or 0.25 mg dispersible tablets PO, single dose + Neoral ± corticosteroids ± azathioprine	**not reviewed** planned 24 5 in 1-3 yo, 7 in 5-9 yo, 12 in 10-16 yo
B351	to evaluate the safety, efficacy, and multiple dose PK of everolimus in pediatric patients	multi-center (6 US, 8 Europe, 1 Brazil), open-label, single-arm	19 (9/10) pediatric patients with *de novo* renal transplant 11W, 2B, 6O 1 – 16 yr	~ 0.8 mg/m^2 BSA as 0.1 or 0.25 mg dispersible tablets PO, BID, day 7 and month 3 + Neoral + corticosteroids	**not reviewed** planned 40 10 in 1-9 yo, 9 in 10-16 yo

Report No.	Objectives	Design	Subjects. M/F, Race, Age	Dose, Dosage Form, Route, Duration	Remarks
Exposure-Response Relationship					
B157	To explore exposure-response relationships between everolimus AUC vs. safety and efficacy parameters to determine the effect of multiple dose everolimus on the steady state PK of cyclosporine	multicenter, randomized, double-blind, dose-finding	101 *de novo* renal transplant recipients 65/36 82W, 9B, 1A, 2O 17 – 69 years	0.5, 1, or 2 mg as 0.25 and 1 mg tablets PO, BID, for 1 yr	**Not reliable data** at 0.5-mg dose due to insensitive assay (LOQ = 2-3 ng/mL)
B201	To explore exposure-response relationships between everolimus trough concentrations *vs.* safety and efficacy parameters To assess the population PK of everolimus during steady-state administration of Neoral	multicenter, randomized, double-blind, parallel-group	588 *de novo* renal transplant recipients 380/208 529W,24B,15A,20O 19 – 67 yr	0.75 or 1.5 mg as 0.25 or 0.5 mg tablets (MF), or 1 g MMF PO, BID each + CsA + corticosteroids	also for basic PK, Population PK, variability, dose proportionality, CsA-everolimus interaction
B251	To explore exposure-response relationships between everolimus trough concentrations *vs.* safety and efficacy parameters To assess the population PK of everolimus during steady-state administration of Neoral	multicenter, randomized, double-blind, parallel-group	583 *de novo* renal transplant recipients 365/218 395W,98B,11A,79O 16 - 71 yr	0.75 or 1.5 mg as 0.25 or 0.5 mg tablets (MF), or 1 g MMF PO, BID each + CsA + corticosteroids	also for basic PK, Population PK, variability, dose proportionality, CsA-everolimus interaction
B253	to explore exposure-response relationships between everolimus trough concentrations *vs.* safety and efficacy parameters To assess everolimus PK during steady-state administration of Neoral	multicenter, randomized, double-blind, parallel-group	634 *de novo* heart transplant recipients 519/115 566W,45B,8A,15O 16 – 69 yr	0.75 or 1.5 mg as 0.25 or 0.5 mg tablets (MF), or azathioprine PO, BID + CsA	also for basic PK, variability, dose proportionality, CsA–everolimus interaction
Population Pharmacokinetics (See Studies B201 and B251)					

MF, market formulation; FMI, final market image; PO, per oral; W, white; B, black; A, Asian; O, other; D, day; M, month; BSA, body surface area; MMF mycophenolate mofetil; CsA, cyclosporine, DDI, drug-drug interaction

6.3. Pharmacometrics Review

PHARMACOMETRIC REVIEW

NDA number:	21-560, 21-561, 21-682, 21-631
Submission date:	12-19-02
Product:	0.25, 0.5, 0.75 and 1.0 mg tablet for adults
	0.1 and 0.25 mg dispersible tablet for pediatrics
Brand name:	Certican
Generic name:	Everolimus
Sponsor:	Novartis Pharmaceuticals Corp.
Type of submission:	PM consult/Population Pharmacokinetic Analysis
Primary Reviewer:	Jang-Ik Lee, Pharm.D., Ph.D.
PM reviewer:	Jenny J Zheng, Ph.D.

A population pharmacokinetic analysis was included in NDA 21-560. The objectives of this analysis were to characterize the population pharmacokinetics of everolimus and the associated inter-individual and intra-individual variability and to identify and quantify the impact of demographic covariates on the pharmacokinetics of everolimus.

A total of 5,260 everolimus concentrations at steady state from 673 subjects were pooled from two phase 3 trials. Both study RADB251 and RADB201 were randomized, double-blind, multicenter trials comparing the efficacy of two oral everolimus dose regimens (0.75 mg bid and 1.5 mg bid) with mycophenolate mofetil 1 g bid when these were added to a baseline regimen of cyclosporine and corticosteroids.

The findings of the analysis are the following:
- The apparent clearance (CL/F) of everolimus in a kidney allograft recipient (44- year old Caucasian with body weight of 71 kg) was 8.8 L/h in the presence of cyclosporine.
- Covariate analyses indicated that no significant difference in apparent clearance was detected for *Asians (n = 17)*. *Blacks (n = 65)*, however, had an average 20 percent higher apparent clearance and may therefore need a higher everolimus dose to achieve similar systemic exposure as non-blacks.
- Patients concomitantly receiving *erythromycin or azithromycin (n = 9)* had an average 20 percent lower apparent clearance. One patient receiving itraconazole had a 74 percent reduction in apparent clearance. These observations indicate that potent inhibitors of CYP3A may decrease the metabolism of everolimus and increase its blood concentrations.

COMMENTS:

1. Trough plasma samples were collected at day 2, week 1, week 2, month 1, month 2, month 3 and month 6. In a sub-population, 4 samples were collected at three occasions including month 2, 3, and 6. The collected data should be sufficient for estimating occasion variability but the sponsor did not estimate that in the analysis.
2. The data used in this analysis could not support the two compartment model which was suggested by the other studies. Therefore, a one compartment model was used. Due to this reason, caution needs to be taken in the interpretation of the results from this analysis. The estimation of CL/F might be reasonable but the estimation of other parameters such as volume and derived half-life were not

interpretable. The estimated half life from this analysis was about 8.7 hours which was much shorter than the half-life of 20 hours estimated from other study.

3. The pharmacokinetic characterization obtained from this analysis represents the pharmacokinetics of everolimus in the presence of cyclosporine.

4. As an exploratory tool, this analysis is useful to screen potential drug-drug interactions but not recommended to be used as a confirmatory analysis.

5. This analysis suggested that P450 3A inhibitors such as erythromycin and itroconazole inhibit the metabolism of everolimus, subsequently increasing the exposure of everolimus. A drug interaction study should be conducted to further evaluate the effect of itroconazole/ketoconazole on the exposure of everolimus.

6. In a separate study (Study 157), the two stage method was used to test the covariate effect on the exposure of everolimus. The results showed that renal function has no effect on the pharmacokinetics of everolimus.

RECOMMENDATION:

The population pharmacokinetic analysis suggested that co-administration of erythromycin or itroconazole with everolimus increases the exposure of everolimus. The sponsor should conduct a drug-drug interaction study to further evaluate the effect of P450 inhibitors on the exposure of everolimus.

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Jenny J Zheng, Ph.D.
Office Clinical Pharmacology/Biopharmaceutics,
Division of Pharmaceutical Evaluation III

OBJECTIVE:
- characterize the population pharmacokinetics of everolimus and the associated interindividual and intraindividual variability
- identify and quantify the impact of demographic covariates on the pharmacokinetic variability of everolimus
- explore the effects of concomitant medications on the pharmacokinetics of everolimus

STUDY DESIGN:
Pharmacokinetic data from the two pivotal phase 3 studies RADB251 and RADB201 were pooled for this population pharmacokinetic analysis of everolimus in *de novo* kidney allograft recipients. Both studies were randomized, double-blind, multicenter trials comparing the efficacy of two oral everolimus dose regimens (0.75 mg bid and 1.5 mg bid) with mycophenolate mofetil 1 g bid when these were added to a baseline regimen of cyclosporine and corticosteroids. Everolimus pre-dose trough blood samples were obtained from all patients at all protocol-specified visits: day 2; weeks 1 and 2; and months 1, 2, 3, and 6. Blood samples for the everolimus *pharmacokinetic profile* were collected at selected centers at months 2, 3 and 6.
The profiles consisted of samples pre-dose and 1, 2, 5, and 8 h post-dose.

DATA: For population pharmacokinetic analysis, everolimus concentration-time data up to and including the 6-month visit were used. In addition, only steady-state everolimus concentrations were considered in the analysis. A steady-state condition was assumed if a specific dose of everolimus had been administered to a patient for at least five consecutive days before the blood sample was obtained. The final pooled data consisted of 5,260 everolimus concentration records in 673 patients: 338 from RADB201 and 335 from RADB251. The majority of the 5,260 records (91.3%) were associated with the scheduled doses: 0.75 mg bid (46.2%) and 1.5 mg bid (45.1%). The remaining 8.7% of the total records were obtained when patients were temporarily at reduced everolimus doses for safety reasons.

METHOD:
A nonlinear mixed effect model was used to characterize the individual patient's pharmacokinetic parameters from their blood concentrations. For model fitting, all calculations were performed using a PC with the double precision version of NONMEM (version V, level 1.0).

Structure model:
The pharmacokinetic structural model used to describe the everolimus concentration-time relationship was a one-compartment first-order input and output model with dose-dependent bioavailability. The possible departure from dose proportionality was quantified by modeling *fa* as a function of dose, namely,
$$fa = 1 - \theta_4 \, ln(dose/0.75 \, mg)$$
where *dose* was the twice-daily dose in mg. Thus, bioavailabilities were relative to a reference bioavailability at 0.75 mg bid.

Covariate model:
Before adding demographic covariates to the pharmacostatistical model, diagnostic plots were constructed of the random effects from pharmacokinetic parameters of the initial model versus demographic characteristics. Inspection of these plots suggested that further examination of the effects of age, weight, and race on *CL* and the effects of weight and race on *Vc* were warranted.
For continuous variable such as age and body weight, both additive and multiplicative effect models were assessed.

Additive model: TVP= $\theta_1 + \theta_2 \bullet (COV - median_{cov})$
Multiplicative model: TVP= $\theta_1 \bullet (COV/median_{cov})^{\wedge}\theta_2$

Where TVP is the typical value of parameters such as CL and V. COV is the individual covariate value e.g. age. Median$_{cov}$ is the median value of the covariate of interest such as age in the studied population.

For categorical variable, multiplicative model was used.

To explore the effects of concomitant medications on everolimus pharmacokinetics, co-medications of interest that were used in the studies were grouped into twelve categories as shown below in Table 2. These included the HMG-CoA reductase inhibitors (atorvastatin, pravastatin, simvastatin, lovastatin); gemfibrozil; various antibiotics and antimycotics (quinolones, bactrim, erythromycins, and azole antifungals); and calcium channel blockers (dihydropyridines, diltiazem, and verapamil).

Indicator variables were used to incorporate this information in the data set. Specifically, if a blood sample was obtained during treatment with a given co-medication, then the corresponding indicator variable had a value of 1 and of 0 if the sample was obtained in the absence of this co-medication.

In contrast to demographic variables such as sex and ethnicity which remained constant throughout the study, the co-medication indicator variables changed during the study depending whether the patient was currently taking a given co-medication or not. Therefore, typical diagnostic plots of random effects (s) against variables of interest were not applicable. Instead, plots of weighted residuals versus co-medications were utilized to explore the relationship between everolimus pharmacokinetic parameters and the presence of a co-medication.

Error models:
The inter-subject variability for pharmacokinetic parameters *CL* and *Vc* was considered random effects and were assumed to be multiplicative and independent:
$$CL = TVCL * EXP(ETA(1))$$
$$V = TVV * EXP(ETA(2))$$
The intra-subject variability (or measurement/analytical error) was modeled with both additive and multiplicative components:
$$Y = F*(1+EPS(1))+EPS(2)$$

The absorption rate constant, *k*a, was assumed to be a fixed effect; that is, the same value for all patients:
$$KA = THETA(3)$$
The relative bioavailability, *f*a, with respect to the 0.75 mg bid dose was also assumed to be a fixed effect:
$$F1 = 1 - THETA(4) * LOG(DOSE/0.75)$$

Model selection:
A basic structure model was first selected. The random effects(s) from pharmacokinetic parameters of the initial model versus demographic characteristics were graphically constructed. The to-be-tested covariates would be selected by inspection of these plots. Both additive and multiplicative effect models were tested for age and weight and the multiplicative model was used to test race effect. Separate race factors for *Black* and *Asian* were tested.

After the evaluation of demographic covariates on the pharmacokinetic of everolimus, the effect of co-medication was explored.

All covariates retained in the interim model were statistically significant at the 0.05 level according to likelihood ratio tests.

RESULTS:

Demographics:

Summary statistics of the demographic characteristics of the patients included in the analysis are shown in Table 1.

The concentration-time profile of everolimus after single-dose administration was characterized by a biphasic decline in concentrations as can been seen in the first-in-man study with this compound (RADW101). The steady-state concentration data in the present evaluation were best described by a standard one-compartment pharmacokinetic structural model. This difference likely reflects the fact that the sampling period of 12 h over the dosing interval may not reveal a second phase in the concentration decline. In addition, the relatively sparse blood sampling schedule at steady state may have obscured the biphasic nature of the profile.

The parameter estimates of the final model are shown in Table 3. The population apparent clearance *(CL* or CL/F) of 8.8L/h characterized in this evaluation is slightly lower than the CL determined in the single-dose study RADW101 in stable renal transplant patients averaging 10 L/h. Because cyclosporine can influence everolimus pharmacokinetic parameters, it is note worthy that these parameters were derived in a population receiving everolimus as part of the intended immunosuppressive regimen with cyclosporine and prednisone.

A slight under-proportionality in exposure of 10 percent was noted at the higher everolimus dose used in this study (1.5 mg bid) relative to the lower dose level (0.75 mg bid).

Although there was a statistically significant influence of weight and age on apparent clearance the relationships were very shallow and data showed considerable scatter. Specifically from the final model, a one-kg increase in body weight would result in a 0.44% increase in apparent clearance. Hence, at two extremes of weight, a 40-kg and a 100-kg patient would have clearances of 7.6 and 10.0 L/h, respectively. With respect to age, one-year increase would result in a 0.34% decrease in apparent clearance. Hence, at two extremes of age, a 20-year-old and a 65-year-old patient would have clearances of 9.5 and 8.2 L/h, respectively.

No influence of Asian ethnicity *(n* = 17) on everolimus pharmacokinetics was detected. By contrast, Blacks *(n* = 65) had a significantly higher apparent clearance by 20 percent compared with non-blacks. This could be due to either higher actual clearance and/or lower bioavailability in this sub-population. In the clinical evaluation of the everolimus phase 3 study conducted in North and South America (RADB251), Blacks had significantly poorer efficacy at the lower everolimus dose level compared with the higher dose level. As a result, Blacks may benefit from a higher dose of everolimus relative to the dose recommended for the general kidney transplant population.

Several ***HMG-CoA reductase inhibitors*** were coadministered during the course of these two studies. A total of 133 patients had 574 blood sampling occasions at which either atorvastatin, pravastatin, or simvastatin were coadministered. No influence on everolimus pharmacokinetics from these agents was noted.

With regard to ***antibiotics and antimycotic agent***s, quinolones as a group (124 patients) and bactrim (450 patients) had no detectable influence on everolimus. By contrast, co-administration of erythromycin or azithromycin (9 patients) and of itraconazole (1 patient) were associated with a significantly decreased apparent clearance of everolimus by 20 percent and 74 percent, respectively.

Among the **calcium channel blocking agent**s, dihydropyridines were coadministered in 267 patients, diltiazem in 22 patients, and verapamil in 5 patients at pharmacokinetic visits. No detectable influence on everolimus was found.

After accounting for covariates, the remaining interindividual variability for apparent clearance was moderate at 27 percent and for apparent distribution volume was high at 36 percent. The combined intraindividual and assay error in everolimus blood concentrations over time of 31 percent was quantified.

CONCLUSION:

- Population analysis of the pooled phase 3 studies using a one-compartment structural model yielded the following pharmacokinetic parameters for a reference kidney allograft recipient (44-year-old Caucasian weighing 71 kg) receiving everolimus as part of a cyclosporine-prednisone immunosuppressive regimen: the *apparent clearance* (CL/F) was 8.8 L/h; and the *apparent central distribution volume* (Vc/F) was 110 L.
- Covariate analyses indicated that no significant difference in apparent clearance was detected for *Asians (n = 17). Blacks (n = 65)*, however, had an average 20 percent higher apparent clearance and may therefore need a higher everolimus dose to achieve similar systemic exposure as non-blacks
- Patients concomitantly receiving *erythromycin or azithromycin (n = 9)* had an average 20 percent lower apparent clearance. One patient receiving itraconazole had a 74 percent reduction in apparent clearance. These observations indicate that potent inhibitors of CYP3A may decrease the metabolism of everolimus and increase its blood concentrations.
- No influence on everolimus disposition was detected during coadministration of atorvastatin, pravastatin, simvastatin, gemfibrozil, quinolone antibiotics, bactrim, dihydropyridines, diltiazem, verapamil, or fluconazole.

COMMENTS:

1. Trough plasma samples were collected at day 2, week 1, week 2, month 1, month 2, month 3 and month 6. In sub-population, 4 samples were collected at three occasions including month 2, 3, and 6. The collected data should be sufficient for estimating occasion variability but the sponsor did not estimate that in the analysis.
2. The data used in this analysis could not support the two compartment model which was suggested by the other studies. Therefore, a one compartment model was used. Due to this reason, caution needs to be taken to interpret the results from this analysis. The estimation of CL could be reasonable but the estimation of other parameters such as volume and derived half-life were not interpretable. The estimated half life from this analysis was about 8.7 hours which was much shorter than about 20 hours estimated from other study.
3. The effect of model mis-specification, e.g. using one compartment model to fit steady state data that were obtained from two compartment model, was briefly assessed. The mean concentrations vs time data after 0.75 mg oral dose in Study RADW101 was fitted first by two compartment model. The steady state data were generated by the two compartment model. Then simulated steady state data was fitted by one compartment model. The results showed that the apparent CL was similar. The estimated CL/F was 9.12 L/h and 9.63 L/h when two compartment model was used to fit single dose data and one compartment model was used to fit the steady state data, respectively. However, the volume estimates and absorption rate constant were very different. Using two compartment model and one compartment model, the estimated central volume of distribution was 51.1 L and 96.3 L, respectively, indicating that the volume estimates are very different. The absorption rate constants were 0.734 h^{-1} for two compartment model and 2.48 h^{-1} for one compartment model.

4. The pharmacokinetic characterization obtained from this analysis represents the pharmacokinetics of everolimus in the presence of cyclosporine.
5. As an exploratory tool, this analysis is useful to screen for potential drug-drug interaction but not recommended to be used as confirmatory analysis.
6. This analysis suggested that P450 3A inhibitors such as erythromycin and itroconazole inhibit the metabolism of everolimus, subsequently increasing the exposure of everolimus. A drug interaction study should be conducted to further evaluate the effect of itroconazole/ketoconazole on the exposure of everolimus.
7. The covariate effects, namely, the effect of age, body weight, gender, ethnicity and renal function on the AUCτ/Dose at steady state (AUCτ,ss), were also tested in Study 157. Please refer to Dr. Jang-Ik Lee's review for the details of study design. The results of the analysis showed that age (ranged from 17 to 69 years old) and body weight (49 to 106 kg) have no effect on the exposure of everolimus. Similarly, the exposure between males (n=60) and females (n=31) or between white (n=74) and non-white (n=17) are not different. For renal function test, creatinine clearance (CLcr) was calculated by the Cockcroft-Gualt equation using serum creatinine measurements at day 14. The apparent clearance of everolimus was also estimated by the concentration data at day 14. The calculated creatinine clearance in the population on day 14 ranged from 10.8 to 106.5 mL/min. The categorization was 11 patients with normal renal function (80.4 – 106.5 ml/min), 38 patients with mild renal impairment (50.0 – 77.1 ml/min), 24 patients with moderate impairment (31.3– 49.7 ml/min), and 8 patients with severe impairment (10.8 – 27.7 ml/min). The regression analysis between steady state exposure (AUCτ,ss) and CLcr was no statistically significant $(p = 0.08)$ and yielded a <u>negative</u> shallow slope of –0.12 L/h per ml/min. A negligible amount of variability in everolimus clearance could be explained by creatinine clearance: 3.9 percent.

Table 1. Demographic characteristics

	RADB201	RADB251	Pooled
N	338	335	673
Gender:			
Male	204	206	410
Female	134	129	263
Race:			
Caucasian	313	225	538
Black	10	55	65
Asian	9	8	17
Other	6	47	53
Weight (kg):			
Median	70	74	71
Mean ± SD	70.6 ± 14.1	76.2 ± 17.9	73.4 ± 16.4
Age (years):			
Median	45.5	44	44
Mean ± SD	44.6 ± 11.6	43.6 ± 12.1	44.1 ± 11.8

Table 2. Comedications

6.3.1.1.1.1 Comedication	Patients	Concentration records
Atorvastatin	74	289
Pravastatin	41	221
Simvastatin	18	64
Lovastatin	3	8
Gemfibrozil	10	76
Quinolones	124	267
Bactrim	450	3093
Erythromycins	9	21
Azole antimycotics	17	47
Dihydropyridines	267	1298
Diltiazem	22	116
Verapamil	5	7

Patients were included if they had at least 1 blood sample obtained during treatment with a comedication.
Quinolones: ciprofloxacin, levofloxacin, norfloxacin, ofloxacin.
Dihydropyridines: amlodipine, isradipine, nifedipine.
Erythromycins: erythromycin, azithromycin.
Azole antimycotics: fluconazole, itraconazole.

Table 3. Parameter estimates: final model

Parameter		Estimate	Standard error	Population variation
CL [L/h] = {θ_1 + $\theta_5 \times$ (Wt-71) +	θ_1	8.82	0.242	27%
	θ_5	0.0391	0.0139	
$\theta_6 \times$ (Age-44)} $\times$	θ_6	-0.0300	0.0146	
(θ_7 $^{is.Black}$) $\times$	θ_7	1.20	0.0805	
(θ_9 $^{is.CoMed1}$)	θ_9	0.806	0.0815	
Vc [L] = {θ_2 + $\theta_8 \times$ (Wt-71)}	θ_2	110	5.31	36%
	θ_8	1.14	0.228	
ka [1/h]	θ_3	6.07	0.703	
Fa =1-θ_4 log(Dose/0.75 mg)	θ_4	0.145	0.0487	
Residual random effect =	Var(ε_1)	0.0930	0.0132	
(Predicted $\times$ ε_1) + ε_2	Var(ε_2)	1.56	0.846	

Notation: is.Black =1 if race is Black; = 0 otherwise
is.CoMed1 = 1 if erythromycins was administered; = 0 otherwise

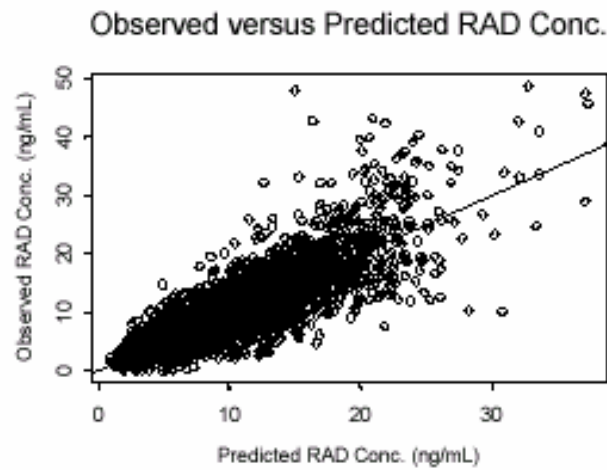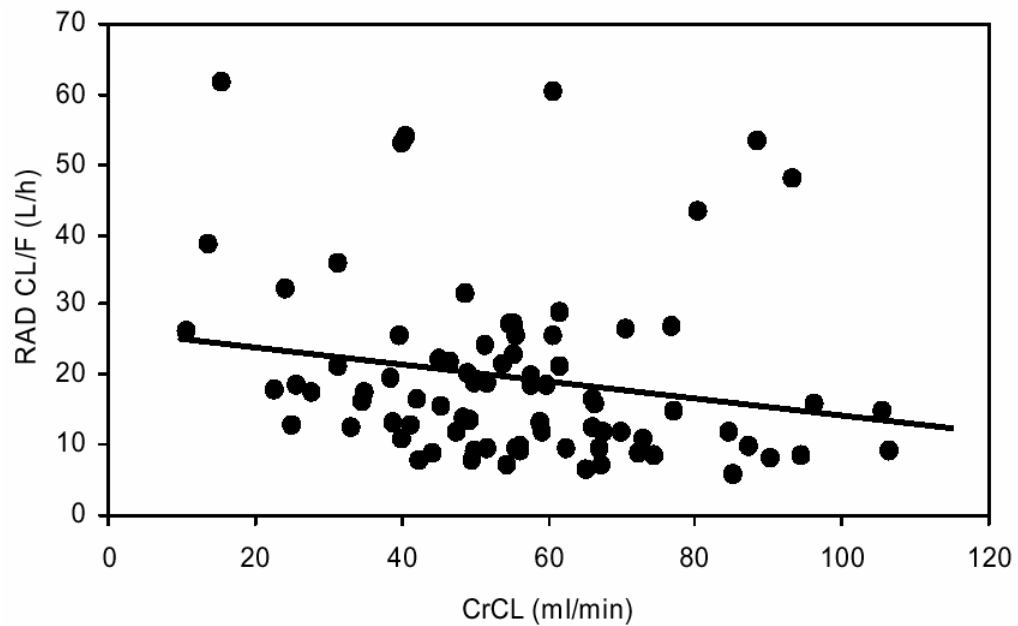Figure 1. Observed versus predicted RAD Concentration

Figure 2. Scatter plot of creatinine clearance on day 14 vs RAD clearance with linear regression line

6.4. Dissolution Review

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA	**21-560, 21-561, 21-628, 21-631**
Submission Date(s)	**10/4/02 (chemistry only), 12/20/02, 2/14/03, 6/10/03, 7/9/03, 7/18/03**
Brand Name	**Certican**
Generic Name	**Everolimus (code name; RAD001, SDZ RAD)**
Primary Reviewer	**Jang-Ik Lee, Pharm.D., Ph.D.**
Supporting Reviewers	**Jenny J. Zheng, Ph.D. (Pharmacometrics)**
	Seong Jang, Ph.D. (Dissolution)
Team Leader	**Philip Colangelo, Pharm.D., Ph.D.**
OCPB Division	**DPE III (HFD-880)**
OND Division	**ODE IV DSPIDP (HFD-590)**
Sponsor	**Novartis Pharmaceuticals Corp.**
Relevant IND(s)	**52,003**
Submission Type; Code	**Original, 1S (NME)**

Formulation; Strength(s):
  *N21-560, N21-628***: Immediate Release Tablets; 0.25, 0.5, 0.75, 1.0 mg**
  *N21-561, N21-631***: Dispersible Tablets; 0.1, 0.25 mg**

Proposed Indication
  *N21-560, N21-561***: Prophylaxis of organ rejection in allogeneic kidney transplant patients**
  *N21-628, N21-631***: Prophylaxis of organ rejection in allogeneic heart transplant patients**

Proposed Dosage and Administration: **Oral doses of 0.75 mg b.i.d. or larger to maintain whole blood concentrations of everolimus ≥ 3.0 ng/mL in combination with cyclosporine and corticosteroids**

This is the review of dissolution data of the above application. The original submissions, dated on 10/4/02 and 2/14/03, include dissolution data from one tablet batch for each strength. The proposed dissolution methods for Certican immediate release tablets and dispersible tablets were reviewed based on these data; the relevant raw data are attached in the Appendix 1 at the end of this review. However, because the dissolution data were provided for only one tablet batch for each strength, this was insufficient to adequately determine the dissolution specification for both products. Thus, the Chemistry and Clinical Pharmacology and Biopharmaceutics reviewers requested the sponsor submit additional dissolution data from at least three batches of all dose strengths, dated on 10/1/03. The corresponding additional dissolution data were submitted on 10/8/03; the relevant raw data are attached in the Appendix 2 at the end of this review. The recommendation for dissolution specifications of Certican immediate release tablets and dispersible tablets was based on these data.

Recommendation: The sponsor's proposed dissolution methods for Certican immediate release tablets and dispersible tablets are acceptable. However, it is recommended that the dissolution specifications be tightened more than what the sponsor has proposed. Thus, we recommend the final dissolution specification for each product as follows:

Immediate release tablets: *Q=*   *in 30 min*
                                                (
Dispersible tablets: *Q=*   *in 15 min*

I.   Dissolution of Certican Immediate Release tablets

In vitro dissolution of Certican tablets was evaluated in 11 different media using paddle or basket methods. The paddle speed was 50 rpm. The results are illustrated in Figure 1.
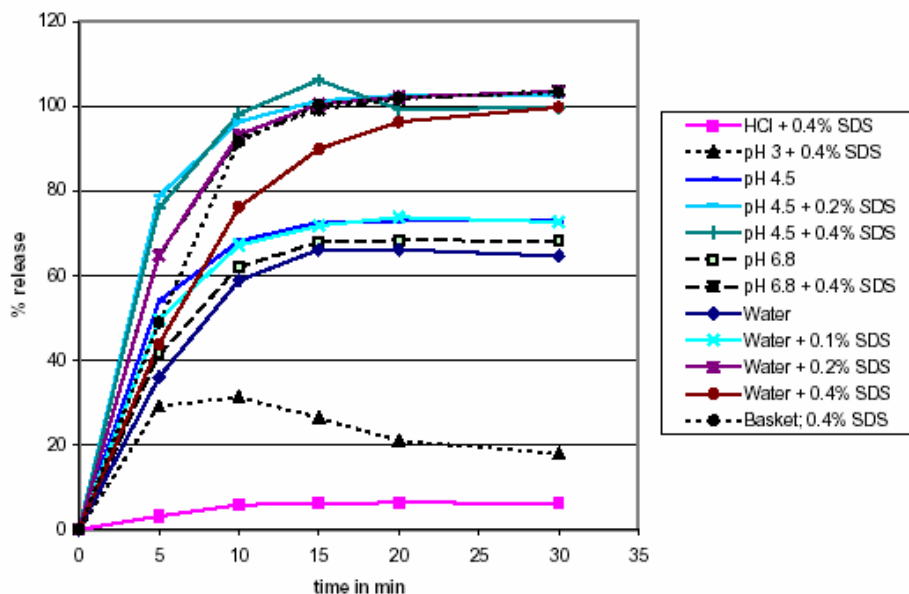
Figure 1. Dissolution profiles of 1 mg Certican tablets, batch X341 1099, in different media.

Sodium dodecylsulfate (SDS) was used to enhance dissolution of drug substance. To enable complete dissolution, the addition of 0.2% SDS (critical micelle concentration) was necessary. The drug substance is not stable in strong acidic media, up to pH 3. In weak acidic media (buffer pH 4.5 and 6.8 in water), the drug substance is stable. However, the increase in pH with 0.4% SDS has no advantage over water with 0.4% SDS. **Unexpectedly greater solubility and dissolution were achieved with 0.2% SDS compared to 0.4% SDS, resulting in less discriminating dissolution profiles with 0.2% SDS compared to 0.4% SDS.** Thus, the sponsor was requested to test dissolution of Certican tablets at lower paddle speed. The results are illustrated in Figure 2.
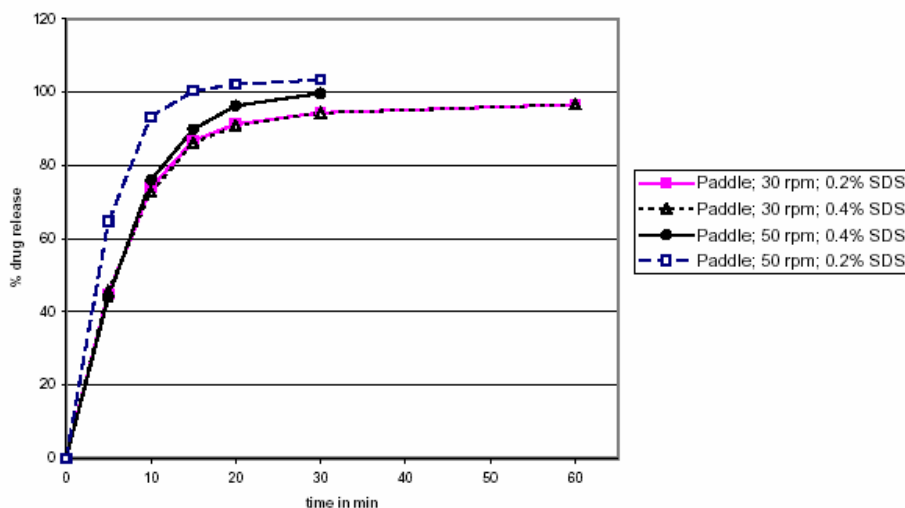
Figure 2. Dissolution profiles of 1 mg Certican tablets, batch X341 1099, with paddle. The sponsor proposed a paddle method at 50 rpm in water with 0.4% SDS based on the following rationale.

Method: the basket method does not provide slower profile than the paddle method. Since the paddle method is preferred to the basket method, Certican tablets will be tested by means of the paddle method.

Rotation: a routine method for the purpose of batch release is best under control, if it is carried out at 50 rpm rather than 30 rpm, because the dissolution bath calibration with USP calibrator tablets is performed at 50 and 100 rpm. The operating conditions should preferably lie within this range.

Medium: regarding the run-to-run-variability, the medium of water with 0.4% SDS is considered more robust and therefore more adequate than the medium of water with 0.2%. The critical micelle concentration (CMC) of SDS is 0.2%. Below the CMC, the surface tension like other physical parameters is variable; above the CMC, the surface tension becomes almost constant. As consequence, a dissolution test performed with a medium which contains SDS in a concentration distinctly above the CMC is considered robust, whereas in contrast, at the CMC the operating conditions are borderline and therefore the test can be expected to be easily influenced by random variables.

Discriminatory power of the method: In order to compare the discriminatory power of different test conditions, the sponsor made an additional tablet batch RH1-60.8 which is without one excipient, i.e., crospovidone, resulting in slower dissolution compared to normal tablet batches. Dissolution of batch RH1-60.8 was tested under three different conditions. The results are illustrated in Figures 3 and 4.
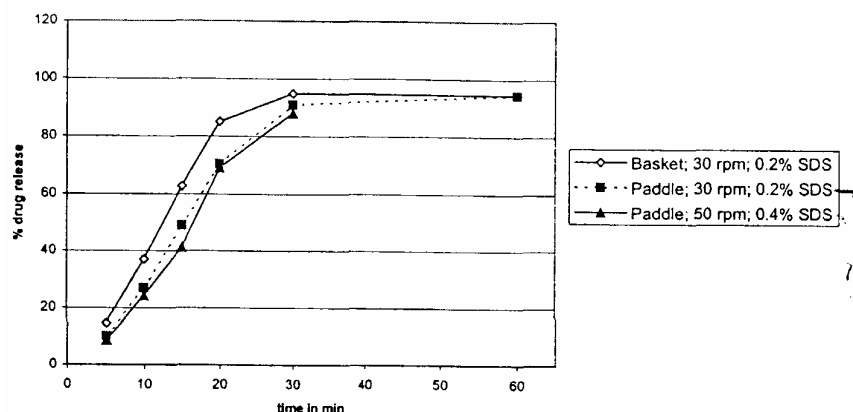
Figure 3. Dissolution of Certican tablets, batch RH 1-60.8
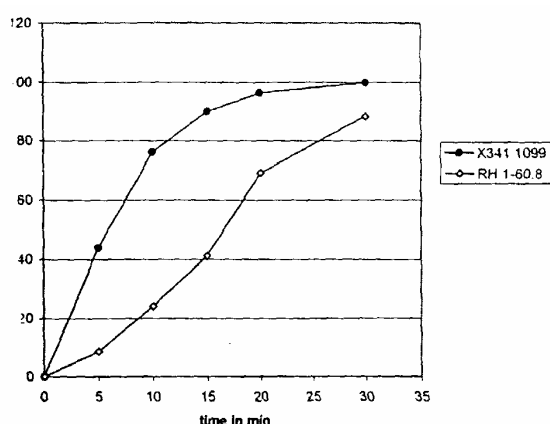
Figure 4. X341 1099 and RH 1-60.8 with paddle; 50 rpm in water with 0.4% SDS
The similarity factors, f2, between batch X341 1099 and RH 1-60.8 were 27, 22, and 19 for [basket; 30 rpm; 0.2% SDS], [paddle; 30 rpm; 0.2% SDS], and [paddle; 50 rpm; 0.4% SDS], respectively.

The sponsor proposed the following as the final dissolution method and specification for Certican® tablets.

Apparatus:	Paddle method (USP: apparatus 2)
Media:	water + 0.4% sodium dodecylsulfate
Volume:	500 mL
Speed of rotation:	50±2 rpm
Dissolution specification:	Q=

Reviewer's comment: The sponsor's proposed dissolution method for Certican immediate release tablets is acceptable. However, according to additionally submitted dissolution data obtained from three tablet batches of each strength (See Appendix 2), we found that the dissolution specification can be tightened more than the sponsor proposed. Thus, we recommend the sponsor use **Q**= *as the final dissolution specification for immediate release tablets.*

II.   Dissolution of Certican Dispersible tablets

The dissolution method for the tablets was used for the dispersible tablets as well. As the release profiles of the dispersible tablets is very fast, the rotation speed was reduced to 30 rpm. Two SDS concentrations, i.e., 0.2% and 0.4% were tested using basket and paddle methods. The results are shown in figure 4.

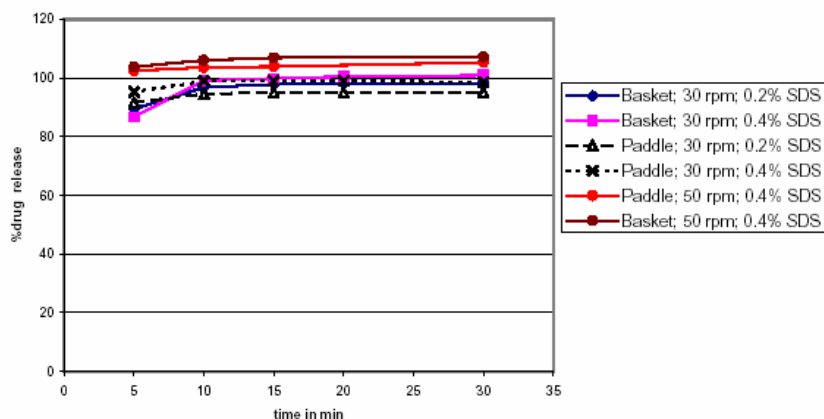**Figure 5.** Dissolution profiles of 0.25 mg Certican dispersible tablets, batch X077 0200.

The changes in the apparatus and the rotation speed from 50 rpm to 30 rpm did not reduce the fast dissolution rate of this dosage form. The fast release is the part of the design of the "dispersible tablets". None of the other parameters were able to slow down the dissolution rate. Thus, the sponsor proposed the same method for the immediate release and dispersible tablets. The proposed method and specification for the dispersible tablets are as follows:

Apparatus: Paddle method (USP: apparatus 2)
Media: water + 0.4% sodium dodecylsulfate
Volume: 500 mL
Speed of rotation: 50±2 rpm
Dissolution specification: Q=

*Reviewer's comment: The sponsor's proposed dissolution method for Certican dispersible tablets is acceptable. However, according to additionally submitted dissolution data obtained from three tablet batches of each strength (See Appendix 2), we found that the dissolution specification can be tightened more than the sponsor proposed. Thus, we recommend the sponsor use **Q=** as the final dissolution specification for dispersible tablets.*

III.   Reviewer's comments

The sponsor's proposed dissolution methods for Certican immediate release tablets and dispersible tablets are acceptable. However, according to additionally submitted dissolution data obtained from three tablet batches of each strength, it is recommended that the dissolution specification be tightened more than what the sponsor has proposed. Thus, we recommend the final dissolution methods and specifications for each product as follows:

Certican Immediate Release Tablets

Apparatus:	*Paddle method (USP: apparatus 2)*
Media:	*water + 0.4% sodium dodecylsulfate*
Volume:	*500 mL*
Speed of rotation:	*50±2 rpm*
Dissolution specification:	*Q=*

Certican Dispersible Tablets:

Apparatus:	*Paddle method (USP: apparatus 2)*
Media:	*water + 0.4% sodium dodecylsulfate*
Volume:	*500 mL*
Speed of rotation:	*50±2 rpm*
Dissolution specification:	*Q=*

IV.   Recommendation

Please convey Clinical Pharmacology and Bipharmaceutics reviewer's comments to the sponsor and the medical officer.

Seong H. Jang, Ph.D.
Reviewer
Clinical Pharmacology and Biopharmaceutics

DPEIII/OCPB

Concurrence

Phil Colangelo, Pharm.D., Ph.D.
Team Leader
Clinical Pharmacology and Biopharmaceutics

DPEIII/OCPB

6.5. OCPB Filing Review Form

<table>
<tr><td colspan="4" align="center">***Office of Clinical Pharmacology and Biopharmaceutics***
New Drug Application Filing and Review Form</td></tr>
<tr><td colspan="4" align="center">**General Information About the Submission**</td></tr>
<tr><td></td><td>Information</td><td></td><td>Information</td></tr>
<tr><td>**NDA Number**</td><td>**21-560, 21-628**</td><td>**Brand Name**</td><td>**Certican**</td></tr>
<tr><td>**OCPB Division (I, II, III)**</td><td>**III**</td><td>**Generic Name**</td><td>**everolimus**</td></tr>
<tr><td>**Medical Division**</td><td>**HFD-590**</td><td>**Drug Class**</td><td>**immunosuppresive**</td></tr>
<tr><td>**OCPB Reviewer**</td><td>**Jang-Ik Lee**</td><td>**Indication(s)**</td><td>**prophylaxis of kidney or heart transplant rejection**</td></tr>
<tr><td>**OCPB Team Leader**</td><td>**Philip Colangelo**</td><td>**Dosage Form; Strengths**</td><td>**Tablets; 0.25, 0.5, 0.75, 1.0 mg**</td></tr>
<tr><td>**Date of Submission**</td><td>**12/19/02**</td><td>**Dosing Regimen**</td><td>**0.75 mg twice daily**</td></tr>
<tr><td>**Estimated Due Date of OCPB Review**</td><td>**8/31/03**</td><td>**Route of Administration**</td><td>**oral**</td></tr>
<tr><td>**PDUFA Due Date**</td><td>**10/20/03**</td><td>**Sponsor**</td><td>**Novartis Pharmaceuticals**</td></tr>
<tr><td>**Division Due Date**</td><td>**9/20/03 (tentative)**</td><td>**Priority Classification**</td><td>**1S**</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
</table>

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
Clin. Pharm. and Biopharm. Information				
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	?			
I. Clinical Pharmacology				
Mass balance:	X	1	1	
Isozyme characterization:	X	(2)	(2)	One study for P-gp
Blood/plasma ratio:	X	(1)	(1)	
Plasma protein binding:	X	(2)	(2)	
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	2	2	
multiple dose:		0	0	
Patients-				
single dose:	X	2	1	
multiple dose:	X	6	5	
Dose proportionality -				
fasting / non-fasting single dose:	X	4	4	
fasting / non-fasting multiple dose:	X	5	4	
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	3	3	
In-vivo effects of primary drug:	X	2	2	
In-vitro:	X	(3)	(3)	
Subpopulation studies -				
ethnicity:	X	3	3	
gender:	X	3	3	
pediatrics:	X	3	3	partially reviewed
geriatrics:	X	2	2	
renal impairment:				determined using population pharmacokinetics
hepatic impairment:	X	1	1	
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1	1	
Phase 3 clinical trial:	X	3	3	Pop PK and ER studies

Population Analyses -				
Data rich:	**X**	**3**	**3**	
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:	**X**	**3**	**3**	
replicate design; single / multi dose:				
Food-drug interaction studies:	**X**	**2**	**2**	
Dissolution:	**(X)**	**(1)**	**(1)**	
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Analytical validation	**(X)**	**(4)**	**(3)**	
Literature References	**X**			
Total Number of Studies		**24 (9)**	**19 (8)**	**()** *in vitro* **studies**

Filability and QBR comments		
	"X" if yes	**Comments**
Application fileable?	**X**	Reasons if the application <u>is not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)	**see QBR section**	
Other comments or information not included above		
Primary reviewer Signature and Date	**Jang-Ik Lee (1/31/03)**	
PM reviewer Signature and Date	**Jenny Zheng**	
Secondary reviewer Signature and Date	**Philip Colangelo**	

CC: NDA 21-385, HFD-850 (P. Lee), HFD-540 (CSO), HFD-880 (TL, DD, DDD), CDR

End of Document

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 /s/
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Jang-Ik Lee
10/17/03 04:35:55 PM
BIOPHARMACEUTICS



Phil Colangelo
10/17/03 04:48:56 PM
BIOPHARMACEUTICS
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