

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**22-334**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Afinitor (everolimus)

**Date:** March 24, 2009

**To:** File for NDA #22-334

**From:** John K. Leighton, PhD, DABT  
Associate Director for Pharmacology  
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review, memoranda and labeling provided by Drs. Luan Lee and Haleh Saber. I concur with their conclusions that Afinitor may be approved. No additional pharmacology or toxicology studies are necessary for the proposed indication.

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/s/

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John Leighton  
3/24/2009 03:32:48 PM  
PHARMACOLOGIST

## MEMORANDUM

**Date:** March 23, 2009  
**From:** Haleh Saber, Ph.D.  
Pharmacology Acting Team Leader  
Division of Drug Oncology Products  
**To:** File for NDA #22,334  
Afinitor® (everolimus) tablets  
**Re:** Approvability for Pharmacology and Toxicology

Afinitor® (everolimus) is indicated for the treatment of patients with advanced renal cell carcinoma after disease progression following treatment with sunitinib or sorafenib. Nonclinical studies that investigated the pharmacology and toxicology of everolimus were provided in support of the NDA. Everolimus, an ether derivative of sirolimus, is an inhibitor of the mTOR pathway. mTOR is a serine-threonine kinase downstream of PI3K/AKT pathway, which acts as a growth factor and nutrient sensor. Inhibition of mTOR by everolimus was shown to reduce cell proliferation, angiogenesis, and glucose uptake in *in vitro* and/or *in vivo* studies. In the Highlight section of the label, the pharmacologic class of Afinitor is defined as “kinase inhibitor” to be consistent with other products in this class. Of note, everolimus is approved in Europe as an immunosuppressive agent, under the name Certican®.

Pharmacology, safety pharmacology, pharmacokinetic/ ADME, and toxicology studies supporting the marketing application of Afinitor for the proposed indication were conducted in *in vitro* systems and in animal species. Based on the general toxicology studies, toxicities associated with everolimus were comparable to those reported for temsirolimus, another mTOR inhibitor approved for the treatment of renal cell carcinoma. Everolimus-related findings included effects in the male reproductive system, coagulation pathway, GI tract, lungs, metabolism/endocrine system, and lymphoid tissues. Nonclinical studies in rodents indicated that everolimus and/or its metabolites crossed the blood-brain barrier and the placenta, and were excreted into milk of lactating animals.

Everolimus was negative for evidence of genetic toxicity in the standard battery of tests described by ICH S2. Everolimus was negative for evidence of carcinogenicity in two rodent studies. Reproductive toxicology studies conducted with everolimus included the male fertility, embryo-fetal toxicity and prenatal/ postnatal development toxicity studies. Everolimus resulted in infertility in male rats, with partial recovery after 10-13 weeks of treatment-free period. Oral doses of everolimus in female rats resulted in increased pre-implantation loss, suggesting that the drug may reduce female fertility. Everolimus was embryo-fetal toxic when administered to pregnant rats; toxicities included decreased number of fetus, malformation (e.g., sternal cleft) and retarded skeletal development. In rabbits, embryo-fetal toxicity was evidenced as increased resorption. Because of the embryo-fetal effects, Pregnancy Category D is recommended for everolimus. The prenatal and postnatal development study in rats at doses tested showed no adverse

effects on delivery and lactation and no drug-related effects on the developmental parameters in the offspring (morphological development, motor activity, learning, or fertility assessment). However, reduced body weight and slight reduction in survival were noted in offspring.

The nonclinical studies were reviewed in detail by Dr. Shwu-Luan Lee. The nonclinical findings are summarized in the "Executive Summary" and "Discussion and Conclusions" of the review, and reflected in the product label.

**Recommendation:** I concur with Dr. Lee's conclusion that pharmacology and toxicology data support the approval of NDA 22,334 for Afinitor. There are no outstanding non-clinical issues related to the approval of Afinitor for the proposed indication.

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/s/

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Haleh Saber  
3/23/2009 03:48:54 PM  
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-334
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	6/30/2008
DRUG NAME:	Afinitor® (everolimus)
INDICATION:	Treatment of advanced renal cell carcinoma
SPONSOR:	Novartis Pharmaceutical Corporation
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Drug Oncology Products
PHARM/TOX REVIEWER:	Shwu-Luan Lee, Ph.D.
PHARM/TOX SUPERVISOR:	Haleh Saber, Ph.D.
DIVISION DIRECTOR:	Robert Justice, M.D., M.S.
PROJECT MANAGER:	Christy Cotrell

Date of review submission to Division File System (DFS): 3/12/09

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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

- A. Recommendation on approvability  
There are no pharmacology/toxicology issues which preclude approval of everolimus (Afinitor<sup>®</sup>) for the requested indication.
- B. Recommendation for nonclinical studies  
No additional non-clinical studies are required for the proposed indication.
- C. Recommendations on labeling  
Recommendations on labeling have been provided within team meetings and communicated to the sponsor.

### **II. Summary of nonclinical findings**

- A. Brief overview of nonclinical findings  
Everolimus (RAD001) is a hydroxyethyl derivative of rapamycin. Everolimus binds to FKBP-12, an intracellular protein, resulting in an inhibitory complex formation. The complex binds to mTOR (mammalian target of rapamycin) and inhibits mTOR kinase activity.

The oral bioavailability of everolimus was low, e.g. approximately 5% in mice and 6% in monkeys. Everolimus binding to plasma proteins was ~75% in human, 85% in monkey, 93% in rats, and >99% in mouse plasma, at concentrations tested. In rats, tissue concentration of radioactivity was generally highest in the GI tract, pancreas, lungs, kidneys, liver, and tissues/organs of the immune system. Tissue elimination of radioactivity was slow; at Hr 144 (Day 6 post-dose) radioactivity was detectable in some tissues/organs, e.g. in liver, kidneys, and GI tract. Everolimus penetrated the brain dose dependently, with a brain: blood concentration ratio of 3:1 after an intravenous dose of 30 mg/kg. Everolimus is extensively metabolized. There appears to be an appreciable first-pass effect contributing to low oral bioavailability of the parent drug. Cytochrome P450 isoenzyme 3A4 (CYP3A4) was the major enzyme involved in the biotransformation of everolimus in human liver microsomes. Excretion of everolimus and/or its metabolites was mostly via the bile; urinary excretion was negligible. In the rat, elimination was mainly via the bile (~71% in bile duct cannulated rats following i.v. administration) and feces (69% to 82% of the dose). Everolimus and its metabolites penetrated the placenta and were excreted into milk in rats; the milk: blood AUC ratio of radioactivity was 3.5:1. After repeated administration, evidence of drug accumulation was observed only in rats at relatively high and toxic doses (in 4 week studies, no accumulation was found in the 26 week study). In monkeys, dose-normalized exposure tended to decrease with increasing doses (except at very high doses).

Everolimus showed minimal or no effect in safety pharmacology studies when evaluated for effects on the cardiovascular, central nervous, or respiratory systems. However, myocarditis was observed in several toxicology studies. In safety pharmacology studies, orally

administered everolimus at doses up to 50 mg/kg in mice induced slight increases in total urinary potassium and chloride levels, but no effects on gastrointestinal transit time were seen.

Repeat dose toxicology studies conducted in mice, rats, minipigs and monkeys have identified the following toxicities:

- Reproductive organs: males (e.g. atrophy in testes, epididymis, and prostate; ↓ sperm count and motility), females (ovary: reduced follicular development, uterus: atrophy)
- Hematopoietic/lymphoid system:
  - ↓ lymphocytes, ↑ neutrophils, ↑ monocytes, ↑ % band cells, associated with lymphoid atrophy in lymph nodes, thymus and/or spleen (in minipigs and monkeys)
  - ↑ RBC, HGB, and Hct in rats, associated with ↓ serum iron and hemosiderosis in the spleen. ↑ RBC, HGB, and Hct: also in mice and minipig, but decreased in monkeys.
  - ↓ Platelets, ↑ fibrinogen, ↑ aPTT
  - Sternum hypocellularity was found in minipigs.
- GI tract: stomach (acute inflammation at glandular tissue and mucosal hyperplasia/hypertrophy in rats), intestine (small and large intestines: erosion and mucosal atrophy in minipigs, aggregation of macrophages and mucosal inflammation in monkeys). The GI lesions may have contributed to ↓ food consumption, weight loss, malnutrition and related clinical chemistry findings (↓ albumin, A/G ratio, and phosphorus, in all species)
- Lung: accumulation of foamy alveolar macrophages (all species)
- Skin: ulceration, scabs, lesions (all species)
- Metabolism/endocrine: ↑ cholesterol and triglyceride (all species)
- Pancreas: necrosis and exocrine cell vacuolation in minipigs, degranulation of exocrine cells and degeneration of pancreatic islet cells in monkeys; ↑ amylase and lipase
- Heart: in rats and/or monkeys: myocarditis, myocardial degeneration and/or fibrosis
- Eye (mainly in lens): swelling and disruption of fibers in the anterior cortex (rat only)
- Kidney: ↑ BUN and creatinine, increased incidence of hydronephrosis and pigment in renal tubular epithelium in rats, tubular degeneration with karyomegaly, interstitial inflammation and basophilic tubules in mice

Everolimus did not show mutagenic activity in bacterial Ames test in *Salmonella* or in TK mutation test in L5178Y mouse lymphoma cells. Everolimus did not demonstrate clastogenic activity in a chromosome aberration test in V79 Chinese hamster cells, and did not increase micronucleus formation in mice after oral doses up to 500 mg/kg (1500 mg/m<sup>2</sup>).

Everolimus was not carcinogenic in the rodents. In the 104 week studies, oral treatment with everolimus at up to 0.9 mg/kg induced toxicities comparable to findings in other repeat dose toxicology studies. The 0.9 mg/kg dose in mice and rats corresponded respectively to 4.3 and 0.2 times the estimated clinical exposure at the recommended human dose of 10 mg/day. No drug-related neoplastic findings were observed.

Everolimus was shown to have adverse effects on male and female reproductive organs. See above for effects observed in repeat-dose toxicology studies. In a 13 week male fertility study in rats, an oral dose of 5 mg/kg (30 mg/m<sup>2</sup>, corresponding to 81% of the AUC<sub>0-24h</sub> at the recommended human dose of 10 mg/day), resulted in male infertility. The adverse effect was not fully recovered after a 10-13 week treatment-free period. When administered to female rats, prior to mating and continued to gestation day 16, everolimus at oral doses  $\geq$  0.1 mg/kg (0.6 mg/m<sup>2</sup>,  $\sim$  4% the AUC<sub>0-24h</sub> at recommended human dose of 10 mg/day) induced embryo-fetal toxicities including increased pre- and post-implantation loss and resorptions, decreased numbers of live fetuses and malformations (e.g., sternal cleft) and retarded skeletal development. In rabbits, embryo-fetal toxicity was evidenced by increased resorption at 0.8 mg/kg (9.6 mg/m<sup>2</sup>,  $\sim$  1.6 times the recommended human dose on body surface area basis). In a prenatal and postnatal development study in rats, oral treatment of everolimus in pregnant females (F<sub>0</sub>) from gestation day 6 to lactation day 21 did not induce maternal toxicities or adverse effects on delivery and lactation parameters. The F<sub>1</sub> pups of dams treated at 0.1 mg/kg (0.6 mg/m<sup>2</sup>, approximately 10% of the recommended human dose based on the body surface area) had reduced body weights (up to 9% reduction from the control) and showed a slight reduction in survival. There were no drug-related effects on the developmental parameters (morphological development, motor activity, learning, or fertility assessment) in the F<sub>1</sub> generation.

#### B. Pharmacologic activity

##### Primary pharmacology:

Everolimus (RAD001) is a derivative of rapamycin. Similar to rapamycin (sirolimus) and temsirolimus, the antiproliferative activity of everolimus results from the inhibition of mTOR (**m**ammalian **t**arget **o**f **r**apamycin) activity. mTOR a serine-threonine kinase, downstream of PI3K-AKT pathway, is implicated in protein synthesis and cell cycle control. Everolimus has been shown to be an inhibitor of tumor growth in xenograft models of various human cancer cell lines. In xenograft models tested, the down-regulation of p70 S6 kinase (S6K), a kinase downstream from mTOR and involved in protein translation, has been directly related to everolimus anti-tumor activity.

##### Secondary pharmacology:

The immunosuppressive effect of everolimus was demonstrated in an *in vitro* assay, where everolimus blocked lymphocyte proliferation in response to a mitogenic stimulus. In Europe, everolimus is used as an immunosuppressant in organ transplant patients to prevent transplant rejection. Additionally, in *in vitro* assays, everolimus exhibited a direct inhibitory effect on mouse and human osteoclast formation and activity, and to a lesser extent osteoblast differentiation.

#### C. Nonclinical safety issues relevant to clinical use

The toxicities in the target organs identified in the animals, e.g., metabolic-endocrine (e.g. hyperlipidemia), GI tract (stomatitis), lung (pneumonitis), kidney ( $\uparrow$  serum creatinine) and inflammation in skin and mucus (oral mucositis, mouth ulcers), were also reported in the patients.

All toxicities of everolimus reported in animals, including those seen in the eye, kidney, liver, pancreas, reproductive organs, and embryo/fetus should be considered as potential risks to humans.

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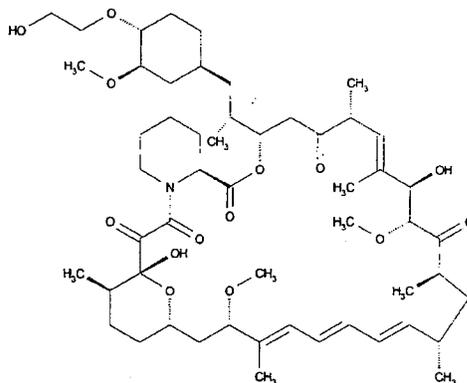
## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 22-334  
**Review number:** 1  
**Sequence number/date/type of submission:** 000/June 30, 2008/NDA  
**Information to sponsor:** Yes ( ) No (x)  
**Sponsor and/or agent:** Novartis Pharmaceuticals Corporation  
One Health Plaza  
East Hanover, NJ 07936-1080  
**Manufacturer for drug substance:** Novartis Pharma Stein AG Schaffhauserstrasse  
CH-4332 Stein Switzerland  
**Reviewer name:** Shwu-Luan Lee  
**Division name:** Division of Drug Oncology Products  
**Review completion date:** February 27, 2009

**Drug:**

Trade name:	AFINITOR®
Generic name:	Everolimus
Code name:	RAD001, SDZ RAD, SDZ 222-666
Chemical name:	40-O-(2-hydroxyethyl)-rapamycin (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- methoxy- cyclohexyl]-1-methylethyl}-19,30- dimethoxy- 15,17,21,23,29,35-hexamethyl-11,36- dioxo-4- aza-tricyclo[30.3.1.0"]hexatriaconta- 16,24,26,28- tetraene-2,3,10,14,20-pentaone
CAS registry number:	159351-69-6
Molecular formula:	C <sub>53</sub> H <sub>83</sub> NO <sub>14</sub>
Molecular weight:	958.22 gm/mole
Structure:	



Relevant INDs/NDAs/DMFs: IND 66279; NDAs (not approved) 21628, **b(4)**

**Pharmacologic class:** kinase inhibitor  
**Mechanism of action:** mTOR inhibitor

**Intended clinical population:** for the treatment of advanced renal cell carcinoma

**Clinical formulation:** tablets: 5 and 10 mg everolimus, with

Excipients:

butylated hydroxytoluene (Ph.Eur., USP/NF)  
 magnesium stearate (Ph.Eur., USP/NF)

croscopovidone (Ph.Eur., USP/NF)  
 lactose (Ph.Eur., USP/NF)

**b(4)**

**Route of administration:** Oral

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

Study Number	Study Title
Pharmacology	
Primary pharmacodynamics	
<i>In vitro</i> studies	
	[Bjornsti M-A, Houghton P J (2004)] The TOR pathway: A target for cancer chemotherapy. Nature Reviews Cancer; 4:335-48.
	[Abraham RT and Gibbons JJ (2007)] The Mammalian Target of Rapamycin Signaling Pathway: Twists and Turns in the Road to Cancer Therapy. Clin Cancer Res 13; 3109-14.
RD-2004-00475	Synthesis and <i>in vitro</i> biological profile of the RAD metabolite NVP-ATG181-NX-1, a RAD choline-ester

Study Number	Study Title
RD-2001-01088	Enzymatic profile of RAD001: <i>In vitro</i> inhibition of protein kinases
	[Sarbasov DD, Ali SM, Kim D-H, et al, 2004] Rictor, a Novel Binding Partner of mTOR, Defines a Rapamycin-Insensitive and Raptor-Independent Pathway that Regulates the Cytoskeleton Current Biol; 14:1296 – 302.
	*[Kim D-H, et al, (2002)] mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110: 163-175.
	*[Kim D-H, et al, (2002)] mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Mol. Cell 11: 895-904.
	*[Hara K, et al, (2002)] Raptor, a binding partner of target of rapamycin (TOR), mediate TOR action. Cell 110: 177-189.
RD-2002-03223	<i>In vitro</i> antiproliferative activity of RAD001 against a broad panel of tumor cell lines
RD-2006-02213	The <i>in vitro</i> antiproliferative activity of RAD001 against a panel of breast, NSCLC and renal tumor lines
RD-2000-02544	Downregulation of m TOR targets in tumor cell lines <i>in vitro</i> (comparison with CCI-779)
RD-2000-02151	RAD001 inhibits the growth factor-stimulated activation of p70 S6 kinase
RD-2003-03495	Molecular analysis of a panel of tumor cell lines with known response to RAD001 <i>in vitro</i>
	[Gingras AC, Raught B, Sonenberg N (2001)] Regulation of translation initiation by FRAP/mTOR. Genes Dev; 15:807-26.
RD-2002-03252	Comparison of the antiproliferative activity of RAD001 with activation of the PTEN/P1-3 kinase/Akt/m TOR pathway in tumor cell lines
	[Majumder PK, Febbo PG, Bikoff R, et al (2004)] mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med; 10:594-601.
RD-2001-00852	RAD001: effects on endothelial and fibroblast cell proliferation
	[Shinohara ET, Cao C, Niermann K, et al, (2005)] Enhanced radiation damage of tumor vasculature by mTOR inhibitors. Oncogene; 24(35):5414-22
<i>In vivo</i> studies	
RD-2000-02541	Downregulation of m TOR targets in tumors and skin derived from KB-31 human epidermoid carcinoma xenograft studies
RD-2001-00450	Prolonged inactivation of in tumors and skin derived from CA20948 pancreatic tumor-bearing rats
RD-2000-02545	Inhibition of the mTOR target in rat peripheral lymphocytes
RD-2002-03817	Prolonged inactivation of p70S6K in peripheral blood mononucleocytes derived from CA20948 pancreatic tumor-bearing rats and non-tumor-bearing rats
	[Mabuchi S Altomare DA, Connolly DC, et al, (2007a)] RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. Cancer Res; 67(6) 2408-13.
RD-2002-03707	Effect of the rapamycin derivative, RAD001, in the syngeneic CA20948 rat pancreatic tumor model
RD-2000-02547	Studies on the tolerability of athymic BALB/c <i>nu/nu</i> (nude) mice to RAD001
RD-2000-02548	Evaluation of the antitumor activity of RAD001 in experimental xenograft tumor models of pancreatic cancer
RD-2000-02549	RAD001 is an effective antitumor agent in experimental KB-31 xenograft tumor models of epidermoid cancer
	[Pende M, Kozma SC, Jaquet M, et al (2000)] Hypoinsulinaemia, glucose intolerance and outcome among endocrine treated patients. Br J Cancer; 86:540-5.
RD-2004-01547	<sup>18</sup> Fluoro-deoxy-glucose ( <sup>18</sup> FDG) tumour metabolism detected by positron emission tomography (PET) imaging in mice: RAD001 decreases uptake of <sup>18</sup> FDG by B16/BL6 melanoma metastases and H596 human tumour xenografts
RD-2006-01383	RAD001 does not decrease uptake of <sup>18</sup> FDG by human tumour xenografts with low sensitivity <i>in vitro</i> to RAD001

Study Number	Study Title
RD-2001-00853	RAD001: effects on angiogenesis-induced by growth factor- impregnated, subcutaneous implants in mice
RD-2001-00854	RAD001: effects in an orthotopic B16/BL6 melanoma model in C57BL/6 mice
	[Mabuchi S, Altomare DA, Connolly DC, et al, (2007a)] RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. <i>Cancer Res</i> ; 67(6) 2408-13.
	[Manegold PC, Paringer C, Kulka U et al (2008)] Antiangiogenic therapy with mammalian target of rapamycin inhibitor RAD001 (everolimus) increases radiosensitivity in solid cancer. <i>Clin Cancer Res</i> ; 14(3):892-900.
Secondary pharmacodynamics:	
Bone remodeling	
RD-2002-03782	The effect of RAD001 on mouse and human osteoclast formation and activity
Immunosuppression (kidney transplant)	
	[Saunders RN, Metcalfe MS, Nicholson ML (2001)] Rapamycin in transplantation: A review of the evidence. <i>Kidney Int</i> ; 59:3-16.
	[Schuler W, Sedrani R, Cottens S, et al (1997)] SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. <i>Transplantation</i> ; 64:36-42.
	[Kovarik JM, Kaplan B, Tedesco Silva H, et al (2002)] Exposure-response relationships for everolimus in de novo kidney transplantation: defining a therapeutic range. <i>Transplantation</i> ; 73:920-5.
Safety Pharmacology	
Neurological, gastrointestinal and renal effects	
RAD 02-c	General pharmacology of SDZ RAD
PKF-93-02177	Primary observation test of RAD001
Cardiovascular effects	
0120037-DITU 1014	Effect of FTY720 and RAD N BHT on HERG Currents Recorded from Stably Transfected HEK293 Cells
0770800	Electrophysiological Safety Measurements of hERG Currents in Stably Transfected HEK293 Cells
982042	Effect of RAD N BHT on action potential parameters in sheep isolated cardiac Purkinje fibres.
RD-2000-01460	Cardiovascular effects of RAD in anesthetized pigs
Pulmonary effects	
RD-2000-01492	Effect of RAD on lung function in the guinea-pig
Pharmacokinetics/ ADME	
DMPK(CH) R98-707	Pharmacokinetics and excretion after single intravenous and peroral administration (0.9 mg/kg) of <sup>3</sup> H-labeled RAD001 to mice
DMPK(CH) R00-874	Absorption, disposition and excretion in cynomolgus monkeys after single intravenous (1 mg/kg) and oral (5 mg/kg) administration of [ <sup>3</sup> H]SDZ RAD
DMPK(CH) 1997/287	Quantitative determination of rapamycin and SDZ RAD in blood samples after single and multiple administration in human and monkey
DMPK(CH) R00-1253	<i>In vitro</i> blood distribution, plasma protein binding and stability of RAD001 in mouse plasma
DMPK(CH) R00-1253-01	RAD001: Stability in mouse, monkey and human plasma. Addendum 1 to report: <i>In vitro</i> blood distribution, plasma protein binding and stability of RAD001 in mouse plasma.
DMPK(CH) R00-1253-02	Addendum 2 to report: <i>In vitro</i> blood distribution, plasma protein binding of RAD001 in rat plasma.
DMPK(CH) 1997/515	Distribution and excretion of total radioactivity in rats after peroral administration of 1.5 mg/kg [ <sup>14</sup> C]-labeled SDZ RAD

Study Number	Study Title
DMPK(CH) R98-194	Whole-body autoradioluminography in albino and pigmented rats after po and iv doses of [ <sup>3</sup> H]RAD001
DMPK(CH) R98-732	Embryofetal transfer in pregnant rats on Day 13 and Day 17 of gestation after po administration of [ <sup>3</sup> H]RAD001
DMPK(CH) R98-708	Galactogenic transfer, kinetics, and metabolism in milk and blood after single peroral administration (0.9 mg/kg) of <sup>3</sup> H-labeled RAD001 to lactating rats
DMPK(CH) R00-2214	Dose-dependent brain penetration in rat
DMPK(CH) R98-706	Disposition in rats after single and repeated once daily peroral administration (0.5 mg/kg/day) of <sup>3</sup> H-labeled RAD001 for 21 consecutive days
DMPK(CH) R00-1806	Biotransformation in mice following a single oral and intravenous dose (0.9 mg/kg) of [ <sup>3</sup> H]RAD001
303-013	Absorption, distribution, metabolism, and excretion in rats after single intravenous (1 mg/kg, 10 mg/kg) and oral (1.5 mg/kg, 15 mg/kg) administration of [ <sup>3</sup> H]SDZ RAD 666
DMPK(CH) R98-1404	Biotransformation in cynomolgus monkey following a single oral dose of [ <sup>3</sup> H]RAD001
DMPK(CH) R99-2448	Inhibition of RAD001 <i>in vitro</i> metabolism by ketoconazole, itraconazole, and fluconazole
Toxicology	
Single dose toxicology	
393131	Acute oral toxicity study – mice
393118	Acute oral toxicity study – rats
Repeat dose toxicology	
95/SPM052/0888	SDZ RAD: Toxicity study by oral gavage administration to Hanlbm Wistar rats for 4 weeks followed by a 2 week reversibility period
96/SPM090/0404	SDZ RAD: A repeat toxicity study by oral gavage administration to Hanlbm Wistar rats for 4 weeks followed by a 2 week reversibility period
96/SPM083/1130 and 0770978 †	SDZ RAD: Toxicity study by oral gavage administration Hanlbm Wistar rats for 26 weeks followed by a four-week reversibility period (†: histopathological examination of slides of archived kidney samples from 96/SPM083/1130)
971033	SDZ RAD: 4-week oral (gavage) toxicity study in minipigs
95/SPM049/1008	SDZ RAD: Toxicity study by oral gavage administration to cynomolgus monkeys for 4 weeks followed by a 2-week reversibility period
96/SPM078/1067	SDZ RAD: Toxicity study by oral (gavage) administration to cynomolgus monkeys for 26 weeks
1534-1463-045	SDZ RAD: 52-week oral (gavage) toxicity study in the cynomolgus monkey
Special repeat dose studies	
617951	SDZ RAD: A comparative 2-week oral (gavage) toxicity study in the rat with a micro-emulsion and a solid dispersion
Impurity investigation	
96/SPM091/0532	Comparative toxicity study in Hanlbm Wistar rats with batches differing in by-product content
991094	4-week oral toxicity study in rats (batch comparison)
634676	A comparative 2-week oral (gavage) toxicity study in the rat with two different batches
Genotoxicity	
<i>In vitro</i> studies	
Mut.Bakt. 27/95	SDZ RAD 666: Mutagenicity test using <i>Salmonella typhimurium</i>
Z 59	SDZ RAD 666: Chromosome aberration test with V79 Chinese hamster cells
1463/4-1052	SDZ RAD: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells using the microtitre® fluctuation technique
Impurity investigation	
Mut.Bakt. 66/96	SDZ RAD (solid dispersion): Mutagenicity test using <i>Salmonella typhimurium</i>
Z63	SDZ RAD (solid dispersion): Chromosome aberration test with V79 Chinese hamster cells
001801	Mutagenicity test using <i>Salmonella typhimurium</i> (Batch control)

Study Number	Study Title
001831	Chromosome aberration test with V79 Chinese hamster cells
<i>In vivo</i> study	
MK 36	SDZ RAD 666 (SDZ 222-666): Mouse bone marrow micronucleus test by the oral route
Carcinogenicity	
SPM118/973229	SDZ RAD: Oncogenicity study by oral gavage administration to CD-1 mice for 104 weeks
SPM113/973228	SDZ RAD: Oncogenicity study by oral gavage administration to Hanlbm Wistar rats for 104 weeks
Reproductive and developmental toxicity	
Fertility and early embryonic development	
7073R	An oral 13-week investigative fertility study in male rats with 13 weeks recovery
Embryofetal development	
3074R	SDZ RAD (SDZ 222-666): An oral fertility and embryo-fetal development study in female rats
4070K	SDZ RAD (SDZ 222-666): An oral embryo-fetal development study in rabbits
Prenatal and postnatal development	
987105	RAD001: An oral pre- and postnatal development study in rats
Local tolerance	
246781	Primary skin irritation/corrosion study with RAD001 in the rabbit
246792	Assessment of contact hypersensitivity to RAD001 in the albino guinea pig
Other toxicity	
96/SPM098/0796	Comparative study of ophthalmic toxicity by oral gavage administration to CD rats and Hanlbm rats for 4 weeks
27EXR	A 2-week oral (gavage) mechanistic toxicity study in rats

\* Articles not submitted by the sponsor; however, found relevant by the reviewer, Dr. T. Palmby to the pharmacology discussion (Section 2.6.2). Similarly, the following articles were used in the pharmacology section: Cejka D, et. al. (2008) *Cancer Biol Ther*; 7: [Epub ahead of print]; Fujishita T, et. al. (2008) *PNAS*; 105: 13544-9; Johansson G, et. al. (2008) *Mol Cancer Ther*; 1237-45; Lu CH, et. al. (2007) *Clin Cancer Res*; 13: 5883-88; Rossi F, et. al. (2006) *PNAS*; 103: 12843-8.

Studies not reviewed within this submission:

✓

b(4)

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1   Page(s) Withheld

Trade Secret / Confidential (b4)

Draft Labeling (b4)

Draft Labeling (b5)

Deliberative Process (b5)

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## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

An ether derivative of rapamycin, everolimus (RAD001) targets and inhibits mTOR (mammalian target of rapamycin) and thereby has the potential to inhibit cell proliferation, glycolysis and angiogenesis, the cellular processes regulated by this signaling pathway. Everolimus binds to FKBP-12, resulting in an inhibitory complex formation. The complex binds to mTOR and inhibits mTOR kinase activity. mTOR is a regulator of protein synthesis primarily through phosphorylation and activation of p70S6 kinase (S6K) and 4E-BP1.

Inhibition of S6K and 4E-BP1 activity by everolimus often occurred along with a dose-dependent growth inhibition in multiple human tumor cell lines *in vitro*. In addition, inhibition of tumor growth in multiple human tumor cell line xenograft models correlated with *in vivo* decreases in S6K activity and 4E-BP1 phosphorylation. Everolimus also inhibited growth of tumors in xenograft models from a panel of human tumor cells never passaged *in vitro*. Everolimus sensitivity to inhibition of tumor cell proliferation correlated with AKT phosphorylation status, a protein upstream of mTOR and subject to alterations in some cancers. Finally, as mTOR has been linked to glycolysis and angiogenesis, everolimus inhibited glucose uptake in tumor cells and angiogenesis in tumor models.

### 2.6.2.2 Primary pharmacodynamics

#### Mechanism of action:

Everolimus (RAD001) is a rapamycin derivative that targets the mTOR signal transduction pathway. mTOR is a serine-threonine kinase, which acts as a nutrient sensor and controls cellular metabolism by regulating protein synthesis, thereby stimulating cell growth and proliferation. Studies have shown that the mTOR pathway is deregulated in some tumors, making mTOR a candidate for inhibition in an attempt to control tumor-cell growth (Bjornsti M-A, Houghton PJ (2004) *Nature Reviews Cancer*; 4:335-48; Abraham RT and Gibbons JJ (2007) *Clin Cancer Res* 13; 3109-14).

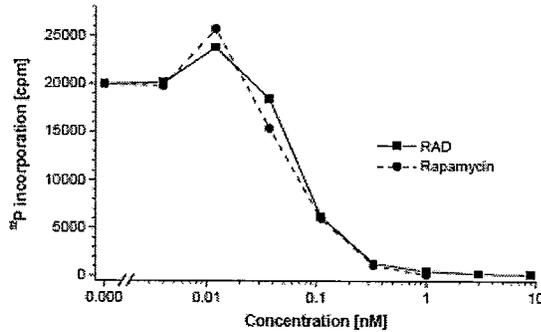
Rapamycin and everolimus bind to the intracellular protein, FKBP-12, with similar affinities ( $IC_{50} = 3.3$  nM and 5.3 nM, respectively) [Report RD-2004-00475]. *In vitro* kinase assays demonstrated the lack of activity of everolimus toward 10 different protein kinases at or below 10 uM concentrations [Report RD-2001-01088]. Rapamycin and FKBP-12 form a complex that binds to and inhibits mTOR-raptor Complex 1 (mTORC1) (Sarbasov DD, Ali SM, Kim D-H, et al, (2004) *Current Biol*; 14:1296-302). Two distinct mTOR complexes exist in mammalian cells involving the binding to different cellular components. The mTOR-raptor complex 1 (mTORC1) a rapamycin-sensitive complex is a nutrient-sensitive complex that signals to cell growth machinery (Kim, D.-H., et al, (2002) *Cell*; 110:163-75; Kim, D.-H. et al, (2003) *Mol. Cell*; 11:895-904; Hara, K., et al, (2002) *Cell*; 110:177-89). In addition, mTOR can form a distinct complex (mTORC2), which does not regulate the serine/threonine kinase p70S6 kinase or bind to FKBP12-rapamycin (Sarbasov DD, Ali SM, Kim D-H, et al, (2004) *Current Biol*; 14:1296-302).

mTOR regulates protein translation through the downstream phosphorylation and activation of the serine/threonine kinase p70S6 kinase (S6K). The main phosphorylation target of S6K is the ribosomal protein S6, which after stimulation forms complexes involved in ribosomal function. mTOR also phosphorylates 4E-BP1, which releases inhibition of eIF-4E and allows for cap-dependent protein translation. It is the stimulation of these two effector pathways downstream of mTOR that is thought to mediate the cell growth and proliferation response (Bjornsti M-A, Houghton PJ (2004) *Nature Reviews Cancer*; 4:335-48). A panel of human tumor cell lines were tested for their response to everolimus in *in vitro* cell proliferation assays [Report RD-2002-03223; Report RD-2006-02213]. Everolimus induced changes in S6K kinase activity and 4E-BP1/eIF-4EB association in both mTOR pathway sensitive or insensitive cell lines at concentrations that did not inhibit proliferation in the insensitive lines. Results of this study indicate that the inhibition of these two pathways may not predict cellular

response to everolimus [Report RD-2000-02544; Report RD-2000-02151; Report RD-2003-03495].

[Report RD-2000-02151]

#### Inhibition of p70 S6 kinase activity by everolimus



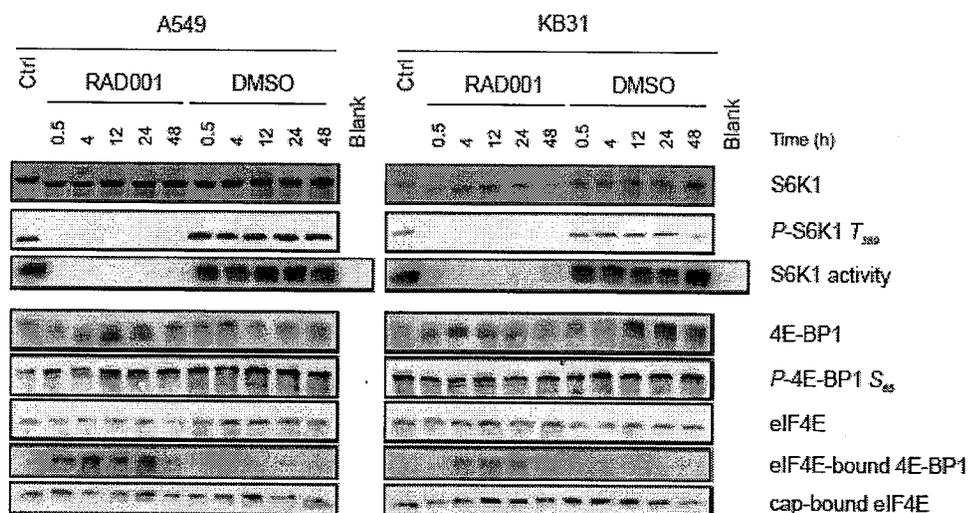
Increasing concentrations of RAD001 or rapamycin were added to 10ml aliquots of B13-29-15 cells ( $1 \times 10^5$  /ml) which were continuously grown in the presence of 02.ng/ml IL-6. After 1 hour cultivation at 37°C, cells were quickly chilled and harvested. p70 S6 kinase was precipitated from the cell lysate supernatants (containing 50µg total protein) using the p70 S6 kinase-specific polyclonal antibody M5. The S6 kinase activity was then determined with these immunoprecipitates using purified ribosomal 40S subunits as substrate. After the kinase reaction, the 40S ribosomal proteins were electrophoretically separated on an SDS polyacrylamide gel, the S6-containing band cut-out from the gel and incorporation of <sup>32</sup>P into the S6 band quantified by Cerenkov counting.

*(excerpted from sponsor's package)*

[Report RD-2003-03495]

#### Time-dependent effects of everolimus on p70S6K1 and 4E-BP1 pathways in A549 and KB31 cells

Appears This Way  
On Original



RAD001-sensitive A549 lung carcinoma and -relatively resistant KB-31 epidermoid cells were treated for the indicated times with 20 nM RAD001 or DMSO as a vehicle control. Thirty micrograms of tumor cell extracts were electrophoretically resolved by 10 to 15 % denaturing SDS-PAGE. After transfer to polyvinylidene difluoride membranes by semi-dry blotting, the protein levels or phosphorylation states (i.e. activation states) of the indicated proteins were revealed with the appropriate antibodies (see Methods section 2.3). Extracts were assayed for S6K1 activity using 40S ribosomal subunits as an *in vitro* substrate (autoradiographs of [ $\gamma$ -<sup>32</sup>P]phosphate incorporation into S6 protein are presented), and for 4E-BP1 binding activity to the eIF4E translation initiator factor on cap-coupled sepharose beads (see Methods section 2.4 and 2.5, respectively).

(*excerpted from sponsor's package*)

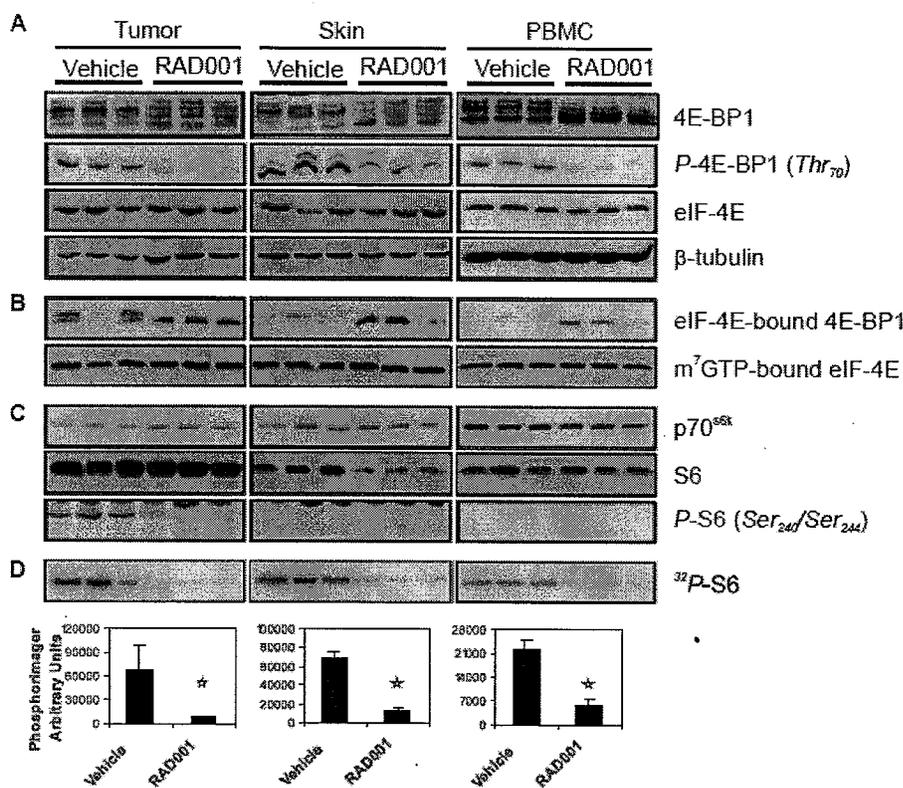
Multiple examples of S6K and 4E-BP inhibition have been shown for *in vivo* models of everolimus administration. Mice with epidermoid KB-31 tumors were treated with 7.5 mg/m<sup>2</sup>/day everolimus for 35 days. Tumor and skin tissues were harvested 1 hour after the last dose was administered. Tumors that were growth inhibited in response to everolimus often had an inactivation of S6K [Report RD-2000-02541]. Similarly, treatment of pancreatic CA20948 tumor-bearing rats with a single 30 mg/m<sup>2</sup> dose of everolimus resulted in inactivation of S6K in both tumors and skin sample that were harvested 12 hours after the last dose administration [Report RD-2001-00450]. The data in these studies indicate that everolimus treatment results in prolonged S6K inhibition in tumors and skin in tumor-bearing animals, providing support for a mechanistic explanation of anti-tumor potency of an intermittent dosing schedule. Peripheral blood mononucleocytes (PBMCs) isolated 2 hours and 12 hours after administration of a single 30 mg/m<sup>2</sup> dose in normal rats had an inhibition of S6K activity, which was sustained for up to 72 hours [Report RD-200-02545; Report RD-2002-03817]. Importantly, the tumor tissue from CA20948 tumor-bearing rats treated with a single 30 mg/m<sup>2</sup> dose harvested 24 hours later had a reduced S6 phosphorylation (substrate of S6K), which paralleled S6K activity [Report RD-2002-03817].

Previously, rapamycin was demonstrated to promote a decrease in phosphorylation of 4E-BP1, which led to an increase in 4E-BP1/eIF-4E complex formation, a transcriptionally repressive association (Gingras AC, Raught B, Sonenberg N (2001) Genes Dev; 15:807-26). Similar effects were seen with everolimus. Everolimus treatment of multiple cell lines resulted in 4E-BP1 dephosphorylation and increased association with eIF-4E *in vitro* [Report RD-2000-02544; Report RD-2003-03495]. Likewise, 4E-BP1 phosphorylation at threonine 70 and its association with eIF-4E was reduced 24 hours after pancreatic CA20948 tumor-bearing

rats were treated with one 30 mg/m<sup>2</sup> everolimus dose (assay was done in both skin and PBMCs) [Report RD-2002-03817].

[Report RD-2002-03817]

**Effects of a single everolimus administration (30 mg/m<sup>2</sup>) on S6 ribosomal protein phosphorylation, 4E-BP1 phosphorylation/mobility and 4E-BP1/eIF-4E association in rat PBMCs, skin and CA20948 tumors**



CA20948 tumor-bearing rats received a single administration of an efficacious dose of RAD001 (5 mg/kg) or Neoral vehicle, and were sacrificed 24 hours post-administration (3 rats per group). Tumors, skin and peripheral blood mononucleocytes (PBMC) were individually prepared and extracted. Results from individual rats are presented. Panels A and C: total protein (equalized for protein) was subjected to immunoblot analysis for 4E-BP1 protein, phospho-threonine 70 4E-BP1 [P-4E-BP1(Thr70)], eIF-4E protein, S6 40S ribosomal protein and phospho-serine 240/244 S6 [P-S6(Ser240/Ser244)] levels. Panel B: the level of 4E-BP1 bound to eIF-4E was measured by purification of 4E-BP1/eIF-4E complexes on 7-methyl guanosine triphosphate (m<sup>7</sup>GTP)-Sepharose, followed by western blot analysis.  $\beta$ -tubulin levels were analyzed as an internal loading control (Panel A). Panel D: p70<sup>S6k</sup> activity was measured using 40S ribosomal subunits as an *in vitro* substrate. An autoradiograph of [ $\gamma$ -<sup>32</sup>P]phosphate incorporation into S6 protein (<sup>32</sup>P-S6) is shown. Graphs represent phosphorimager quantification of the S6 kinase assays. Data are  $\pm$  SD of n = 3/group. Stars represent p < 0.05 versus untreated controls (Dunnett test).

(excerpted from sponsor's package)

**Drug activity related to proposed indication:**

The mTOR pathway has been described to be a potential therapeutic pathway for treatment of malignancies, including those that have become resistant to traditional or other targeted therapies (Bjornsti M-A, Houghton PJ (2004) Nature Reviews Cancer; 4:335-48; Abraham RT and Gibbons JJ (2007) Clin Cancer Res 13; 3109-14). The ability of everolimus to block mTOR signaling and, thereby, tumor growth, has been investigated in non-clinical studies

(Cejka D, et. al (2008) Cancer Biol Ther; 7: [Epub ahead of print]; Fujishita T, et. al (2008) PNAS; 105: 13544-9; Johansson G, et. al (2008) Mol Cancer Ther; 1237-45; Lu CH, et. al (2007) Clin Cancer Res; 13: 5883-88; Mabuchi S, et. al (2007) Cancer Res; 67: 2408-13; Rossi F, et. al (2006) PNAS; 103: 12843-8). The studies with everolimus showed that a range of *in vitro* growth inhibition was observed on a panel of human tumor cell lines.

**[Report RD-2002-03223][Report RD-2006-02213]**

**Anti-proliferative IC50s for everolimus on human cell lines**

Tumor origin	Cell line	IC50 (nM)	Tumor origin	Cell line	IC50 (nM)
Breast	BT549	0.7 ± 0.2 (3)	Lung	A549	2.4 (2)
	ZR-75-1	1.9 ± 1.1 (3)		NCI-H-460	44 ± 20 (3)
	MCF7	0.6 ± 0.1 (3)		NCI-H-1299	10.5 ± 3.1 (3)
	MDA-MB231	7.2 ± 4.1 (3)		NCI-H-596	5 ± 2 (4)
	HCC1395	1.2 ± 1.2 (3)		NCI-H-1650	>2500 (3)
	MDA-MB435	4.5 ± 3.2 (3)		NCI-H-1975	1.3 ± 0.5 (4)
	MDA-MB436	>2500 (3)		NCI-H-838	0.6 ± 0.3 (4)
	MDA-MB468	1.5 ± 0.7 (3)		NCI-H-1838	>2500 (3)
SKBR3	0.7 ± 0.3 (4)	NCI-H-2122	>2500 (3)		

Tumor origin	Cell line	IC50 (nM)	Tumor origin	Cell line	IC50 (nM)	
	BT474	0.6 ± 0.1 (4)		NCI-H-226	>2500 (3)	
Renal	786-O	0.4 (2)		NCI-H-522	>2500 (3)	
	SKRC-01	0.5 ± 0.1 (3)		NCI-H-322M	>2500 (3)	
	SKRC-52	0.4 ± 0.2 (3)		NCI-H-23	>2500 (3)	
	A-498	2.8 ± 0.7 (4)	Glioblastoma	BS125H.2	3 ± 0.6 (3)	
	769-P	0.2 ± 0.1 (4)		BS153	3.9 ± 1.2 (3)	
	G402	0.3 ± 0.1 (3)		LN229	21 ± 6 (3)	
	RCC4	1.4 ± 0.1 (3)		LN401	1.5 ± 0.3 (3)	
	RPTEC	0.3 ± 0.1 (3)		LN428	327 ± 206 (3)	
Caki-1	>2500 (3)	LN751		24 ± 14 (4)		
Prostate	DU-145	10.3 ± 2.2 (7)			U87	5.0 ± 0.6 (3)
	PC3M	149 ± 46 (6)		Colon	HCT15	65.2 ± 23 (3)
	LNcap	0.7 (2)	HCT116		4125 ± 1853 (3)	
Epidermoid	KB -31	1778 ± 800 (7)	Pituitary	GH3 (rat)	2.1 ± 1.8 (3)	
	KB-8511	1489 ± 806 (4)	Melanoma	B16 (murine)	0.7 ± 0.2 (3)	

Cell lines were incubated for 24 hr before RAD001 treatment and the cells re-incubated for 3 days. Methylene blue staining was performed on day 4 and the amount of bound dye (proportional to the number of surviving cells) determined. IC50s were determined using the SoftmaxPro program. Number of separate experiments performed in parentheses. Except for three cases, results are limited to where 3 or more experiments were performed [Report RD-2002-03223; Report RD-2006-02213].

(*excerpted from sponsor's package*)

*In vivo* studies showed that administration of 7.5 mg/m<sup>2</sup>/day everolimus for 35 days resulted in reduced tumor growth (T/C = 0.25) [Report RD-2002-03707], as did treatment of CA20948 tumor-bearing rats with intermittent doses of everolimus (30 mg/m<sup>2</sup> 1-2 times/week) [Report RD-2001-00450]. At doses 1/6 or below the established MTD [Report RD-2000-02547], everolimus inhibited tumor growth of experimental tumor xenografts previously characterized as both mTOR pathway sensitive or insensitive *in vitro* [Report RD-2000-02548][Report RD-2000-02549].

Anti-tumor activity of everolimus was tested on a series of 58 different tumor xenograft models established from human tumors that were only passaged *in vivo*. The tumor cells consisted of breast (5 lines), colorectal (9 lines), gastric (3 lines), lung (22 lines), melanoma (6 lines), ovarian (4 lines), pancreatic (3 lines) and renal (6 lines) cells. Overall, everolimus produced a mean T/C (change in tumor growth) of 0.27 and the growth inhibitory effect was quantitatively similar in all 58 models, when mice were administered daily oral dose of 30 mg/m<sup>2</sup> [Report RD-2007-01486]. A separate study focused on 6 renal cell carcinoma cell lines. Results show regressions in 50% of the everolimus treated mice with T/C values between 0.03 and 0.63 [Report RD-2008-00256]. In addition, well-vascularized CA20948 tumors in Lewis rats responded to everolimus in a dose-dependent manner, at doses up to 15 mg/m<sup>2</sup>/day [Report RD-2002-03707].

The PTEN/AKT pathway is deregulated in cancers, e.g. by mutation or by growth factor-induced activation. To test whether sensitivity to everolimus may be due to PTEN status or AKT activity, a study was performed comparing IC<sub>50</sub> values for inhibition of tumor cell proliferation, PTEN status and phosphorylation of AKT. Sensitivity to everolimus did not seem to be affected by PTEN status. However, increased phosphorylation of AKT (at serine 473) seemed to correlate significantly with increased anti-proliferative activity of everolimus [Report RD-2002-03252].

The regulation of the mTOR pathway, and more specifically S6K1, has been linked to glucose homeostasis (Pende M, et. al (2000) Nature; 408: 994-7). Everolimus has been shown to block expression of several HIF-1 $\alpha$  target genes, including enzymes involved in the glycolytic pathway and Glut-1, a major glucose transporter (Majumder PK, et. al (2004) Nat Med; 10: 594-601). <sup>18</sup>F-deoxyglucose-PET imaging was performed on mice bearing B16/BL6 tumors after treatment with 30 mg/m<sup>2</sup>/day everolimus. Everolimus was found to cause a 40 % decrease in FDG uptake compared to control treated animals [Report RD-2004-01547]. In a similar study done with tumor cells, HCT-116 and KB-31, that were insensitive to growth inhibition by everolimus *in vitro*, there was no decrease in FDG uptake by the tumor cells in everolimus treated animals [Report RD-2006-01383].

Angiogenesis is thought to be another mode of tumor inhibition for rapamycin and other drugs inhibiting mTOR. Everolimus was shown to inhibit HUVEC proliferation stimulated by VEGF (IC<sub>50</sub> = 120  $\pm$  22 pM) and FGF (IC<sub>50</sub> = 842  $\pm$  396 pM) [Report RD-2001-00852]. *In vivo* anti-angiogenic activity of everolimus included inhibition of VEGF dependent angiogenic response in a growth factor-impregnated, sub-cutaneous implant mouse model at a dose which inhibited tumor growth in xenograft models [Report RD-200100853]. In a similar orthotopic model for murine melanoma where B16/BL6 cells were injected intra-dermally in the ears of mice, everolimus dose-dependently inhibited primary tumor growth and metastases to cranial lymph node at 3 to 15 mg/m<sup>2</sup>/day. Blood vessel density in the primary and metastatic tumors was also reduced by oral everolimus administration at 15 mg/m<sup>2</sup>/day [Report RD-2001-00854]. In a separate study, everolimus decreased VEGF production by KB-31 cells *in vitro*. Additionally, everolimus decreased VEGF production in tumor xenografts in mice concurrent with a decrease in tumor growth. A significant reduction in blood vessel density was seen with a decrease in tumor growth in a similar study with HCT-116 xenografts treated for 18 days with 30 mg/m<sup>2</sup>/day everolimus. Both of these cell lines were growth insensitive to everolimus *in vitro* [Report RD-2006-01383]. Several published reports have documented anti-angiogenic effects of everolimus *in vitro* and in decreasing blood vessel density in animal tumor models (Mabuchi S, et. al (2007) Cancer Res; 67(6): 2408-13;

Mabuchi S, et. al (2007) Clin Cancer Res; 13(14): 4261-70; Shinohara ET, et. al (2005) Oncogene; 24(35): 5414-22; Manegold PC, et. al (2008) Clin Cancer Res; 14(3): 892-900).

### 2.6.2.3 Secondary pharmacodynamics

Everolimus is an immunosuppressive agent and is approved in Europe under the name Certican® for prevention of rejection, in heart and kidney organ transplant recipients. When everolimus was used in preventing transplant rejection, maximum effectiveness was achieved with 5-10 ng/mL whole blood trough levels in small animals (Saunders RN, et. al (2001) Kidney Int; 59: 3-16). In rodent models of autoimmune diseases and allotransplantation, everolimus was effective at oral doses of 1-5 mg/kg/day (Schuler W, et. al (1997) Transplantation; 64: 36-42). It has been proposed that daily dosing in patients is necessary to reduce risk of rejection by keeping blood levels over 3 ng/mL, but manage adverse events by keeping it under 15 ng/mL (Kovarik JM, et. al (2002) Transplantation; 73: 920-5). Conversely, weekly administration of everolimus has been shown to have similar effects as daily administration.

Another secondary pharmacological effect of everolimus is on bone remodeling. Everolimus was tested in assays for mouse and human osteoclast formation and activity and for differentiation of mouse osteoblasts. Everolimus inhibited osteoclast activity ( $IC_{50} = 0.6$  nM), and to a lesser extent, osteoclast and osteoblast formation ( $IC_{50} = 10.5$  nM and 13.5 nM, respectively). Everolimus also inhibited human osteoclast activity in resorbed area ( $IC_{50} = 3.4$  nM) and collagen fragment release ( $IC_{50} = 4.0$  nM), and human osteoclast formation ( $IC_{50} = 7.7$  nM). Together, this data suggests everolimus can inhibit mouse and human osteoclast formation and activity, and to a lesser extent osteoblast differentiation [Report RD-2002-03782].

### 2.6.2.4 Safety pharmacology

Reviewer's note:

- This section was reviewed by Dr. S. Kunder for NDA            (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is re-formatted to fit the current NDA
- Everolimus was referred to as RAD (for SDZ RAD, RAD001).

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#### Neurological effects:

The Irwin primary observation test (for effects on motor activity, locomotion, behavioral stimulation, behavioral depression, muscle tone, neurologic activity, autonomic activity, pupilar diameter, rectal temperature and lethargy) was performed in rats with oral doses of 2, 20 and 50 mg/kg [Study #PKF-93-02177]. Motor activity was increased at all doses within 5 min of dosing. Rats receiving 20 mg/kg showed an increase in flight reaction. Pupil diameter was increased at 50 mg/kg for at least 23 h after dosing. Food consumption in the 50 mg/kg group also decreased. RAD appears to exert slight CNS effects at doses of  $\geq 20$  mg/kg.

#### Cardiovascular effects:

In an i.v. study in anesthetized pigs, doses of 0.01, 0.1, 1.0 and 10 mg/kg were used to determine the effects of RAD on blood pressure, heart rate, blood flow, respiratory rate and electrocardiogram. No cardiovascular effects were seen at doses  $\geq 10$  mg/kg i.v. in pigs [RD-2000-01460]. In the *in vitro* assay on hERG currents recorded from stably transfected HEK293 cells, RAD did not inhibit HERG currents [Study #0120037-DITU 1014 and #0770800].

Everolimus (0.1-10 µg/mL) was incubated with isolated sheep Purkinje fibers, and no changes of action potential duration (ADP) or other parameters to indicate effects on ECG parameters or the potential of QT interval prolongation [Study #982042].

Pulmonary effects:

Pulmonary effects of RAD were examined in anesthetized, ventilated guinea pig model at doses of 0.3, 3 and 30 mg/kg i.v. to determine effects on airway resistance and dynamic compliance. No effects were seen at these doses. There was a reduction in airway reactivity to histamine at 3 and 30 mg/kg, indicating some antihistaminic activity [RD-2000-01492].

Renal effects:

In saline-treated mice, at RAD doses of 15 and 50 mg/kg p.o., slight increases in total excretion of urinary potassium and chloride were seen [Study #RAD 02-c].

Gastrointestinal effects:

The effect of RAD on intestinal transit time with charcoal was studied in mice at doses up to 50 mg/kg, p.o. No effect on gastrointestinal transit time was seen [Study #RAD 02-c].

Abuse liability: None

Other: None

#### 2.6.2.5 Pharmacodynamic drug interactions

been reviewed.

Therefore these studies have not

#### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

See tables provided in Section 2.6.2.

#### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

*Reviewer's note:*

- This section was reviewed by Dr. S. Kunder for NDA [redacted] (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is modified as well as re-formatted to fit the current NDA template.
- Everolimus was referred to as RAD (for SDZ RAD, RAD001).

##### 2.6.4.1 Brief summary

Preclinical pharmacokinetics were studied in mice, rats and cynomolgus monkeys following oral and intravenous administration. Oral absorption of single dose radiolabeled RAD was determined in the mouse (12%), rat (39-43%), monkey (18%), and humans (11%). Oral bioavailability was determined in the mouse (5%), rat (14-26%), and monkey (6%). Plasma protein binding was similar among the monkey (16% free), the rat (7.6% free), and humans (25% free), while in the mouse extensive protein binding occurred (0.1% free). Tissue distribution was determined by radiolabel studies in the rat in which tissue levels following i.v. dosing were

highest in the liver and kidney after 5 minutes. Half-lives of label in most tissues were between 1.4 and 1.9 days, except in brain (10 days), testes (13 days) and epididymides (5 days). Following oral dosing, the greatest amounts of label were found in the heart, liver, lung, kidney, spleen, thyroid and adrenal glands after 2 hours. RAD was extensively metabolized. Parent drug is the predominant form found in blood, with parent compound averaging in mice, rats and humans between 31 and 63% of total radioactivity, and 12% in monkeys. Five major metabolites are found *in vivo* in human, monkey, rat and mouse. These metabolites are typically the result of conjugation with fatty acids and hydrolytic and hydroxylated products. The related compound rapamycin may arise as a metabolite but was found in small amounts (~5%) of total AUC in clinical pharmacokinetic studies. Hydrolytic and hydroxylated metabolites were studied *in vitro* for immunosuppressive activity and were approximately 60- to 500 fold less active than the parent, RAD. The majority of radioactivity was eliminated in the feces in mouse (95-99%), rat (68-89%), monkey (66-75%), and humans (79%). Urinary excretion was consistently low (<7%). Bile duct cannulated rats demonstrated approximately 71% biliary excretion. Everolimus and the metabolites can penetrate blood brain barrier and placenta, and transfer into milk in rats. The major enzyme involved in metabolism of RAD is CYP3A4. CYP3A4 inhibitors ketoconazole and itraconazole were shown to inhibit biotransformation of RAD while fluconazole, a weak CYP3A4 inhibitor, did not.

#### 2.6.4.2 Methods of Analysis

[See under individual study reviews]

#### 2.6.4.3 Absorption

**Study title:** Pharmacokinetics and excretion after single intravenous and peroral administration (0.9 mg/kg) of <sup>3</sup>H-labeled RAD001 to mice

**Study no.** DMPK(CH) R98-707

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** Nov, 1998-Jan 1999

**GLP (no)**

**Dose & formulation:** [<sup>3</sup>H]- RAD001, specific activity = 49.8 MBq/mg

**Animals:** male CD-1 mice

**Protocol:** Mice were administered <sup>3</sup>H-labeled RAD001 by intravenous route (0.9 mg/kg) and oral gavage (0.9 mg/kg). Blood was collected at 0.083, 0.5, 1, 3, 8, 24 and 72 h post dosing. Urine and feces were collected daily up to 72 h post dosing. Blood, fecal and urine levels were analyzed by liquid scintillation counting.

#### Results:

The pharmacokinetic values for this study:

Dose (mg/kg)	route	<sup>3</sup> H t <sub>max</sub> (h)	RAD t <sub>max</sub> (h)	<sup>3</sup> H C <sub>max</sub> (ng-eq/mL)	<sup>3</sup> H AUC (ng-eq•h/mL)	RAD C <sub>max</sub> (ng/mL)	RAD AUC (ng•h/mL)	Dose absorbed (%)	RAD Bioavailability (%)
0.9	i.v.	1		3814	36701		18720		
0.9	p.o.	0.5	1	489	4207	108	1013	12	5

**RAD: SDZ RAD, everolimus**

- The oral bioavailability of everolimus was ~5% after oral dosing in mice.
- Approximately 12% of the dose administered was absorbed.

*The reviewer's note:* The result pertinent to excretion is reported in Section 2.6.4.6. "Excretion". A related study #DMPK(CH) R00-1806 was reviewed in Section 2.6.4.5. "Metabolism".

**Study title:** Pharmacokinetics in mice after intravenous bolus administration (0.9 mg/kg) with RAD001

**Study no.** DMPK(CH) R00-874

**Study facility:** Novartis Pharma AG  
Preclinical Safety/Drug Metabolism  
ADE Section  
4002 Basel Switzerland

**Date of study:** 25-28 Jan 2000

**GLP (no)**

**Dose & formulation:** placebo for Sandimmune/saline

**Animals:** mice, CD-1, males

**Protocol:** Mice were administered a single i.v. injection (0.9 mg/kg) of RAD 001. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48 and 72 h after dosing. Blood samples were analyzed by LC-APCI-MS.

Results:

- AUC(everolimus) = 18.7  $\mu\text{g}\cdot\text{h}/\text{mL}$ ; radioactivity:  $C_{\text{max}}$ : 3814 ng-eq/mL,  $\text{AUC}_{0.083-72\text{h}}$ : 36701 ng-eq $\cdot\text{h}/\text{mL}$ ;  $t_{1/2}$  = 9.83 h; clearance = 0.79 mL/min/kg,  $V_{\text{ss}}$  = 0.37 L/kg
- Blood levels of RAD in this i.v. study were maintained near injection levels for 8 h, then declined steadily from 8 to 72 h.

**Study title:** Quantitative determination of rapamycin and SDZ RAD in blood samples after single and multiple administration in human and monkey

**Study no.** DMPK(CH) 1997/287

**Study facility:** Novartis Pharma AG  
Preclinical Safety/Drug Metabolism  
ADE Section  
4002 Basel Switzerland

**Date of study:** Aug 4-5 1997

**GLP (no)**

**Dose & procedure:** samples used in this study were obtained from patients receiving a single dose of RAD (25 mg), multiple doses of 0.75 mg in stable renal transplant patients and cynomolgus monkeys receiving 4-week multiple dose 0.5 mg/kg/day treatment. Existing blood samples (patient and monkey) had rapamycin and RAD concentrations determined by HPLC

Results: Human blood samples: 25 mg single dose, rapamycin/RAD = 3.0-5.4%

0.75 mg multi-dose, rapamycin/RAD=3.8-5.2%

Cynomolgus monkey: 0.5 mg/kg/day, 4-weeks, rapamycin/RAD=7.8-10.7%

Conclusions: Rapamycin was found in blood samples of patients and cynomolgus monkeys receiving RAD in amounts ranging from 3.0-5.2% in patients to 7.8-10.7% in monkeys.

#### 2.6.4.4 Distribution

**Study title:** *In vitro* distribution, plasma protein binding and stability of RAD001 in mouse plasma.

**Study no.** DMPK (CH) R00-1253

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** 15-26 May 2000

**GLP (no)**

**Drug:** <sup>3</sup>H-RAD001 batch 98902, 25.3 MBq/mL

**Animals/samples:** pooled blood from CD-1 mice

**Protocol:** Plasma protein binding was determined using mouse blood erythrocytes by the erythrocyte partitioning method. Plasma dilutions from 0.1 to 60 % were used with <sup>3</sup>H-RAD001/RAD001 at a concentration range of 5-5000 ng/mL.

#### Results:

- The majority of <sup>3</sup>H-RAD001 was located in the plasma fraction (= 98±4%) from 5 to 5000 ng/mL.
- Bound fraction in plasma= 99.9% at 10 ng/mL

Conclusions: RAD001 was highly bound to mouse plasma proteins (99.9%). This is greater than the plasma binding seen in other species ranging from 75 (rats and humans) to 84% (monkeys).

**Study title:** Stability in mouse, monkey and human plasma. Addendum to the study: *In vitro* distribution, plasma protein binding and stability of RAD001 in mouse plasma (above)

**Study no.** DMPK(CH) R00-1253-01

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** May to Sept 2000

**GLP (no)**

**Drug & formulation:** <sup>3</sup>H-RAD001 batch RA 910-7, 100 ng/mL added to plasma

**Species/samples:** pooled plasma from mouse (CD-1), cynomolgus monkey, healthy human volunteers

**Protocol:** <sup>3</sup>H-RAD001 was added to mouse, cynomolgus monkey and human plasma to determine the stability of RAD001. Incubation was at 37 °C. RAD001 levels in plasma were

determined by HPLC from samples collected after 0.5, 1, 2, 6 and 24 h following addition to plasma. Structures of degradation products were determined.

**Results:** Plasma stability: The stability half-life of RAD was:  
19.7 h in mouse plasma  
1.9 h in cynomolgus monkey plasma  
4.0 h in human plasma

Degradants of RAD001 in this study consisted mainly of the \_\_\_\_\_

**Conclusions:** RAD001 has a short half-life in monkey and human plasma at 37 °C. RAD001 in mouse plasma has a longer half-life, almost 20 h. \_\_\_\_\_ were the primary degradation products in this assay.

**Study title:** *In vitro* blood distribution and plasma protein binding of RAD001 in rat plasma.  
Addendum to the study: *In vitro* distribution, plasma protein binding and stability of RAD001 in mouse plasma (above)

**Study no.** DMPK(CH) R00-1253-02

**Study facility:** Novartis Pharma AG  
Preclinical Safety/Drug Metabolism  
ADE Section  
4002 Basel Switzerland

**Date of study:** 15-26 May 2000

**GLP (no)**

**Dose & formulation:** <sup>3</sup>H-RAD001 batch RA 919-7, specific activity 87.7 MBq/mg, RAD, batch 98902, total concentrations 5, 50, 100, 500, 1000 and 5000 ng/mL

**Animals/samples:** pooled blood and plasma from rats, HAN/WIST

**Protocol:** Erythrocytes were suspended in plasma solutions, incubated with RAD001 and the resulting supernatant collected. RAD001 concentration was determined by scintillation counting. Protein binding of RAD001 added to rat plasma was determined by the erythrocyte partitioning method. A shortened (5 min) RAD001 incubation time (compared with previous assays) was used due to the findings of the stability study (above).

**Results:** The blood distribution of RAD001, or fraction in plasma, was concentration dependent, ranging from 33.6% (5 ng/mL) to 85.5% (5000 ng/mL). The bound fraction in plasma was 92.4%.

**Conclusions:** RAD bound to plasma proteins in a concentration-dependent manner as seen with rat, monkey and human plasma. The bound fraction in rat plasma was 92.4%

**Study title:** Distribution and excretion of total radioactivity in rats after per oral administration of 1.5 mg/kg <sup>14</sup>C-labelled SDZ RAD

**Study no.** DMPK(CH) 1997/515

**Study facility:** Novartis Pharma AG  
 Preclinical Safety/Drug Metabolism  
 ADE Section  
 4002 Basel Switzerland

**Date of study:** 10 May 1998

**GLP (no)**

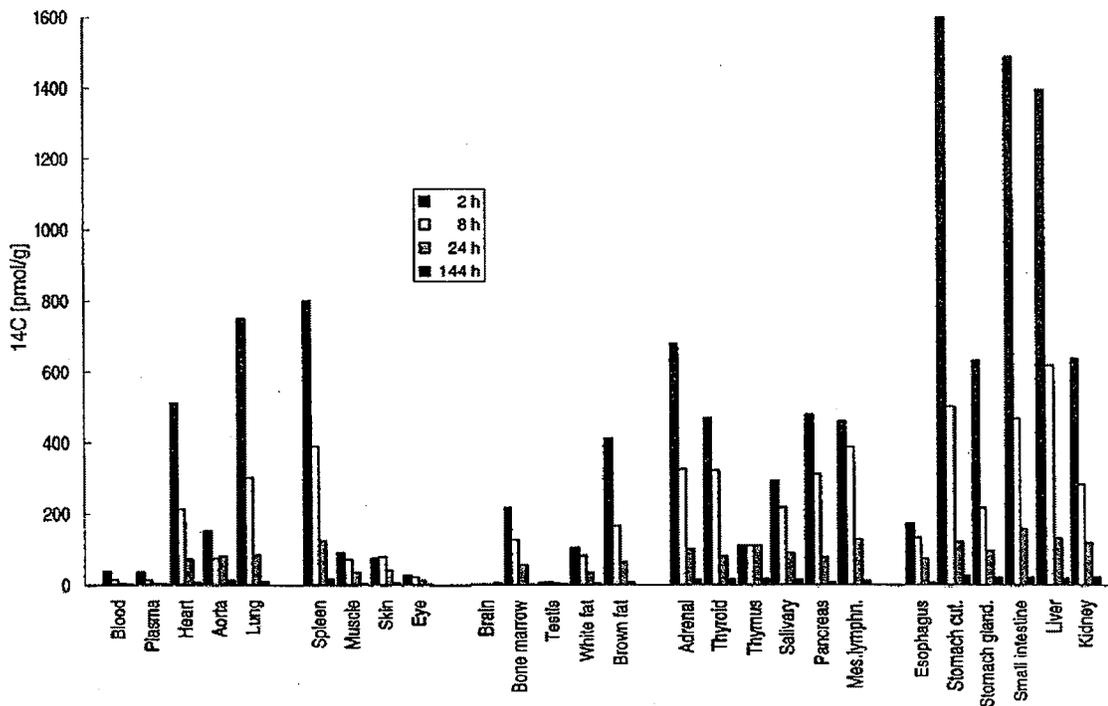
**Dose & formulation:** 1.5 mg/kg <sup>14</sup>C-labelled SDZ RAD, batch RSE 009-1, specific activity 115 µCi/mg (4.27 MBq/mg)

**Animals:** rats, male HAN Wistar

**Protocol:** Rats were orally administered 1.5 mg/kg <sup>14</sup>C-labelled SDZ RAD. Rats were sacrificed at 2, 8, 24 and 144 h after dosing (n=3 rats/time point). Tissue specimens were processed and analyzed by scintillation counting (expressed as <sup>14</sup>C-concentration (pmol/g)). Other rats were placed in cages to collect urine and feces for determination of <sup>14</sup>C-radioactive substance elimination.

Results:

The tissue distribution of radioactive substance(s) at various time points is summarized in the figure below (from the sponsor)



The radiolabeled substance was extensively distributed in the organs of treated rats after 2 h, mostly at concentrations greater than that of blood. Highest concentrations were in the gastrointestinal tract and liver. At 8 and 24 h, tissue concentrations were greatly reduced but similar in distribution pattern. The greatest tissue concentrations were in liver, mesenteric lymph node, thymus, spleen and gastrointestinal tract. Radioactivity in the testis and brain remained at low concentrations. At 144 h, overall tissue concentrations decreased, with greatest concentrations located in kidney, liver, and gastrointestinal tract.

The excretion of radioactive substance(s) in urine and feces following a single oral dose of 1.5 mg/kg [<sup>14</sup>C]RAD (table from the sponsor):

Excretion in % of administered radioactivity									
Time interval [ h ]	Urine				Faeces				Urine and faeces
	RA13	RA14	RA15	Mean	RA13	RA2	RA3	Mean	Mean
0- 24	1.13	1.29	1.12	1.18	90.26	89.96	86.90	89.04	90.22
24- 48	0.12	0.14	0.21	0.16	2.30	3.31	3.56	3.06	3.22
48- 72	0.03	0.05	0.07	0.05	0.73	0.81	1.01	0.85	0.90
72- 96	0.02	0.02	0.03	0.02	0.41	0.44	0.50	0.45	0.47
96-120	0.01	0.01	0.02	0.01	0.23	0.25	0.27	0.25	0.26
120-144	0.01	0.01	0.01	0.01	0.16	0.15	0.16	0.16	0.17
144-168	0.00	0.01	0.01	0.01	0.10	0.11	0.13	0.11	0.12
0-168	1.33	1.52	1.47	1.44	94.20	95.04	92.54	93.92	95.36

A total of 0.5% of the original dose was estimated to remain in the carcass at 144 h. Excretion studies showed 89% of the dose eliminated in feces in 24 h. After 7 days, 94% elimination via feces had occurred, with only 1.4% via urine.

**Conclusions:** Extensive distribution of radioactivity after administration of [<sup>14</sup>C]RAD was demonstrated in the rat, with highest concentrations in liver, gastrointestinal tract and tissues of the immune system. Rapid elimination was seen predominantly by the fecal route.

**Study title:** Whole-body autoradioluminography in albino and pigmented rats after p.o. and i.v. doses of [<sup>3</sup>H] RAD001

**Study no.** DMPK(CH) R98-194

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** 19 Nov 1998

**GLP (no)**

**Dose & formulation:** 1.5 mg/kg p.o., 1 mg/kg i.v., <sup>3</sup>H-RAD001 batch RA 919-6, specific activity 36.1 MBq/mg in 1:20 RAD placebo/0.9% saline

**Animals:** rats, male HAN:WIST (albino) and Long Evans (pigmented)

**Protocol:** Intravenously dosed rats were sacrificed at 5 min, and 168 h after injection and orally dosed rats were sacrificed at 2 and 168 h following dosing and immediately frozen. Sagittal sections of the carcasses were exposed on tritium sensitive imaging plates for image development, then digitally scanned and processed and quantified.

**Results:** In both albino and pigmented rats tissue distribution was similar; highest concentrations were found in the gastrointestinal tract, liver and kidney. The melanin containing tissues of the pigmented rats did not retain radioactivity with greater affinity than non-melanin containing tissues of the albino rats.

**Conclusions:** After oral and intravenous dosing to albino and pigmented rats, radiolabeled RAD and/or its metabolites were distributed similarly among tissues and had no affinity for melanin in tissues of the pigmented rats. The distribution was mainly in the extravascular compartment.

**Study title:** Embryofetal transfer in pregnant rats on day 13 and day 17 of gestation after p.o. administration of [<sup>3</sup>H] RAD001

**Study no.** DMPK(CH) R98-732

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** 2 Sept –12 Nov 1998

**GLP (no)**

**Drug & formulation:** <sup>3</sup>H-RAD001 batch RA 919-6, specific activity 36.1 MBq/mg; [<sup>3</sup>H]RAD001 was suspended in a 1:20 RAD placebo/0.9% saline mixture to give the intended dosage. The specific activity of [<sup>3</sup>H]RAD001 in this suspension was 35.7 MBq/mg (corresponding to the radioactivity dose of 816-1045 μCi/kg) and 35.6 MBq/mg (corresponding to the radioactivity dose of 872-919 μCi/kg) for treatment in rats on gestation days 13 and 17, respectively.

**Animals:** rats, female HAN:WIST dosed on day 13, 17 of gestation

**Protocol:** Pregnant female rats, on gestation day 13 or gestation day 17, were administered approximately 0.9 mg/kg [<sup>3</sup>H] RAD001 by oral gavage (2-1.8 mL/kg). The major maternal organs, the fetuses and the placenta from rats treated on gestation day 13, were collected and processed at 0.5, 2, 6 and 24 h after dosing. Radioactivity was determined by liquid scintillation counting (LSC). The radioactivity concentrations in maternal and fetal tissues, placentas, amnion and amniotic fluid of rats on day 17 of gestation were determined by quantitative whole body radioluminography (QWBAL). The sacrifice time points were the same as the above (0.5, 2, 6 and 24 h after dosing).

**Results:**

- Gestation day 13 rats showed highest maternal radioactivity concentrations in the intestine, liver, adrenal, spleen, kidney, lung and thyroid. At 0.5 h postdose, the highest radioactivity concentrations were in intestine and liver (~900 and 240 pmol/g), while intestine, adrenal, salivary gland, kidney, liver and thyroid were tissues with highest radioactivity (20-30 pmol/g) at 24h post dosing. Fetal radioactivity was detected at all time points at levels lower than maternal blood. The fetal radioactivity concentrations were low and similar, i.e., 2-3 pmol/g at 0.5-24 h postdose. In comparison, the radioactivity concentrations in the placenta were 11, 19, 21 and 15 pmol/g at 0.5, 2, 6 and 24 h postdose, respectively.
- Gestation day 17 rats showed radioactive tissue peaks in the lung, pituitary gland, adrenal cortex, spleen and thyroid (~1300 pmol/g) at 6 h post dosing. Radioactivity was detected

in the fetus (66 pmol/g) at 6 h postdose at levels similar to maternal blood. Placenta had detectable radioactivity at all time points, i.e., 40, 110, 600 and 30 pmol/g at 0.5, 2, 6, and 24 h postdose, respectively.

**Conclusions:** Fetal transfer of [<sup>3</sup>H] RAD001 and/or its metabolites was seen in pregnant rats administered an oral dose (0.9 mg/kg) on days 13 and 17 of gestation.

**Study title:** Galactogenic transfer, kinetics and metabolism in milk and blood after single per oral administration (0.9 mg/kg) of <sup>3</sup>H-labeled RAD001 to lactating rats

**Study no.** DMPK(CH) R98-708

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**Date of study:** April, 1999- July, 2000

**GLP (no)**

**Dose & formulation:** 0.9 mg/kg, <sup>3</sup>H-RAD001 batch RA 919-7, specific activity 0.15 MBq/mg

**Animals:** rats, HAN:WIST, lactating rats were treated at Day 9 after parturition

**Protocol:** Female rats were administered <sup>3</sup>H-RAD001 by oral gavage. Oxytocin (4 UI/kg) was administered intraperitoneally 15 min prior to milking. Milk was collected by vacuum milking device. Milk and blood samples were collected at 0.5, 2, 4, 8, 24, 48, 72 and 96 h postdose (the lactating rats were divided into two groups, n=4/group, with one group sampled at 0.5, 4, 24, 72 hr and the other at the rest of time points). <sup>3</sup>H radioactivity in the milk and blood samples was measured by liquid scintillation; concentrations of <sup>3</sup>H substances were converted from pmol/g to nmol/L, assuming a density of 1 g/mL for blood and milk. Blood samples were also subjected to HPLC analysis for parent drug and metabolites.

#### Results:

➤ Kinetics of <sup>3</sup>H-radioactivity in blood and milk

The following pharmacokinetic parameters for <sup>3</sup>H-RAD001 were seen in this study:

Dose (mg/kg)		<sup>3</sup> H t <sub>max</sub> (h)	<sup>3</sup> H C <sub>max</sub> (nmol/L)	<sup>3</sup> H AUC (nmol•h/L) 0-24h/0-96 h
0.9	blood	0.5	21.3	114/191
0.9	milk	2.0	25.7	416/634

The peak radioactivity in milk was 1.5 hours later than that observed in blood, but an apparent plateau lasted until 8 hr postdose. The milk-to-blood radioactivity concentration ratio was > 1 over the course of investigation (up to 96 hr postdose), indicating extensive transfer of the parent drug and/or metabolites into the milk.

➤ Metabolism

Metabolite peaks found in blood, i.e., P36, P40, P42, P50, P57, and P147 (corresponding approximately 2-7% of AUC<sub>0-24h</sub> of radioactivity for individual peaks), included fatty acid conjugation, hydrolytic and hydroxylated products typical of those seen in other metabolism studies. The parent drug, RAD001, represented 31% of the AUC. The main two peaks in the

HPLC profiles of milk were RAD001 (~15% of AUC<sub>0-24h</sub>) and the lipophilic conjugate P147 (~17% of AUC<sub>0-24h</sub>).

**Conclusions:** <sup>3</sup>H-RAD001 was rapidly excreted to milk. Radiolabel was concentrated in milk compared to blood, which may be due to the lipid content of milk and the lipophilic nature of RAD001.

**Study title:** Dose-dependent brain penetration in rats

**Study no.** DMPK(CH) R00-2214

**Study facility:** Novartis Pharma AG

Preclinical Safety/Drug Metabolism

ADE Section

4002 Basel Switzerland

**GLP (no)**

**Dose & formulation:** 0.1, 0.3, 1, 3, 10, 30 mg/kg (n=3/dose), i.v.; <sup>3</sup>H-RAD001 batch RA 910-2, specific activity 58.2 mCi/mg (2.15GBq/mg) (corresponding a radio dose of 88 µCi/kg)

**Animals:** rats, male Wistar

**Protocol:** Rats were administered <sup>3</sup>H-RAD001 by i.v. infusion over 0.5 min. At 2 hours postdose, rats were sacrificed, blood samples drawn and brains dissected. In a second part of the study, rats (n=15) received a bolus injection (0.17 min) of 1 mg/kg <sup>3</sup>H-RAD001 (~ 200 µCi/kg). Rats were sacrificed at 0.08, 2, 8, 24 and 168 h (n=3/time point) after dosing. Blood samples were taken and brains dissected. Radioactivity in tissues was measured by liquid scintillation counting.

#### Results:

➤ Dose dependency of <sup>3</sup>H-RAD001 brain penetration

Brain-blood distribution of <sup>3</sup>H RAD001 was nonlinear and dose-dependent, i.e., a low brain/blood ratio up to 1 mg/kg (brain/blood RAD001 ratio ~0.3), followed by an increase ratio (up to 3) at 30 mg/kg. On the other hand, the brain/blood radioactivity concentration ratio was consistent (~0.3) at 0.1-30 mg/kg. The latter may suggest that the radioactive metabolites of RAD001 do not enter brain as much as the parent drug.

➤ Time dependency of <sup>3</sup>H-RAD001 brain and blood distribution

At 2 hr postdose, parent drug/total radioactivity in brain remained close to unity (0.8-1) independent of the dose (0.1-30 mg/kg), indicating the parent drug taken up by brain was not much metabolized at this point. At 168 h RAD001 concentrations were higher in brain (~6 ng/g), whereas blood concentrations (~0.2 ng/g in whole blood) were barely detectable. The parent drug/total radioactivity ratio in brain decreased with time, i.e., 1 at 0.08 h postdose and 0.4 at 168 h postdose.

**Conclusions:** Examination of parent drug and total radioactivity concentrations in the brain appears to indicate that the parent drug crosses the brain-blood barrier better than metabolites.

**Study title:** Disposition in rats after single and repeated once daily peroral administration (0.5 mg/kg/day) of <sup>3</sup>H-labeled RDA001 for 21 consecutive days

**Study no.** DMPK(CH) R98-706

**Study facility:** Novartis Pharma AG  
Preclinical Safety/Drug Metabolism  
ADE Section  
4002 Basel Switzerland

**Date of study:** 20 June 2001

**GLP (no)**

**Dose & formulation:** 0.5 mg/kg, <sup>3</sup>H-RAD001 batch RA 919-7, specific activity 51.3 MBq/mg

**Animals:** rats, males, HAN:WIST (albino)

**Protocol:** Rats were administered <sup>3</sup>H-RAD001, 0.5 mg/kg/day by oral gavage in the following groups:

Group	Sample collection	No. of doses
PK+ metabolism	Day 1: 2, 4, 8, 24h post dose, day 6, 13, 18, 20, 24 h post dose; day 21, 2, 4, 8, 24, 72, 120, 168 h post dosing	21
Excretion	Urine, feces collected daily, to 168 h after last dose (i.e., Day 28)	21
Distribution	Day 1, day 21 autoluminography of carcass	1, 21
Metabolite profiling	Day 21, 24 h after dose	21

**Results:**

➤ **Absorption:**

Plasma PK parameters (mean ± SD) following a single dose (Day 1) or multiple oral doses (Day 21):

	Day 1	Day 21
C <sub>max</sub> (nmol/L)	8.61 ± 1.4	13.81 ± 1.22
AUC <sub>0-24h</sub> (nmol•h/L)	100 ± 19	245 ± 28

The exposures indicated accumulations of RAD001 and/or its metabolites following repeat administration.

➤ **Distribution:**

Tissue distribution showed the highest concentrations in the nasal turbinates, esophagus, glandular stomach mucosa, and intestinal wall throughout the sampling period. Accumulation was seen in multiple versus single dose administration, with the thymus, seminal vesicle and

lacrimal gland showing higher concentrations later in the multiple dose samples. The tissue distribution of radioactivity was mainly extravascular, following single or multiple administrations.

➤ **Metabolism:**

Hydroxylated, hydrolytic and fatty acid conjugate metabolites predominated in metabolites recovered from excretions, and tissues as seen in other rats metabolism studies. The main metabolite peaks in blood were P42, P50 and P57, i.e., products of hydroxylation of the parent drug. In blood, the extent of accumulation (2-4 fold) of these metabolites and RAD001 was comparable to the accumulation of radioactivity. Other complex, unresolved metabolites were seen in feces and urine. These metabolites were mainly of more polar nature than those observed in blood.

➤ **Excretion:**

Excretion of  $^3\text{H}$ -radioactivity was mostly through the fecal route, with small traces excreted as parent drug. After day 8, fecal excretion was 90%. Excretion was nearly complete within 168 h of the last dose; only <0.1% of the cumulative dose was found in the carcass, GI tract and bone.

Conclusions: Rats dosed with 0.5 mg/kg  $^3\text{H}$ -RAD001 showed predominantly fecal excretion as seen in other studies. Tissue distribution was concentrated in the digestive/gastrointestinal tract throughout the 21 day treatment period. Accumulation was seen after multiple dose administration compared to the single dose administration. Metabolic patterns of fatty acid conjugates and hydroxylated/hydrolytic metabolites were seen both in tissues and excretions.

#### 2.6.4.5 Metabolism

**Study title:** Biotransformation in mice following a single oral and intravenous dose (0.9 mg/kg) of  $^3\text{H}$ -RAD

**Study no.** DMPK(CH) R00-1806

**Study facility:** Novartis Pharma AG  
Preclinical Safety/DMPK/ADME  
Basel Switzerland

**Date of study:** Aug-Oct 2000

**GLP (no)**

**Dose & formulation:** 0.9 mg/kg (n=3/route),  $^3\text{H}$ -RAD (specific activity 80.77 MBq/mg) in Sandimmune placebo solution (Batch #Y0450497)/0.9% sodium chloride (for i.v.), or in KZI/saline (for oral)

**Animals:** mice, male CD-1

**Protocol:** Mice were administered  $^3\text{H}$ -RAD by either oral or i.v. route. Animals were housed in metabolic cages to collect urine and feces for 24 h following dosing. Blood was collected 0.083, 1, 3, 8, and 24 h post dose. Concentrations of  $^3\text{H}$ -RAD in blood and excretion samples were determined by liquid scintillation counting. Blood samples were analyzed by HPLC to determine metabolite patterns.

Results: Oral administration resulted in a blood  $C_{\text{max}}$  of 178 pmol/mL at a  $t_{\text{max}}$  of 1 h. AUC for the oral dosing was 1693 pmol•h/mL. Intravenous dosing did not result in detectable blood

levels of  $^3\text{H}$ -RAD. The major component from blood in the oral dosing study was parent RAD (63%). Fecal excretion accounted for 73.4% of elimination. Metabolites included hydrolysis/dehydration and hydroxylation forms.

**Conclusions:** Fecal elimination was the major excretory route for orally administered  $^3\text{H}$ -RAD in mice. Metabolism was through hydrolysis/dehydration and hydroxylation forms as seen in human and monkey studies, i.e., with P40, P42, P50 and P57 as the major metabolite peaks. The metabolite P36 was found in human blood but not (or in trace only) in the blood of mice.

**Study title:** Absorption, distribution, metabolism, and excretion in rats after single intravenous (1 mg/kg, 10 mg/kg) and oral (1.5 mg/kg, 15 mg/kg) administration of [ $^3\text{H}$ ]SDZ RAD 666

Reviewer's note: This study was reviewed by Dr. K Hastings for IND. A group of rats (n=5), intravenously administered [ $^3\text{H}$ ] SDZ RAD 666 at 1 mg/kg (i.v. bolus), were bile canulated. The pertinent results in absorption, metabolism and excretion are summarized (tables from the sponsor, Module 2, Section 2.6.5. "Pharmacokinetics tabulated summary).

b(4)

➤ Absorption:

Route	Blood (parent drug) (mean $\pm$ SD, n = 4-5) <sup>a</sup>			
	intravenous infusion		oral	
	Dose	1 mg/kg	10 mg/kg	1.5 mg/kg
PK parameters				
$C_{\max}$ (ng/mL)	90.5	1347	16.7 $\pm$ 10.4	210 $\pm$ 36
$t_{\max}$ (h)	2	2	1.6 $\pm$ 1.5	2.2 $\pm$ 1.1
AUC (ng·h/mL)	818 $\pm$ 90	5169 $\pm$ 429	176 $\pm$ 126	2034 $\pm$ 244
$t_{1/2, \lambda_1}$ (h)	3.7 $\pm$ 3.0	3.0 $\pm$ 1.2	not measured	not measured
$t_{1/2}$ (h)	60 $\pm$ 23	60 $\pm$ 27	61 $\pm$ 11	47 $\pm$ 7

Route	Blood (total radioactivity) (mean $\pm$ SD, n = 4-5) <sup>a</sup>			
	intravenous infusion		oral	
	Dose	1 mg/kg	10 mg/kg	1.5 mg/kg
PK parameters				
$C_{\max}$ (ng-eq/mL)	202	4523	34 $\pm$ 15	1411 $\pm$ 281
$t_{\max}$ (h)	2	2	4.1 $\pm$ 4.6	1.8 $\pm$ 0.4
AUC (ng-eq·h/mL)	2105 $\pm$ 379	40900 $\pm$ 1529	1245 $\pm$ 292	26091 $\pm$ 3240
$t_{1/2}$ (h)	76 $\pm$ 21	80 $\pm$ 21	96 $\pm$ 39	96 $\pm$ 6

<sup>a</sup> = To obtain pmol-units, multiply ng-units by 1.04357.

<sup>b</sup> = A body weight of 0.3 kg was assumed.

The oral bioavailability (% dose) following the dose of 1.5 mg/kg and 15 mg/kg was approximately 14% and 26%, respectively, based on dose-normalized AUCs.

➤ Metabolism:

Peak (component)	Exposure to metabolite peaks in blood <sup>a,b</sup>					
	1 mg/kg, intravenous		1.5 mg/kg, oral		15 mg/kg, oral	
	AUC(0-26h) ng-eq-h/mL	Radioactivity % of total	AUC(0-24h) ng-eq-h/mL	Radioactivity % of total	AUC(0-24h) ng-eq-h/mL	Radioactivity % of total
P6	196	17	216	65	5922	59
P17					167 <sup>d</sup>	1.5
P23			nd		65 <sup>e</sup>	0.6
P28 (PKF229-255)	100 <sup>c</sup>	8	19	6	2007	18
P33 (PKF226-320)	190	16	15	4	1169	11
P47	160	14	18	5	872	8
P56 (RAD001)	531	45	63	19	1087	10
Sum of peaks	1178	100	330	100	11315	100

<sup>a</sup> = The AUCs were separately calculated by the linear trapezoidal rule from the data of the report.

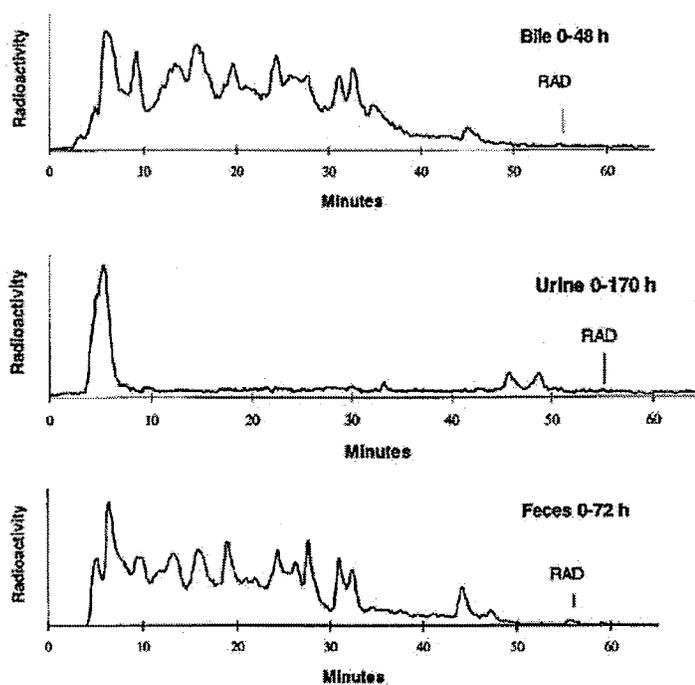
<sup>b</sup> = Multiply ng-eq-h/mL-units by 1.04357 to obtain pmol-h/mL-units.

<sup>c</sup> = AUC(0-10h).

<sup>d</sup> = AUC(0-8h).

<sup>e</sup> = AUC(0-2h).

**Representative metabolite patterns in bile, urine, and feces after 1 mg/kg, intravenous**



Metabolite patterns of urine and feces were similar following oral or i.v. administration. The major excretion route for metabolites was via bile.

➤ **Excretion:**

Excretion of [<sup>3</sup>H] radioactivity within 168 hr (mean ± SD, n=4 -5)

Route Dose	intravenous infusion			oral	
	1 mg/kg	10 mg/kg	1 mg/kg (bolus)	1.5 mg/kg	15 mg/kg
% in bile			71.4 ± 5.9		
% in urine	4.5 ± 0.5	4.2 ± 1.4	3.3 ± 1.0	5.6 ± 1.9	4.8 ± 0.9
% in feces	68.8 ± 6.4	82.0 ± 1.2	4.6 ± 2.7	73.8 ± 8.5	79.1 ± 4.9
% cage wash	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	1.6 ± 2.4	0.1 ± 0.2
% in carcass	3.3 ± 0.6	3.8 ± 0.6	9.0 ± 4.0	4.2 ± 0.7	3.0 ± 0.9
Recovery	76.8 ± 7.2	90.1 ± 2.0	88.6 ± 2.4	85.2 ± 4.2	87.0 ± 5.4
<sup>3</sup> H-water	6.8 ± 1.0	7.5 ± 1.0	7.9 ± 1.4	10.9 ± 1.3	8.6 ± 2.6
<b>Pooled samples<sup>a</sup></b>					
	Sampling item or period		% of dose in sample		
Blood (1 mg/kg, i.v.)	2, 3, 10, 26, and 170 h		-		
Blood (1.5 mg/kg, p.o.)	0.5, 2, 8, 24, and 168 h		-		
Blood (15 mg/kg, p.o.)	0.5, 2, 8, 24, and 168 h		-		
Urine (1 mg/kg, i.v.) <sup>b</sup>	0-170 h		3.3 ± 1.0		
Feces (1 mg/kg, i.v.) <sup>b</sup>	0-72 h		4.6 ± 2.7		
Bile (1 mg/kg, i.v.) <sup>b</sup>	0-48 h		67.5 ± 6.2		
Urine (10 mg/kg, i.v.) <sup>c</sup>	0-170 h		4.2 ± 1.4		
Feces (10 mg/kg, i.v.) <sup>c</sup>	0-74 h		77.5 ± 2.6		
Urine (15 mg/kg, p.o.) <sup>c</sup>	0-168 h		4.8 ± 0.9		
Feces (15 mg/kg, p.o.) <sup>c</sup>	0-72 h		71.7 ± 5.8		

<sup>a</sup> = Sample pools of 2-5 animals were prepared; one animal's results were not included in the bile mean and SD.

<sup>b</sup> = Values are from the bile fistula rats.

<sup>c</sup> = Samples chromatographically investigated, but not shown in the report.

**Study title:** Biotransformation in cynomolgus monkey following a single oral dose of <sup>3</sup>H-RAD

**Study no.** DMPK(CH) R98-1404

**Study facility:** Novartis Pharmaceuticals Corporation

Absorption Distribution Metabolism & Excretion

East Hanover, NJ USA

**Date of study:** Nov 1998-June 2000

**GLP (no)**

**Dose & formulation:** 5 mg/kg, <sup>3</sup>H-RAD, batch RA 919-6, specific activity 1.35 mCi/mg

**Animals:** cynomolgus monkey, males (n=2)

**Protocol:** Cynomolgus monkeys were administered a single dose of <sup>3</sup>H-RAD001, 5 mg/kg, by orogastric tube. Blood was collected at 1, 2, 4, 7 and 24h post dose. Urine and feces were collected for the intervals 0-24, 24-48, 48-72 and 72-96 h postdose. Radioactivity of blood and excretions were measured by liquid scintillation counting. Blood was also analyzed by HPLC for metabolite content.

### Results:

Metabolic profiles in the blood: The AUC values (ng-eq•h/mL)\* of parent drug and major metabolite peaks

	RAD001	P36	P40	P42	P50	P57	Unassigned	Total
AUC <sub>0-24h</sub>	768	509	591	349	699	410	1759	6420
% of total <sup>3</sup> H	12	7.9	9.2	5.4	10.9	6.4	27.4	100

\* AUC: multiply ng-units by 1.04357 to obtain pmol-unit

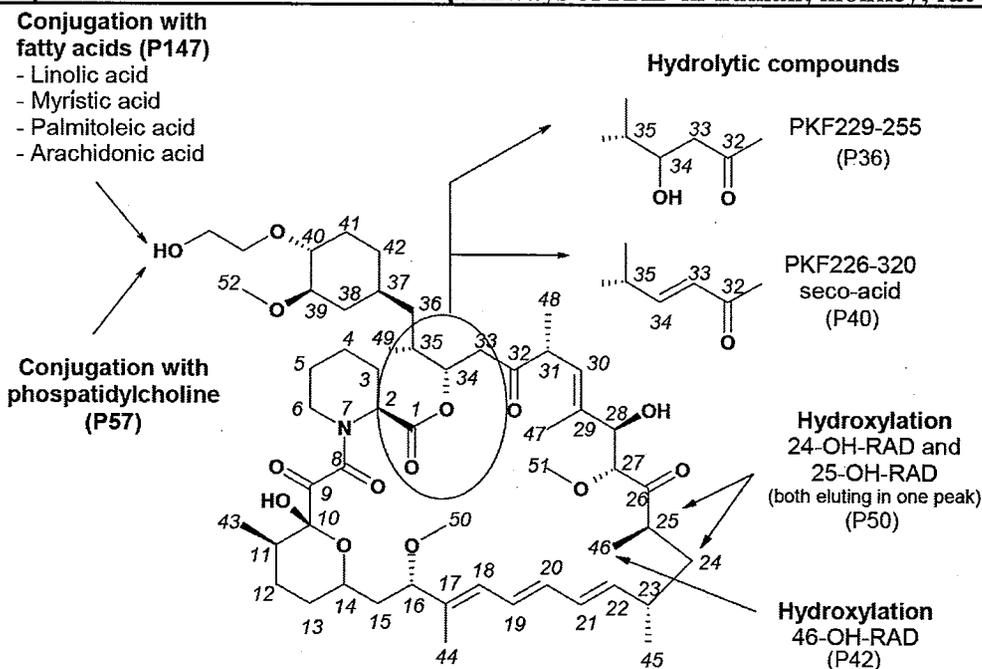
The peaks contained the following major metabolites:

P36: PKF229-255 (hydrolyzed RAD001), P40: PKF226-320 (seco-acid of RAD001), P42: 46-HO RAD001, P50: 24- and 25-HO RAD001

The pharmacokinetic parameters determined in this study,  $C_{max}$  = 776 pmol/mL,  $t_{max}$  = 1.5 h, AUC = 6251 pmol·h/mL are similar to those seen in other monkey studies. Excretion was primarily by feces (93.4%, urine 0.8%). Metabolism was through hydrolysis/dehydration and hydroxylation forms.

**Conclusions:** Following verification of  $^3\text{H}$ -RAD pharmacokinetics in cynomolgus monkeys, metabolites were analyzed in blood, feces and urine. Metabolism through ring-opening hydrolysis and hydroxylated forms, are similar to those found in human *in vivo* metabolism.

### Proposed *in vivo* biotransformation pathways of RAD in human, monkey, rat and mouse



#### 2.6.4.6 Excretion

**Study title:** Pharmacokinetics and excretion after single intravenous and peroral administration (0.9 mg/kg) of  $^3\text{H}$ -labeled RAD001 to mice [Study #DMPK(CH) R98-707]

##### Summary:

With either *i.v.* or *p.o.* dosing, radioactivity was excreted rapidly and nearly completely within 48 h (>96%). Excretion was nearly all in feces with *p.o.* or *i.v.* administration (>95%).

See also other studies in the previous sections.

**2.6.4.7 Pharmacokinetic drug interactions**

**Study title:** Inhibition of RAD 001 in vitro metabolism by ketoconazole, itraconazole and fluconazole

**Study no.** DMPK (CH) R99-2448

**Study facility:** Novartis Pharma AG  
Preclinical Safety/Drug Metabolism  
ADE Section  
4002 Basel Switzerland

**Date of study:** 31 May 2000

**GLP (no)**

**Dose & formulation:** RAD 001, batch RAD001-NXB; ketoconazole, itraconazole, fluconazole; each in DMSO

**Samples:** human liver microsomes

**Protocol:** Microsomal preparations were incubated with RAD001 (0.25 mM) alone and with one of either ketoconazole, itraconazole or fluconazole (stock at 0.5 mM and was diluted with DMSO to obtain concentrations of 0.05 to 2  $\mu$ M) for 15 min.

**Analysis:** Supernatants from incubations were measured for RAD by LC-MS analysis

**Results:** Ketoconazole and itraconazole, inhibitors of CYP3A4, inhibited biotransformation of RAD001, with  $IC_{50}$  of  $0.35 \pm 0.04 \mu\text{mol/L}$  and  $0.18 \pm 0.11 \mu\text{mol/L}$ , respectively. Fluconazole, up to 2  $\mu\text{mol/L}$ , did not inhibit RAD biotransformation, an expected result, as fluconazole is a weak inhibitor of CYP3A4.

**Conclusions:** CYP3A4 is believed to be the main enzyme for metabolism of RAD001 in humans. Inhibitors of this enzyme (ketoconazole, itraconazole) were shown to inhibit biotransformation of RAD001.

**2.6.4.8 Other Pharmacokinetic Studies**

None

**2.6.4.9 Discussion and Conclusions**

See the PK/TK summary, Section 2.6.4.1.

**2.6.4.10 Tables and figures to include comparative TK summary**

This section was summarized by Dr. S. Kunder in NDA            (Special Pathogen and Immunologic Drug Products, October 10, 2003). Modifications are made as needed.

b(4)

➤ Absorption parameters of orally administered RAD in animals and humans

Species	Dose mg/kg	$t_{1/2}$ Ra (h)	$t_{1/2}$ RAD (h)	$T_{max}$ Ra (h)	$T_{max}$ RAD (h)	Dose absorbed, %	BA %	RAD (% of Ra-AUC)	Metabolites (% of Ra-AUC)
Mouse	0.9			0.5	1	12	5	63	37
Rat	1.5	96	61	4.1 $\pm$ 4.6	1.6 $\pm$ 1.5	39	14	14	86
	15.0	96	47	1.8 $\pm$ 0.4	2.2 $\pm$ 1.1	43	26	8	92
Monkey	5	46	18	2.0 $\pm$ 0	1.2 $\pm$ 0.8	18	6	12	88
Human*	0.038	81	33	1.7	1.5	11	NA**	40	60

**RAD: SDZ RAD, RAD001, everolimus**

\* Renal transplant patients dosed with one single oral dose of 3 mg [<sup>14</sup>C]everolimus (body weight 78.4±13.9 kg, n=3; CP Study W107)

\*\* : NA, not available, because there is no appropriate IV formulation for the administration in humans.

t<sub>1/2</sub>: apparent terminal elimination half-life

Ra: radioactivity; BA: bioavailability; Ra-AUC: AUC values of radioactivity

◇ RAD blood concentration after oral and intravenous dosing in ADME studies

Species	Dose mg/kg	Route and frequency	C <sub>max</sub> ng/mL	AUC ng•h/mL
Mouse	0.9	P.O., single	108	1013
	0.9	IV, single	1940	20283
Rat	1.5	P.O., single	16.7	2034
	15	P.O., single	210	818
	1	IV, single	90.5	5169
	10	IV, single	1347	43.8
	0.5	P.O., single	3.39	79.4
	0.5	P.O., 21 days	3.75	
Monkey	5	P.O., single	102	1732
	1	IV, single	415	5471
patients	0.038	P.O., single	35.1	426

◇ Excretion of total radioactivity: indicated as % of doses

Species	Dose Mg/kg	route	urine	feces	bile	carcass	Total recovery
Mouse	0.9	IV	0.64	99.5		0.16	100.8
	0.9	P.O.	1.90	95.2		0.05	97.4
Rat	1	IV	4.5	68.8	71.4	3.3	76.8
	1 *	IV	3.3	4.6		9.0	88.6
	10	IV	4.2	82.0		3.8	90.1
	1.5	P.O.	5.6	73.8		4.2	85.2
	15	P.O.	4.8	79.1		3.0	87.0
	0.5	P.O.	1.1	72.8			73.9
	0.5	P.O., 21 days	0.6	89.7		<0.1	90.3
Monkey	1	IV	7.1	66.6			76.7
	5	P.O.	7.2	75.7			84.5
Patients**	0.038	P.O.	5.1	79.5			84.6

\*(bile duct cannulated)

\*\* Renal transplant patients (one single oral dose of 3 mg, CP Study W107)

◇ RAD blood concentrations after multiple oral dosing in toxicokinetic studies

species	Route/duration	Dose mg/kg	C <sub>max</sub> ng/mL m	C <sub>max</sub> ng/mL f	AUC ng•h/mL m	AUC ng•h/mL f
mouse	PO/104 week	0.1	18.5	17.7	169.5	150.5
		0.3	25.4	51.4	412.8	346.4
		0.9	168.5	187.9	1377.8	3084.2

mouse	PO/13 week	0.15	55	48	803	362
		0.5	146	272	1174	1258
		1.5	331	639	3472	4877
		5	4228	1754	19420	11363
		15	4188	5828	45955	43206
rat	PO/2 week Microemul sion Solid dispersion	0.5	39	30	255	155
		1.5	891	907	6890	4693
		5	21	23	218	169
		15	600	591	5770	3743
rat	PO/4 week	0.5	5.9	11.3	--	--
		1.5	44	38.3	117.5	201.6
		5	163	117.0	1415.6	1005.9
		15	400.5	644.5	4618.4	4054.0
rat	PO/4 week	0.1	<LOD	<LOD	<LOD	<LOD
		0.25	<LOD	<LOD	<LOD	<LOD
		0.5	10	6	102	56
		1.5	52	30	514	237
rat	PO/26 week	0.15	1.5	1.1	7.1	8.1
		0.5	7.1	3.7	44.1	13.1
		1.5	14.4	10.6	92.7	50.8
rat	PO/13 wk Fertility males	0.1	2.8		9.9	
		0.5	8.4		52	
		5	40.4		415	
rat	PO/104 week	0.1	0.5	0.4	2.5	1.0
		0.3	2.1	1.2	25.7	11.4
		0.9	8.9	9.7	138.2	42.9
monkey	PO/4 week	1.5	94.5	130.7	973.2	1194.1
		5	332.7	266.0	3130.4	3259.7
		15	941.3	554.0	8099.3	5830.8
monkey	PO/26 week	0.1	20	33	145	223
		0.5	68	59	358	466
		1	146	177	1106	1218
		5	537	383	4913	3322
monkey	PO/52 week	0.1	8.5	10.1	98.0	59.6
		0.5	24.1	20.5	275.6	176.2
		0.9	84.9	47.8	941.3	471.8

<LOD= less than limit of detection

m: male; f: female

### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Additional tables (from the sponsor, Module 2, Section 2.6.4 "Pharmacokinetics written summary"):

**Table 5-1 Metabolites and unchanged everolimus in blood**

Major peaks	Compound	Species	Mouse	Rat	Rat	Cynomolgus	Human
		Dose	0.9 mg/kg p.o.	(lactating) 0.9 mg/kg p.o.	(multiple dose) 0.5 mg/kg p.o.	monkey 5 mg/kg p.o.	0.038 mg/kg 3 mg <sup>a</sup> , p.o.
				Day 1	Day 21		
		AUC <sub>(0-24h)</sub> [ng-eq-h/mL] (% of total AUC <sub>(0-24 h)</sub> radioactivity)					
P36	PKF229-255	traces	3 (2.4)	6 (6.6)	8 (3.4)	509 (7.9)	21 (4.1)
P40	PKF226-320	85 (5.2)	6 (5.2)	9 (9.8)	22 (9.9)	591 (9.2)	33 (6.5)
P42	46-OH-RAD	12 (0.8)	2 (1.9)	7 (6.9)	11 (4.8)	349 (5.4)	63 (13)
P50	24-/25-OH-RAD	117 (7.2)	8 (6.7)	12 (12)	18 (8.3)	699 (11)	62 (12)
P57	ATG181 <sup>c</sup>	43 (2.7)	4 (2.9)	5 (5.6)	16 (7.3)	410 (6.4)	28 (5.6)
Sum of minor peaks		79 (4.8)	26 (21)	13 (14)	68 (31)	1333 (21)	89 (18)
Total of metabolites		336 (21)	49 (41)	52 (54)	143 (64)	3890 (61)	295 (59)
Unchanged everolimus		1014 (63)	37 (31)	44 (46)	79 (36)	768 (12)	199 (40)
Total of everolimus+metabolite peaks		1349 (83)	85 (71)	96 (100)	222 (100)	4658 (73)	494 (99)
Not extractable		272 (17)	35 (29)	(0) <sup>b</sup>	(0) <sup>b</sup>	1759 (27)	5 (1)
Sum		1621 (100)	120 (100)	96 (100)	222 (100)	6417 (100)	499 (100)
Extraction yield (range)		60 - 89	39 - 78	(100) <sup>b</sup>	(100) <sup>b</sup>	62 - 79	98 - 100
Reference:		R00-1806	R98-708	R98-706	R98-1404	W107	

<sup>a</sup>: 3 mg <sup>14</sup>C-radiolabeled everolimus per patient, mg/kg calculated on a body weight of 78.4 ± 14 kg (n=3); the patients were under Neoral<sup>®</sup> treatment (1/4 patients was excluded for opiate use).

<sup>b</sup>: The radioactivity levels in diethylether and methanol extracts in the remaining pellets could not be properly determined due to intensive sample luminescence. Hence, the amounts listed for the rat were determined under the assumption of 100% sample recovery.

<sup>c</sup>: ATG181= phosphatidylcholine-conjugate with everolimus.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

Oral toxicology studies in rats, minipigs and monkeys have identified lymphoid organs (i.e., thymus, spleen, and lymph nodes: immunosuppression), heart (myocardial degeneration/myocarditis), GI (soft feces and diarrhea), bone marrow (leucocytosis, increased percentage of band cells, lymphopenia), kidney (tubular degeneration), pancreas (islet cell degeneration), male reproductive organs (reduced testes and epididymides weight or immature testes), uterus (atrophy), ovary (follicular development abnormalities) and eye (rat specific toxicities: swelling, disruption of cortical fibers of the lens) as target organs of everolimus.

#### Genetic toxicology:

Everolimus was not mutagenic in bacterial Ames test (*Salmonella typhimurium* TA98, TA97a, TA100, TA102 and TA1535), and was not clastogenic in the chromosome aberration test in V79 Chinese hamster cells (CHO), in the presence or absence of S9-mix. Everolimus was not mutagenic in L5187Y mouse lymphoma cells, in the presence or absence of rat liver S9-mix. Orally administered everolimus up to 500 mg/kg/day did not induce micronucleus formation in mouse.

Carcinogenicity:

Studies using mice and rats, up to 104 weeks, have indicated negative carcinogenicity in the rodents.

Reproductive toxicology:

In a 13-week male fertility study in rats, testicular morphology was affected at 0.5 mg/kg and above, and sperm motility, sperm count, and plasma testosterone levels were diminished at 5 mg/kg, which resulted in infertility at 5 mg/kg. After a 10-13 week non-treatment period, 12 out of 20 females mated were confirmed to be pregnant.

Oral administration of everolimus to female rats prior to mating and continued to gestation day 16 induced embryo-fetal toxicities, including increased resorption, pre-implantation and post-implantation loss, decreased numbers of live fetuses, malformation (e.g., sternal cleft) and retarded skeletal development. The effects were seen in the absence of maternal toxicities. Embryo-fetal toxicities occurred at doses  $\geq 0.1$  mg/kg (0.6 mg/m<sup>2</sup>). In rabbits, embryotoxicity was evident as an increase in resorptions at 0.8 mg/kg (9.6 mg/m<sup>2</sup>).

Special toxicology:

Studies were conducted to investigate the general toxicology and genetic toxicology of impurities found in the drug substance. There were no toxicologically relevant findings.

Everolimus was negative in local tolerance studies in rabbits and in guinea pigs.

**2.6.6.2 Single-dose toxicity***Reviewer's note:*

- This section was reviewed by Dr. Kenneth Hastings for IND Special Pathogen and Immunologic Drug Products, November 21, 1996. b(4)

**1. Acute oral toxicity study - mice (Study 203-023)**

[HanIbm:NMRI (SPF) mice; 5/sex; 10 total; single oral (gavage) dose followed by 14 day recovery/observation period; dose used: 2000 mg/kg; performed by \_\_\_\_\_ drug batch g 95903; no controls; GLP study] b(4)

There were no deaths on study. One female demonstrated a slight decrease in body weight. Slight dyspnea and slight to moderate ruffled fur were seen in two females. No other effects were observed.

**2. Acute oral toxicity study - rats (Study 203-024)**

[HanIbm:WIST (SPF) rats; 5/sex; 10 total; single oral (gavage) doses followed by 14 day recovery/observation period; dose used: 2000 mg/kg; performed by \_\_\_\_\_ drug batch g 95903; no controls; GLP study] b(4)

There were no deaths or significant effects.

### 2.6.6.3 Repeat-dose toxicity

*Reviewer's note:*

- This section includes results of studies reviewed by Dr. S. Kunder for NDA [redacted] (Special Pathogen and Immunologic Drug Products, October 10, 2003): b(4)
  - Study #96/SMP078/1067 (SDZ RAD: Toxicity study by oral gavage administration to cynomolgus monkeys for 26 weeks followed by a four week reversibility period) and Study #1463-045 (52-week oral (gavage) toxicity study in the cynomolgus monkey) are modified.
  - Study #971033 (4-week oral (gavage) toxicity study in minipigs) is re-formatted to fit the current NDA template.
- Study # Sandoz Study 96/SPM083/1130 (SDZ RAD: Toxicity study by oral gavage administration to Hanlbn Wistar rats for 26 weeks followed by a four-week reversibility period) is reviewed by Dr. Shwu-Luan Lee.
- Study # 95/SPM052/0888 (203-042), #96/SPM090/0404 (203-050) and Study #95/SPM049/1008 (203-054) was reviewed by Dr. Kenneth Hastings for IND [redacted] b(4)  
(Special Pathogen and Immunologic Drug Products, November 21, 1996).

This study is not reviewed; salient findings are summarized:

- Doses: 0.15, 0.5, 1.5, 5 and 15 mg/kg (n=10/sex/dose), once daily for 13 weeks.
- Dose dependent findings in hematology (↑ RBC, HGB, and Hct; ↓ WBC), clinical chemistry (↑ cholesterol and creatinine; ↓albumin, A/G ratio and phosphorus), and relative (to body weight) organ weights (↑ liver; ↓ thymus, testes, epididymis, and uterus).
- Target organs by histopathological findings: thymus (medullary atrophy), kidney (tubular degeneration with karyomegaly, interstitial inflammation, basophilic tubules), lung (foamy alveolar macrophages), skin (ulcer, inflammation, scabs), male reproductive organs (testes: depletion of germ cells, vacuolation of germinal epithelium, epididymis: decreased sperm counts) and female reproductive organs (ovary: decreased follicular development; uterus: glandular atrophy).

Note: The following two studies of 4-week repeat dose in rats were reviewed by Dr. K Hastings for IND [redacted]

#### Study title:

Toxicity study by oral gavage administration to Hanlbn Wistar rats for 4 weeks followed by a 2 week reversibility period. Study # 203-042 ( [redacted] Report # 95/SPM052/0888); study dated 6-14-96. b(4)

[Hanlbn:Wistar rats; 10/sex/dose with additional 6/sex in the placebo and high-dose groups (recovery group) and 4/sex/dose for toxicokinetic study; 164 total; daily oral (gavage) doses for four weeks with separate recovery group animals held for two week recovery/observation period; doses used: 0.5, 1.5, 5, 15 mg/kg/day; performed by [redacted] b(4)

— drug batch g Y110 0595; dose volume: 5 mL/kg; placebo control (batch # Y111 0595); **b(4)**  
GLP study]

There were no deaths on study. No significant clinical signs were observed. Reductions in mean body weight gains and food consumption were observed in all treatment groups except low-dose group females. Body weight gains were 87%, 67%, 35%, and 19% of concurrent control mean value in males in the 0.5, 1.5, 5, and 15 mg/kg/day groups, respectively. Respective body weight gains in females were 102%, 80%, 59%, and 27% of the concurrent control mean value. Food conversion efficiency was decreased in all but the low-dose groups, indicating that decreased food consumption did not entirely account for the observed body weight gain effects. Ophthalmic examinations demonstrated a high incidence of anterior suture line opacities in the lenses of treated animals ( $\geq 5$  mg/kg/day). This effect persisted in the high-dose recovery group.

Clinical pathology demonstrated signs consistent with hemoconcentration (increased Hct, Hgb, and RBC in all treatment groups, although no dose-relationship was apparent). Mean red blood cell volume (MCV) and hemoglobin (MCH) values were decreased in some treatment groups. Platelet counts were decreased and neutrophil counts were increased, especially in the higher dose groups. A high incidence of spherocytes was observed in high-dose group females. Hematology effects tended to normalize during the recovery period, with the exception of neutrophil and platelet changes. Clinical chemistry effects included slight elevations in BUN (high-dose group), elevated serum cholesterol ( $\geq 5$  mg/kg/day, females;  $\geq 0.5$  mg/kg/day, males), elevated serum triglycerides ( $\geq 1.5$  mg/kg/day, females only), and decreased serum albumin ( $\geq 5$  mg/kg/day, both sexes). Changes in BUN and albumin tended to persist after the recovery period. No significant urinalysis effects were observed.

Necropsy demonstrated low absolute and relative pituitary weights (females only, all dose groups); low absolute and relative epididymes and seminal vesicle weights ( $\geq 1.5$  mg/kg/day); high absolute and relative lung weights (males, all dose groups;  $\geq 5$  mg/kg/day, females); low absolute and relative ovary weights ( $\geq 5$  mg/kg/day); low absolute and relative prostate weights ( $\geq 5$  mg/kg/day); low absolute and relative spleen weights ( $\geq 5$  mg/kg/day, both sexes); low absolute and relative testes weights (15 mg/kg/day); low absolute and relative thymus weights (all treatment groups); and low absolute and relative uterus weights (females in all treatment groups). Sex organ, lung, and pituitary effects tended to persist after the recovery period. Sperm counts were low in males in the high-dose group and this effect persisted in the recovery group. Low sperm motility was observed in all treatment groups although no dose-relationship was apparent.

Histopathology demonstrated thymic medullary atrophy ( $\geq 1.5$  mg/kg/day); chronic myocarditis (males, all treatment groups;  $\geq 1.5$  mg/kg/day, females); swelling and disruption of fibers in the anterior cortex of the lens ( $\geq 5$  mg/kg/day); foamy alveolar macrophages ( $\geq 0.5$  mg/kg/day, males;  $\geq 1.5$  mg/kg/day, females); loss of germ cells (degeneration of pachytene spermatocytes) with degeneration of the testes (spermatid giant cells and vacuolation of the germinal epithelium) ( $\geq 1.5$  mg/kg/day); reduced epididymides content (15 mg/kg/day); seminal vesicle atrophy ( $\geq 1.5$  mg/kg/day); prostate atrophy ( $\geq 5$  mg/kg/day); ovarian interstitial cell hypertrophy ( $\geq 1.5$  mg/kg/day); uterine atrophy ( $\geq 5$  mg/kg/day); and depletion

of salivary gland secretory granules (> 1.5 mg/kg/day). Microvesiculation of the zona glomerulosa and/or zona fasciculata of the adrenal cortex (> 1.5 mg/kg/day) may have been stress related. Hypertrophy of the gastric chief cells in males ( $\geq$  5 mg/kg/day) was also observed, although the sponsor states that this was probably not attributable to drug toxicity. Examination of bone marrow smears did not demonstrate changes in myeloid:erythroid cell ratios. Chronic myocarditis was graded as minimal to slight, with mononuclear cell inflammatory cells and fibroblasts but without mature collagen. Electron microscopic examination of pulmonary macrophages demonstrated evidence of phospholipidosis. Cardiac, ophthalmic, and sex organ effect tended to persist in the recovery period.

The following toxicokinetic parameters were demonstrated, expressed as the mean:

Dose (mg/kg/day)	Males		Females		Combined	
	$C_{MAX}^*$	$AUC_{0-24}^\dagger$	$C_{MAX}$	$AUC_{0-24}$	$C_{MAX}$	$AUC_{0-24}$
0.5 (Day 1)	5.9	-	11.3	-	8.6	-
0.5 (Day 28)	13.1	33.2	3.8	-	8.5	-
1.5 (Day 1)	44.0	117.5	38.3	201.6	41.1	159.6
1.5 (Day 28)	78.0	321.4	101.8	548.6	89.9	435.0
5 (Day 1)	163.0	1415.6	117.0	1005.9	140.0	1210.8
5 (Day 28)	151.0	1686.7	203.0	1249.0	177.0	1467.9
15 (Day 1)	400.5	4618.4	644.5	4054.0	522.5	4336.2
15 (Day 28)	560.0	4912.3	1141.0	7239.4	850.5	6075.9

\*ng/mL

†ng·hr/mL

There was some evidence of drug accumulation, especially in females in the higher dose groups.  $T_{max}$  was consistently ~1 hour, although evidence of prolonged absorption was observed on day 28 and in higher dose groups.

Comment: Previous experience with rapamycin indicates that the rat is very sensitive to macrolide immunosuppressants. Although ocular toxicity was associated with hyperglycemia in rats given rapamycin, the relationship was not substantiated in this study.

**Study title:**

A repeat toxicity study by oral gavage administration to HanIbm Wistar rats for 4 weeks followed by a 2 week reversibility period. Study # 203-050 ( \_\_\_\_\_ Report # 96/SPM090/0404); study dated 8-8-96.

b(4)

[HanIbm:Wistar rats; 10/sex/dose with additional 6/sex in the placebo and high-dose groups (recovery group) and 4/sex/dose for a toxicokinetic study; 164 total; daily oral (gavage) doses for four weeks with separate recovery group animals held for two week recovery/observation period; doses used: 0.1, 0.25, 0.5, 1.5 mg/kg/day; performed by \_\_\_\_\_

b(4)

—; drug batch g Y182 0895; dose volume: 5 mL/kg; placebo control (batch # Y174 0895); GLP study] **b(4)**

This study essentially repeated study 203-042 in order to determine a NOAEL. There were no deaths on study. No clinical signs were observed on study. Body weight gains, food consumption, and food conversion efficiency were decreased in the high-dose group. Ophthalmic effects (anterior suture line opacities of the lens) were demonstrated only in some animals in the high dose group. Hematologic effects (signs of hemoconcentration and lower platelet counts) were seen in males ( $\geq 0.5$  mg/kg/day) and in females ( $\geq 0.25$  mg/kg/day). Clinical chemistry effects (elevated serum cholesterol, triglycerides, and decreased serum albumin) were observed at doses  $\geq 0.5$  mg/kg/day, consistent with previous findings. Serum amylase activity was elevated in males ( $\geq 0.25$  mg/kg/day) and females (1.5 mg/kg/day). Urine volume was slightly decreased in females in the high-dose group. Sperm counts and motility were not affected by treatment.

Necropsy demonstrated the following changes in organ weights: thymus (decreased,  $\geq 0.5$  mg/kg/day); pituitary (decreased, females only,  $\geq 0.25$  mg/kg/day); epididymides and seminal vesicles (decreased, 1.5 mg/kg/day); ovaries (decreased,  $\geq 0.5$  mg/kg/day) and uteri (decreased,  $\geq 0.25$  mg/kg/day). Histopathology demonstrated thymic medullary atrophy ( $\geq 0.5$  mg/kg/day); chronic myocarditis (1.5 mg/kg/day); myocardial fibrosis (one male in the high-dose group); diffuse alveolar macrophages ( $\geq 0.5$  mg/kg/day, males; 1.5 mg/kg/day, females); interstitial cell hyperplasia of the ovaries (1.5 mg/kg/day); uterine and cervical atrophy (1.5 mg/kg/day); and depletion of salivary gland secretory granules (1.5 mg/kg/day).

Plasma drug levels were at or below the limit of quantitation in the 0.1 and 0.25 mg/kg/day groups. Values obtained in the two higher dose groups were reasonably consistent with results obtained previously.

Comment: Elevations in blood amylase observed in this study were not reported in the previous four week oral toxicity study.

**Study title:**

SDZ RAD: Toxicity study by oral gavage administration to HanIbm Wistar rats for 26 weeks followed by a four-week reversibility period

**Key study findings:**

- The SZD RAD (RAD001) related toxicities, included: mortality (1/50 at 1.5 mg/kg), decreased body weight gains/food conversion efficiency, hematological effects ( $\uparrow$  erythroid parameters and neutrophil counts), increased serum cholesterol and/or triglyceride levels.
- The main target organs on microscopic examinations were kidney, lung, lymphoid organs (lymph nodes and thymus), spleen, stomach, thyroid and male reproductive organs.

**Study no.:** Sandoz Study 96/SPM083/1130

**Volume #, and page #:** Electronic, module 4 (SPM/083.pdf)

Conducting laboratory and location: \_\_\_\_\_

b(4)

Date of study initiation: November 7, 1995

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: RAD001 (SDZ RAD), Batch # Y213 1095, purity: 99.0% (HPLC); placebo (for Group 1, the control): #Y214 1095 and #Y174 0895.

**Methods**

Doses: 0 (control), 0.05, 0.1, 0.15, 0.5 and 1.5 mg/kg (free base, see below for Group assignment)

Species/strain: Rats (SPF, HanIbm: WIST; \_\_\_\_\_)

b(4)

Number/sex/group or time point:

Main study: n=20/sex/group

Recovery animals: n=5/sex/group (For Groups 1 and 4 only, see table below)

Satellite groups used for toxicokinetics study: 12/sex/group

Group	Treatment	Dosages (mg/kg/day)#	Number of animals					
			Main study		Reversibility phase		Satellite study	
			Male	Female	Male	Female	Male	Female
1	Control	0	20	20	5	5	12	12
2	SDZ RAD	0.15	20	20	-	-	12	12
3	SDZ RAD	0.5	20	20	-	-	12	12
4	SDZ RAD	1.5	20	20	5	5	12	12
5	Control	0	20	20	-	-	12	12
6	SDZ RAD	0.05	20	20	-	-	12	12
7	SDZ RAD	0.1	20	20	-	-	12	12

# Expressed in terms of the test material as supplied.

Route, formulation, volume: oral gavage at a dose volume of 5 mL/kg

- Formulation: SDZ RAD (2% microemulsion) was diluted with 5% glucose.
- SDZ RAD placebo was diluted with 5% glucose at a concentration equivalent to the highest concurrent SDZ RAD dosage (i.e., 0.3 mg placebo/mL at 5 mL/kg for Group 1 to make for 1.5 mg/kg; and 0.02 mg/mL at 5 mL/kg for Group 5 to make for 1 mg/kg).

Age: ~7-9 weeks

Weight: 132-288 g

Schedule: Once daily for 26 consecutive weeks. The main study was followed by a 4-week (28-day) recovery period.

**Dose justification:** Dose selection was per sponsor's request. Originally only the control and 3 doses were planned (i.e., 0.15-1.5 mg/kg/day). The sponsor then requested to add one more control group and 2 more treatment groups with smaller doses (i.e., 0.05 and 0.1 mg/kg/day). Of note, these two sets of experiments were not conducted simultaneously. The treatment of higher doses (Groups 1-4) started on 11/29/1995, while the other set (Groups 5-7) on 4/10/1996.

**Observation and Times:**

- Clinical signs:** Twice daily for mortality, moribundity and gross abnormality. The highest grade of severity of the symptom in individual animals was recorded daily (Week 1), twice weekly (Weeks 2-4), once each week (Weeks 5-13) and once each fortnight (Week 14 onwards).
- Body weights:** One week before and on the day of treatment, then weekly during treatment and recovery period.
- Food consumption:** Once weekly during the acclimation, dosing and recovery periods. Food consumption was reported as mean weekly consumption per animal for each cage (n=5/cage).
- Food conversion efficiency:** Calculated as the overall group mean BW gain divided by the total food consumption, multiplied by 100. Weekly group mean food conversion efficiencies were calculated for the first 14 weeks of treatment.
- Ophthalmoscopy:** Once prior to the start of treatment in all main and recovery animals and on surviving Groups 1 and 4 main study animals during Weeks 5, 9, 13, 17, 22 and 26 of treatment and Week 4 of recovery period.
- EKG:** Not performed.
- Hematology:** Blood samples were taken (via the retroorbital sinus) from 10 rats/sex/group of the main study animals during Weeks 4, 14 and 26, as well as during Weeks 26 and 30 in all recovery animals.
- Clinical chemistry:** Blood samples were taken (via the retroorbital sinus) from 10 rats/sex/group of the main study animals during Weeks 4, 14 and 26, as well as during Weeks 26 and 30 in all recovery animals.
- Urinalysis:** Urine was collected (for ~ 17 hours) from 10 rats/sex/group of the main study animals during Weeks 4 (males), 5 (females), 12 and 24, as well as during Weeks 24 and 30 in all recovery animals.
- Bone marrow smears:** From the main study and recovery necropsies. Samples were only examined microscopically for those from Groups 1 and 4.
- Gross pathology:** Scheduled sacrifice: Weeks 26 and 30.
- Organ weights:** At scheduled sacrifice. Adrenal, brain, heart, kidney, liver, lung (with mainstem bronchi), ovaries, pituitary, prostate, salivary glands (submandibular), seminal vesicles, spleen, testis (including epididymides), thymus, thyroid (with parathyroids), and uterus (with cervix).
- Histopathology:** Week 26 or Week 30 (main study and recovery groups, respectively). Tissues collected from all animals. For tissues identified microscopically as potential targets (i.e., heart, lungs, stomach, submandibular salivary glands, testes and thymus), the H&E stained slides were examined in male or female animals in Group 2 and 3 of the main study and recovery groups. The following tissues were examined in main study animals (Groups 1-4) only: kidney\*, mesentary lymph nodes, spleen, thyroid (Groups 2 and 3: only males), mandibular lymph nodes (Groups 2 and 3: females only), and eyes (Groups 2 and 3: only in Group 3 males). See inventory list for organs examined.

*Reviewer's note\*:* During the course of the original study, the kidneys of the recovery animals were not examined histopathologically. The sponsor thus conducted a separate study (Study

#0770978 “Examination of kidneys in recovery animals of the 6-month rat study (96/SPM083/1130)”) to investigate the reversibility of renal findings in the main study groups. The histology slides were processed in kidneys from archived wet tissues from original study in 1995. The study was performed at the Department of Preclinical Safety, Pathology, Novartis Pharma AG, Basel, Switzerland, on February 1, 2008.

**Toxicokinetics:** Blood samples were collected from TK animals on Day 1 and during Weeks 13 and 26, at 1, 2, 4, 7, and 24 hours post-dose (n=4/ time point). The analysis was conducted via ELISA (for blood samples of Groups 1, 3 and 4, with LOQ of 2 ng/mL) or via HPLC/MS (blood samples of Group 2 and aqueous humor, with LOQ of 1.3 ng/mL). The TK analysis was not conducted for Groups 5-7, because the assay was not sensitive enough.

### **Results:**

#### Mortality:

There was one treatment-related death (moribund sacrifice, #72) in Group 4 main study males during Week 23. The clinical signs prior to death were mainly lesions at hind feet. Other findings included low hemoglobin, increased total leukocyte count associated with a high neutrophil count, and ulceration and poditis at the microscopic examination.

Another Group 4 male (#166, designated as a recovery animal) was sacrificed during Week 26, due to severe damage around the left eye as a result of blood sampling procedure. The death was not drug related.

Clinical signs: Not remarkable.

#### Body weights and weight gains:

➤ Percent reduction of group mean body weights (%) from the control was summarized:

Group	Males			Females				
	G1 (g)	G4 (g)	G4 (%)	G1 (g)	G3 (g)	G3 (%)	G4 (g)	G4 (%)
No. (a)	20	19	19	20	20	20	20	20
Week 26	425.8	369.8	<b>13*</b>	249.6	237.1	<b>5*</b>	227.6	<b>9*</b>
No. (b)	25	23 (c)	23	25	20	20	25	25
Week 26	434	379	13	258	244	5	235	9

**Bolded printed numbers represent statistically significant changes (\*based on Dunnett's test).**

No. (a): number of animals in main study group, No. (b): number of animals in main study group and recovery group, in the case of G1 and G4.

c: two Group 4 males died at end of 26 weeks.

➤ Group mean body weight gains:

Reductions of overall group mean body weight gains in treated animals (Week 0-26), in comparison with the control, were observed. Reductions of weight gains were more apparent in females than in males, and changes reached statistical significance mainly in females. Findings resolved in the recovery period. The statistically significant changes (% reduction from the control) are summarized in the tables below:

Group	Males			Females						
	G1 (g)	G4 (g)	G4 (%)	G1 (g)	G2 (g)	G2 (%)	G3 (g)	G3 (%)	G4 (g)	G4 (%)
No.	25	25	25	25	20	20	20	20	25	25
Week 0-26	194	134	31	84	75	11	71	29	62	26

Food consumption: Not remarkable.

Food conversion efficiency:

There was a trend of reduction in food conversion efficiency as doses increased from 0.15 to 1.5 mg/kg. No apparent dose-dependence was shown at doses 0.05-0.1 mg/kg/day.

Group	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Week 1-14	6.5	6.5	6.2	5.0	3.9	3.6	3.5	3.2

Ophthalmoscopy: Not remarkable

EKG: Not performed

Hematology:

The % changes (statistically significant) from the vehicle control (Group 1) were summarized in the table below:

Week 4:

Dose Group	Males			Females		
	G2	G3	G4	G2	G3	G4
Number of animals	10	10	10	10	10	10
PCV (Hct) ↑		4	7		5	10
HGB ↑	4	6	8			8
RBC ↑	4	8	12			6
MCV ↓		3	3			
WBC ↓				34	19	
Neutro (Ab) ↑			50	↓ 44		22
Lympho (Ab) ↓				33	24	24
Platelet ↓			23			16
PT ↓					7	5

Week 14:

Dose Group	Males			Females		
	G2	G3	G4	G2	G3	G4
Number of animals	9	10	8	10	10	10
PCV (Hct) ↑			5			7
HGB ↑	4	4	6			7
RBC ↑	4	6	12			6
MCH ↓			6			
MCV ↓			6			
WBC ↓	↑ 19			27		
Neutro (Ab) ↑			47			50
Lympho (Ab) ↓	↑ 21			28	22	20
Platelet ↓		17	20			
APTT ↑	10	10	14		13	

## Week 26 and Week 30 (Group 4 R):

Dose Group	Males				Females		
	G2	G3	G4	G4 R*	G2	G3	G4
Number of animals	15	15	15	4	15	15	15
PCV (Hct) ↑		7	5				7
HGB ↑	5	7	7				5
RBC ↑	6	11	13				4
MCH ↓		4	6				
MCV ↓		3	6				
WBC ↓	↑ 23			↑ 67	44	25	
Neutro (Ab) ↑	75		117				117
Lympho (Ab) ↓			20	↑ 73	47	32	29
Platelet ↓		15	18				17
APTT ↑		13	12				

\* G4R: Group 4 recovery animals (no remarkable findings in G4 recovery females)

## Comment:

- The main hematological effect of SDZ RAD included increased erythroid parameters (PCV, HGB and RBC) and neutrophil counts, as well as reduced platelet counts. These findings were persistent through the treatment period. Males appeared to be more susceptible to the treatment than females.

There were no remarkable findings in bone marrow examination.

Clinical chemistry:

The % changes (statistically significant) from the vehicle control (Group 1) are summarized in the table below:

## Week 4:

Dose Group	Males					Females				
	G6	G7	G2	G3	G4	G6	G7	G2	G3	G4
Dose (mg/kg)	0.05	0.1	0.15	0.5	1.5	0.05	0.1	0.15	0.5	1.5
N	10	10	10	10	10	10	10	10	10	10
Amylase ↑			16	23	38				24	62
Creatinine ↑					8				8	12
Cholesterol ↑		27		22	50			24		35
Triglycerides ↑		43							44	50
A/G ↓				6	13					
Phosphorus ↓	13	20	6	12	16	10	9 (NS)	13	10	17
Iron ↓								20	22	

## Week 14:

Dose Group	Males					Females				
	G6	G7	G2	G3	G4	G6	G7	G2	G3	G4
Dose (mg/kg)	0.05	0.1	0.15	0.5	1.5	0.05	0.1	0.15	0.5	1.5
N	10	10	10	10	10	10	10	10	10	10
Amylase ↑				18	27					41
Lipase ↑										50
Cholesterol ↑					20			22		22
Albumin ↓					8					
A/G ↓					13					
Phosphorus ↓	7	8		15	12	11	11	12	22	9 (NS)

Dose Group	Males					Females				
	G6	G7	G2	G3	G4	G6	G7	G2	G3	G4
Mg ↓							6	9	7	
Iron ↓	20			19 (NS)	18 (NS)		10	15	14	19

Week 26:

Dose Group	Males					Females				
	G6	G7	G2	G3	G4	G6	G7	G2	G3	G4
Dose (mg/kg)	0.05	0.1	0.15	0.5	1.5	0.05	0.1	0.15	0.5	1.5
N	10	10	10	10	15	10	10	10	10	15
Amylase ↑				20	16					17
Lipase ↑						16				
Glucose ↓				8	11	↑ 12				6
CPK ↑								82		75
Cholesterol ↑		28		29	38					
Triglycerides ↑			36					21		
Albumin ↓					8			6		8
A/G ↓					7					
Phosphorus ↓				7	8			19	23	13
Iron ↓	18	19			19			10	10	16

Week 30 (Recovery):

	Males	Females
Dose Group	G4	G4
Dose (mg/kg)	1.5	1.5
N	4	5
Amylase ↓		26
Phosphorus ↑	13	

All findings resolved.

Urinalysis:

Not remarkable; however, the urine volume was increased, thus there was a decrease in the specific gravity, in Groups 2-4; although neither of the changes reached statistical significance.

Gross pathology:

The findings were mainly in Groups 3 and 4:

	Group 1		Group 2		Group 3		Group 4		MR
	M	F	M	F	M	F	M	F	
No. of animals	20	20	10	10	10	10	19	20	4
Kidney Hydronephrosis		1		1	2		2		
Lung Areas of change	1		1		2		16*	1	3*
Thymus Areas of change	1		1		1		3	2	

**R:** recovery animals. There were no remarkable findings in female recovery animals.

**.\*:** statistically significant compared to the control

Findings in the pre-scheduled deaths (both were Group 4 males):

- #72: feet (abrasions, occasional pale and dark areas on hind feet), thymic lymph node (dark)
- #166: eyes (left eye\*: large and misshapen, dark, exophthalmic), mandibular lymph node (dark), skeletal muscle (musculature of left side of head and lower jaw: numerous poorly defined, dark areas, left orbit\*: abnormal content, red fluid and clotted blood). \*: likely due to blood sampling procedures.

Comment:

- Pale areas found in the lung correlated with increased organ weight (see below) and accumulation of alveolar macrophages. Sixteen of Group 4 male rats which showed pale foci in the lung, had accumulation of alveolar macrophages (severity: slight (n=1), moderate (n=12) and marked (n=2)).

**Organ weights:**

Organ weight changes (absolute or relative body weight, % change from the control) are summarized in the following table:

**Males**

Group	G2	G3	G4	G4R	G2	G3	G4	G4R
Parameter	g	g	g	g	% BW	% BW	% BW	% BW
Number of animals	20	20	19	4	20	20	19	4
Brain ↓			4				↑ 12	
Epididymides ↓	↑ 9		22	31			15	28
Kidney ↓			10					
Liver ↑						11	9	
Lung ↑			30			12	50	
Pituitary ↓	13	13	25		20	20	15 (NS)	
Prostate ↓			26				16	
Seminal vesicle ↓			19				14	
Spleen ↑								16
Submandibular SI GI ↓			20					
Testes ↓	↑ 7	↑ 13	14	31		↑ 14		28
Thymus ↓			30	↑ 58			19	↑ 64

**G4R: Recovery animals**

**g: absolute weight, % BW: relative to body weight**

**NS: not statistically significant**

**Females:**

Group	G3	G4	G4R	G3	G4	G4R
Parameter	g	g	g	% BW	% BW	% BW
Number of animals	20	20	5	20	20	5
Brain ↓			6		↑ 6	
Kidney ↓		7				
Liver ↑				9	15	
Lung ↑					15	
Pituitary ↓	20	40			38	
Spleen ↑			19		14	30
Thymus ↓		24			18	↑ 19 (NS)
Uterus ↓		40			34	

Comments:

✧ All Findings resolved.

Histopathological findings:

The following table is the summary of incidence and severity (expressed as incidence/group mean of severity) of drug-related histological findings. No remarkable findings were associated with Groups 5-7 animals.

Week 26 (main study animals):

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
No. of animals	20	a	a	19	20	a	a	20
Adrenal								
Microvesiculation of zona Fasci- Culata								2
Slight		(20)	(20)			(20)	(20)	
Kidney								
Hydronephrosis								
Slight		1	1	3		1		1
Moderate			1	2				
Marked			1		1			
Pigment within tubular epi cells								
Slight			1	12			3	12
Moderate								1
Lung		(20)	(20)			(20)	(20)	
Accumulation of alveolar macrophages								
Minimal	1	3	5		2	2	6	3
Slight	1		6	4		1	4	9
Moderate			1	13				6
Marked				2				1
Interstitial pneumonitis								
Minimal		1	1					
Slight			1	3				
Moderate				1				
Perivascular lymph infiltration								
Minimal						1	1	
Slight			2	9			1	9
Moderate				9				1
Lymph node, mandibular				(18)		(20)	(20)	
Lymphoid atrophy								
Slight							2	6
Lymph node, menstetric		(20)	(20)			(20)	(20)	
Lymphoid atrophy								
Slight			2	4			1	7
Lymphocytolysis								
Slight			1	2				
Sinus histiocytosis								
Slight		1		3				1
Salivary gland, submandibular		(20)	(20)			(20)	(20)	
Degeneration of ducts								
Slight								2
Moderate				1				

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
No. of animals	20	a	a	19	20	a	a	20
Skin	(0)	(0)	(3)	(2)	(0)	(0)	(0)	(0)
Scab(s)								
Slight				1				
Moderate			1	1				
Ulcers								
Moderate				1				
Spleen		(20)	(20)			(20)	(20)	
Hemosiderosis								
Slight			2	8		3	3	5
Moderate							1	8
Stomach		(20)	(20)			(20)	(20)	
Acute inflammation: Glandular region								
Slight		1	1	3		1		1
Mucus cell hypertrophy/hyperplasia								
Slight		1	2	3			3	1
Moderate			1	2				2
Testes		(20)	(20)					
Partial depletion of one or more generations of germ cell								
Minimal				6				
Slight				6				
Severe	1							
Spermatid giant cells								
Minimal				3				
Slight				8				
Tubular vacuolation								
Minimal				3				
Slight				8				
Thymus		(20)	(20)			(20)	(20)	
Hemorrhage								
Slight		1	4	2		1		
Moderate		1	1	1				1
Lymphocytolysis								
Slight				5				
Medullary atrophy								
Slight			10	1		1	10	11
Moderate			1	15		1		9
Marked				3				
Thyroid		(20)	(20)			(20)	(20)	
Follicular cell hypertrophy								
Slight			1	5				
Moderate				2				
Follicular cell vacuolation								
Moderate			1	2				
Reduced intrafollicular colloid								
Moderate			1	2				
Marked				2				

a: not microscopically examined, unless otherwise indicated by numbers in the parenthesis.

Week 30 (recovery animals):

Sex	Males		Females	
Group	1	4	1	4
No. of animals	5	4	5	5
Epididymidis				
Epithelial vacuolation				
Slight		1		
Reduced sperm content				
Present		2		
Kidney*				
Pigment disposition within tubular epithelial cells				
Trace	1	1	3	1
Minimal	1	3		3
Lung				
Accumulation of alveolar macrophages				
Minimal	2	1	1	1
Slight		1		1
Moderate		2		
Perivascular lymph infiltration				
Slight		1		
Stomach				
Mucus cell hypertrophy/hyperplasia				
Slight		1		
Testes				
Almost total depletion of germ cells leaving only sertoli				
Minimal		1		
Slight		1		
Partial depletion of one or more generations of germ cell				
Slight		1		
Moderate		1		
Spermatid giant cells				
Minimal		2		
Slight		2		
Tubular vacuolation				
Minimal		2		
Slight		2		
Thymus				
Hemorrhage				
Slight		1		
Medullary atrophy				
Slight		1		

\* Data also incorporated those obtained from Study 0770978 "Examination of kidneys in recovery animals of the 6-month rat study (96/SPM083/1130)"

Histopathological findings in animals (2 Group 4 males) that died within treatment period:

Animal	#72	#166
Feet		
Peditis: present	+	
Ulcers: marked	+	
Harderian glands		
Hemorrhage: moderate	+	
Kidney		
Acute inflammation of pelvis: slight		+
Pigment within tubular epithelial cells: slight	+	

Animal	#72	#166
Lung		+
Accumulation of alveolar macrophages: minimal		+
Perivascular lymph infiltration: slight		
Lymph node, mandibular		+
Erythrocytes and erythrophagocytosis in sinuses: moderate		
Lymphoid atrophy: moderate	+	
Lymph node, mesenteric		
Lymphoid atrophy: moderate	+	
Lymphocytolysis: slight	+	
Sinus histiocytosis: slight	+	
Lymph node, thymic		
Sinus histiocytosis: slight	+	
Salivary gland, submandibular		
Degeneration of ducts: moderate	+	
Skeletal muscle		
Hemorrhage into muscle: marked		+
Stomach		
Acute inflammation: Glandular region: slight		+
Mucus cell hypertrophy/hyperplasia: moderate	+	
Testes		
Almost total depletion of germ cells leaving only sertoli: minimal		+
Partial depletion of one or more generations of germ cells: slight		+
Spermatid giant cells		
Minimal	+	
Moderate		+
Tubular vacuolation		
Minimal	+	
Moderate		+
Thymus		
Hemorrhage: moderate	+	
Lymphocytolysis: slight		+
Medullary atrophy: moderate	+	+

Adequate Battery: yes (x), no ( )

Peer review: yes (x), no ( )

**Comments:**

- ✧ Oral administration of SZD RAD (RAD001) in rats identified the target organs as lymphoid organs (lymph nodes, thymus and spleen), lung, kidney, stomach, thyroid and reproductive organs (especially males). The incidence and severity of these lesions exhibited a dose-dependent trend; effects were mainly seen at 1.5 mg/kg/day.
- ✧ The findings of lymphoid atrophy and increased hemosiderosis were consistent with decreased thymic weights and increased splenic weights, respectively.
- ✧ Most of the findings were recovered or partially recovered at the end of the 4-week recovery period.

**Toxicokinetics:**

The C<sub>max</sub>, AUC<sub>0-24hr</sub>, and dose-normalized parameters are summarized in the tables below, in TK animals (n=4/sex/group/time point):

## Males:

Group	Day 1			Week 13			Week 26		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	0.15	0.5	1.5	0.15	0.5	1.5	0.15	0.5	1.5
C <sub>max</sub> (ng/mL)	1.8	11.2	69.3	1.2	10.9	35.9	1.5	7.1	14.4
C <sub>max</sub> /dose	12	22.4	46.2	8	21.8	23.9	10	14.2	9.6
AUC (ng•h/mL)	13.3	70.7	618.6	14.1	60.7	278.3	7.1	44.1	92.7
AUC/dose	88.7	141.4	412.4	94	121.4	185.5	47.3	88.2	61.8
T <sub>max</sub> (hr)	2	1	2	2	1	1	1	1	1

## Females:

Group	Day 1			Week 13			Week 26		
	G2	G3	G4	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	0.15	0.5	1.5	0.15	0.5	1.5	0.15	0.5	1.5
C <sub>max</sub> (ng/mL)	0.4	6.2	57.3	0.8	9.2	39.9	1.1	3.7	10.6
C <sub>max</sub> /dose	2.7	12.4	38.2	5.3	18.4	26.6	7.3	7.4	7.1
AUC (ng•h/mL)	4.3	69.7	394.4	11.3	67.6	287.9	8.1	13.1	50.8
AUC/dose	28.7	139.4	262.9	75.3	135.2	191.9	54	26.2	33.7
T <sub>max</sub> (hr)	2	1	1	4	1	1	2	1	1

Comment:

- ✧ The dose-normalized C<sub>max</sub> and AUC levels of SDZ RAD (RAD001) were more than dose-proportional in both males and females on Day 1 and Week 13. In Week 26, these values were approximately dose proportional.
- ✧ Repeated administrations of SDZR RAD did not exhibit evidence of accumulation, since the AUC levels were in fact higher on Day 1 than in Week 13 and in Week 26.
- ✧ AUC values were higher in males in Week 26.

Study summary and discussion:

See Section 2.6.7 "Toxicology tabulated summary".

- Treatment with SDZ RAD (RAD001, everolimus) in rats, dosing from 0.05 to 1.5 mg/kg/day for 26 weeks, induced the following findings: mortality (1/50 at 1.5 mg/kg/day), reduced body weight gain/food conversion efficiency, changes in hematological parameters (increased PCV/hematocrit, RBC and HGB, increased neutrophil counts, and decreased platelets), clinical chemistry (increased cholesterol, triglyceride and amylase, decreased albumin and iron), and changed organ weights (↑ spleen, ↓ thymus). The main target tissues/organs, based on histopathological examinations, were lymphoid organs (mainly lymphoid atrophy in lymph nodes and thymus, and lymphocytolysis in thymus), kidney (hydronephrosis, ↑ pigment within the tubular epithelial cells), lung (accumulation of alveolar macrophages and perivascular lymph infiltration), spleen (hemosiderosis), stomach (acute inflammation at glandular tissue and mucosal hyperplasia/hypertrophy), thyroid (follicular cell hypertrophy and vacuolation and reduced intrafollicular colloid) and male reproductive organs (epididymis and testes: reduced sperm count, depletion of germ cells, partial depletion of one or more generations of germ cells, spermatid giant cells, and tubular vacuolation).

- The cause of moribund sacrifice of #72 (Group 4 male) was lesions in feet. The impaired healing of the lesion was attributable to the drug effect, i.e., immunosuppression, anti-angiogenicity and effects on metabolic functions.
- Reduced food conversion efficiency was associated with reduced weight gains, and possibly also related with inflammation and hypertrophy/hyperplasia in mucous linings of the stomach (possibly due to chronic irritation). The latter may affect digestion and uptake of nutrients.
- The sponsor proposed a hemoconcentration mechanism to support the increased Hct (and decreased MCV and MCH), HGB and RBC. This was particularly noticeable for Group 4 males, because, according to the sponsor, changes in Group 3 males and females were generally within the range expected in the laboratory. As no diarrhea or vomiting was observed, the possible dehydration may be indicated by increased urine volume.
- Reduced plasma iron concentrations and the increased incidence of hemosiderosis in the spleen in Group 4 animals may suggest an increased production of erythrocytes. However, there were no remarkable changes in the cellularity and composition of the bone marrow.
- Changes in clinical chemistry:
  - Increased cholesterol and triglyceride levels were consistent with clinical findings of hyperlipidemia and the effects of other rapamycin (serolimus)-like agents. Comparing with changes of the triglyceride levels, increased cholesterol levels followed a better dose-dependent relationship.
  - Increases of amylase and lipase likely indicate the drug effects on metabolic functions. There were no histopathological findings in liver or pancreas, although lesions in pancreas were observed in monkeys and minipigs.
  - The clinical chemistry data in this study did not indicate renal lesions or hyperglycemia.
- Although no direct histopathological evidences were found in this study to indicate activities of SDZ RAD on hypothalamic-pituitary axis, decreased organ weights in pituitary (absolute and relative) and sex organs (epididymis, prostate, seminal vesicles, and uterus), histopathological findings in thyroid and sex organs (especially testes and epididymis) may suggest minor hormonal alterations due to effects on hypothalamic-pituitary axis.
- According to the sponsor, pigment inclusions within renal tubular epithelial cells in Groups 3 and 4 animals may be due to 1) lysosomal storage of polar lipids following chronic administration of amphiphilic drugs such as SDZ RAD, and 2) inhibition of lysosomal enzymes and resultant intralysosomal accumulation of proteins in the cells.
- There were no remarkable findings in eyes under the conditions of the study, although swelling and disruption of fibers in the anterior cortex of the lens were found in 4-week studies in rats (Study #95/SPM090/0404 at 1.5 mg/kg/day and Study #95/SPM052/0888 at 5 mg/kg/day, reviewed by Dr. K. Hastings).
- Unlike results obtained in other studies in rats (e.g., 4 week studies), there were no remarkable findings in the heart, i.e., myocarditis and/or myocardial fibrosis.
- Toxicokinetics:
  - ✧ The dose-normalized  $C_{max}$  and AUC levels of SDZ RAD were more than dose-proportional in both males and females in Day 1 and Week 13, but approximately dose proportional in Week 26.

- ✧ Repeated administrations of SDZR RAD did not exhibit evidence of accumulation, since the AUC levels were higher on Day 1 than in Week 13 and in Week 26.

**Study title:** 4-week oral (gavage) toxicity study in minipigs

**Key study findings:** Immunosuppressive effects seen as effects on leukocytes and lymphatic organs (spleen, thymus, lymph nodes). Skin had drug-related dermatitis, possibly due to immunosuppression. Pancreas and adrenal gland (cortex and medulla) were affected, as were male (testes) and female (ovaries and uterus) by toxicity.

**Study no:** 971033

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** Novartis Pharma AG  
Basel, Switzerland

**Date of study initiation:** 29 July 1997

**GLP compliance:** yes

**QA report:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** X011 0397, na, 94.1%

**Formulation/vehicle:** 9.1% solid dispersion in water

**Dosing:**

Species/strain: minipig, Gottingen SPF

#/sex/group or time point (main study): 3

Satellite groups used for toxicokinetics or recovery: 2 in high dose for recovery

Age: 3-4 months

Weight: 6.7-8.2 kg

Doses in administered units: 0, 1.5, 5.0, 15.0 mg/kg

Route, form, volume: oral gavage, not provided

**Observations and times:**

Clinical signs: daily

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: all pre-study, control and high dose, week 4

EKG: all pre-study, control and high dose, week 4

Hematology: pretest, study days 25, 43 and 57 (recovery)

Clinical chemistry: pretest, study days 25, 43 and 57 (recovery)

Urinalysis: not performed

Gross pathology: at necropsy

Organs weighed: see histopathology table

Histopathology: at necropsy

Toxicokinetics: day 1 at 1, 3, 7 and 14 h after dosing; day 2, 24h after dosing; day 28 at 1, 3, 7 and 28 h after dosing; day 29, 24 h after dosing

Other: rectal body temperature: day 8, male C12, day 9, male C12

**Results:**Mortality:

- 1 male (15 mg/kg) day 10; 3 males (15 mg/kg) sacrificed (days 8, 9, 10), poor health (sedation, tremor, weak limbs, cyanosis, shallow breathing, bradypnea, feces with blood)
- 1 female (15 mg/kg) sacrificed day 39 (recovery), poor health

Clinical signs:

- 15 mg/kg: diarrhea after day 7, see above sacrificed males; sedation, tremor, 1 male, week 3
- 5 mg/kg group: diarrhea, 1 male day 2-4,
- 1.5 mg/kg: diarrhea, males, starting day 5

Body weights: decreased in all treated males, females (15 mg/kg). After recovery, bodyweight was comparable to pre-study values

Food consumption: reduced in all treated males, females (15 mg/kg)

Ophthalmoscopy: no treatment related effects were seen

Electrocardiography: no treatment related effects seen

Hematology: decreased platelets, lymphocytes in treated males; slightly decreased lymphocytes, treated females

Clinical chemistry: increased BUN, creatinine,  $\alpha$ 2- and  $\beta$ 1-globulins, and cholesterol; decreased phosphorus, alkaline phosphatase,  $\alpha$ 1- and  $\beta$ 2-globulins, A/G ratio were seen in all treated groups

Urinalysis: not performed

Organ weights: lower weights: pituitary, testes, 1.5, 5.0 mg/kg males; pancreas, all treated males; uterus, ovaries, females, 5, 15 mg/kg

Gross pathology: discoloration of gut mucosa in sacrificed males

Histopathology:

Histopathology finding	1.5 mg/kg	5 mg/kg	15 mg/kg
Mucosal atrophy	3/3 m	2/3 m, 1/3 f	3/3 m, 3/3 f
Large intestine: erosion atrophy			3/3 m, 1/3 f 3/3 m, 2/3 f
Spleen: lymphoid depletion		1/3 f	3/3 m
Pancreas: vacuolated cells necrosis		3/3 m, 3/3 f 1/3 f	3/3 m, 3/3 f 2/3 m, 1/3 f
Thymus: lymphocytic phagocytosis atrophy	2/3 m 1/3 f	3/3 m 2/3 m, 3/3 f	3/3 m 3/3 m, 3/3 f

Adrenal cortex-zona fasciculata: Microvacuolation necrosis	2/3 m, 1/3 f 1/3 m	2/3 m, 2/3 f 1/3 f	3/3 m, 3/3 f 2/3 m
Adrenal medulla: Vacuolated cells		1/3 m	3/3 m
Testes: Spermatozoa giant cells Leydig cell hypertrophy	2/3 m	3/3 m	3/3 m 2/3 m
Ovaries: Necrotic follicles	1/3 f	3/3 f	3/3 f
Uterus: Glandular atrophy			3/3 f
Sternum Hypocellularity	2/3 m	3/3 m	3/3 m
Skin: dermatitis	2/3 m, 2/3 f	3/3 m, 3/3 f	2/3 f

Toxicokinetics:

The following parameters were determined, day 28/29:

Dose (mg/kg)	sex	n	Cmax (ng/ml)	AUC (0-24h) (ng.h/ml)
1.5	M	3	145.2	2937.2
	f	3	153.4	2402.3
5.0	M	3	399.7	6163.7
	F	3	346.5	5950.9
15.0	M	1	459.3	7982.6
	F	5	397.5	7729.5

**Summary of individual study findings:**

In this 4-week oral study in minipigs, spontaneous death or early sacrifice occurred at the high dose, 15 mg/kg. These animals had intestinal erosion, possibly due to infection exacerbated by immunosuppression. Signs of immunosuppression were seen in leukocytes and lymphatic organs (spleen, thymus, lymph nodes). Skin also had drug-related dermatitis, possibly due to immunosuppression. Pancreas and adrenal gland (cortex and medulla) were affected, as were male (testis) and female (ovaries and uterus).

Note: The following 4-week repeat dose toxicity study in monkeys was reviewed by Dr. K Hastings for IND \_\_\_\_\_

b(4)

**Study title:** Toxicity study by oral (gavage) administration to cynomolgus monkeys for 4 weeks followed by a 2 week reversibility period. Study # 203-054 \_\_\_\_\_ Report # 95/SPM049/1008); study dated 8-19-96.

b(4)

[3/sex/dose with an additional 2/sex in the placebo and high-dose groups (recovery group); 32 total; daily oral (gavage) doses for four weeks with separate recovery group animals held for two week recovery/observation period; doses used: 1.5, 5, 15 mg/kg/day; performed by \_\_\_\_\_ drug batch # Y110 0595; placebo controls (batch # Y111 0595); GLP study]

b(4)

No deaths occurred on study. Clinical signs observed on study included piloerection and reddening and/or staining of the abdomen in males in the high-dose group and skin lesions in

all treated groups. No significant effects of treatment on body weights or food consumption were observed.

Decreases in red cell parameters (Hct, HGB, RBC) were seen in the mid- and high-dose groups. Increases in leukocytes (due primarily to increases in neutrophils and monocytes) were seen in the mid- and high-dose groups. Plasma fibrinogen levels were increased in all treatment groups. Serum AST and ALT levels were increased in the high-dose group. Other clinical chemistry changes included decreases in serum inorganic phosphorus (all dose groups), albumin (mid- and high-dose groups), and urine sodium (high-dose group). Serum globulin levels (except gamma globulins) were elevated in the mid- and high-dose groups.

Necropsy demonstrated decreased thymus weights in all treatment groups. Pancreatic weights were decreased in some high-dose group animals. Histopathology effects included thymic medullary atrophy and intestinal histiocytosis in mid- and high-dose group animals. Splenic lymphoid atrophy was seen in all dose groups. Minor skin lesions were seen in mid- and high-dose group animals. Most adverse effects were not seen after the recovery period.

The following pharmacokinetic parameters (sexes combined) were determined using blood samples obtained on study:

Dose (mg/kg/day)	AUC <sub>0-24</sub> <sup>*</sup>		AUC/dose <sup>*</sup>		C <sub>max</sub> <sup>†</sup>	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
1.5	1142.4	1085.6	761.6	723.7	110.7	113.1
5	2623.9	3203.7	524.8	640.7	221.8	300
15	4787.6	6978.2	319.2	465.2	314.1	747.9

<sup>\*</sup> ng·hr/mL

<sup>†</sup> ng/mL

There was evidence of accumulation, especially in the high-dose group. Accumulation factors were 2.6 and 2 (C<sub>max</sub>) and 1.5 and 1.4 (AUC) in males and females in the high-dose group, respectively. T<sub>max</sub> values varied from 1 to 3.3 hours.

**Study title:** SDZ RAD: Toxicity study by oral gavage administration to cynomolgus monkeys for 26 weeks followed by a four week reversibility period

**Note:** the study was reviewed by Dr. S. Kunder for NDA   Modifications have been **b(4)** made to the review.

**Key study findings:**

- The SZD RAD (RAD001) related toxicities, included: mortality (2/12 at 5 mg/kg and 2/8 at 1.5 mg/kg), clinical signs (skin lesions and deterioration of health), decreased body weight /food consumption (mainly at 5 mg/kg), hematological effects (↓ HGB and Hct and ↑fibrinogen), organ weights (↓ thymus, testes, spleen in females).

- The main target organs on microscopic examinations were lymphoid tissues (atrophy: thymus, lymph nodes, spleen), GI (small intestine: aggregation of macrophages, large intestine: mucosal inflammation), skin (epidermal necrosis, inflammation, ulceration), heart (myocardial degeneration), pancreas (degranulation of exocrine cells, degeneration of pancreatic islet cells), adrenal (cytoplasmic vacuolation) and ovary (reduced follicular development and increase in follicular atresia).

**Study no:** 96/SMP078/1067 (203-072)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 28 November 1995

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug Lot #** Y213 1095

**Formulation/vehicle:** solution in 5% dextrose

**Methods (unique aspects):** none

**Dosing:**

Species/strain: cynomolgus monkeys, \_\_\_\_\_

b(4)

#/sex/group or time point (1<sup>st</sup> treatment phase): 4, except control and 5 mg/kg: 6/sex/group. *Due to unexpected mortality at 5 mg/kg/day, the dosing at this dose was terminated in 9-10 weeks. No recovery phase was conducted. A second treatment phase was commenced later to include a lower dose (0.1 mg/kg/day) with a concurrent control group (see below).*

2<sup>nd</sup> treatment phase (#/sex/group): controls (2); 0.1 mg/kg (4)

Age: 31-39 months (1<sup>st</sup> treatment phase), 11-15 months (2<sup>nd</sup> treatment phase)

Weight: 1.69-2.47 kg (1<sup>st</sup> treatment phase), 1.28-1.75kg (2<sup>nd</sup> treatment phase)

Doses in administered units: 0.5, 1.5, 5 mg/kg; 0.1 mg/kg (2<sup>nd</sup> treatment phase)

Route, form, volume: oral gavage, 5 mL/kg

**Observations and times:**

Clinical signs: daily

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: prior to treatment, weeks 11/12, 25

EKG: prior to treatment, weeks 1, 12/13, 25/26

Hematology: prior to treatment, weeks 4, 13, 26

Clinical chemistry: prior to treatment, weeks 4, 13, 26

Urinalysis: prior to treatment, weeks 4, 12 24

Gross pathology: at necropsy

Organs weighed: see table

Histopathology: at necropsy, see table

Toxicokinetics: day1, weeks 13, 26 @ 1, 2, 4, 7, 24 h post treatment

Other: not applicable

**Results:**

Dose (mg/kg)	0.1 (2 <sup>nd</sup> phase)	0.5	1.5	5.0 (treatment terminated after week 9)
Mortality			2 M, sacrificed 1 week 14, 1 week 25; both approx. 25% body weight loss	2/12 (1M, 1F) sacrificed week 9, COD: weight loss, deterioration in health, skin lesions (abrasions, ulcerations)
Clinical signs	Skin ulceration	Skin ulceration (M)	Skin ulceration; Piloerection, hunched posture	Skin ulceration; Piloerection, hunched posture
Body weights				5M, 2F: loss of body weight (~10%)
Food consumption				food consumption decreased, after week 3
Ophthalmoscopy	Not remarkable			
ECG	Not remarkable			
Hematology			Decreased hemoglobin, packed cell volume Increased fibrinogen	Decreased hemoglobin, packed cell volume Increased fibrinogen
Clinical chemistry		Slight increased cholesterol	Decreased albumin Slight increased cholesterol	Decreased albumin, A/G ratio Decreased phosphorus Slight increased cholesterol and triglyceride
Urinalysis	Not remarkable			
Organ weights		Decreased weight: Thymus Spleen (F)	Decreased weights: Thymus Testes (slight) Thyroid (slight) Pancreas (slight) Spleen (F)	Decreased weights: Thymus Testes (slight) Pancreas (slight) Spleen (F)
Gross pathology		Skin lesions	Skin lesions Reduced thymic size	Skin lesions Reduced thymic size
Histopathology		Pancreas: degranulation of exocrine cells 1M Small intestines: accumulation of macrophage in villi Spleen : atrophy of germinal centers	Thymus: cortical and medullary atrophy Lymph node atrophy Pancreas: degranulation of exocrine cells 3M Spleen : atrophy of germinal centers Small intestines: accumulation of macrophage in villi Myocardial degeneration (1) Ovaries: reduced follicular development, increased atresia	thymus: cortical and medullary atrophy Lymph node atrophy Spleen : atrophy of germinal centers Small intestines: accumulation of macrophage in villi myocarditis (1) Large intestine: mucosal inflammation Myocardial necrosis (2) Pancreas: degenerated islet cells; degranulation of exocrine cells Adrenals: cytoplasmic vacuolation Ovaries: reduced follicular development, increased atresia
Toxicokinetics AUC (ng•h/mL)				
Males Day 1	83	389	1096	4049
Week 13	53	280	777	4913 (week 9)
Week 26	145	358	1106	NA
Females Day 1	112	564	1133	3504
Week 13	NA	332	779	3322 (week 9)
Week 26	233	466	1218	NA

NA: not applicable

**Summary of individual study findings**

Study findings included thymic cortical and medullary atrophy, lymphoid follicular atrophy and medullary depletion, splenic atrophy (germinal centers), aggregations of macrophages in the small intestine, mucosal inflammation of the large intestine, ulceration and abrasion of skin

with inflammation and epidermal necrosis, myocardial degeneration of the heart and myocardial necrosis, myocarditis, degranulation of the exocrine cells of the pancreas, cytoplasmic vacuolation of the adrenals, reduced follicular development of the ovaries and follicular atresia, and medullary tubular dilation of the kidney. The effects of SDZ RAD on immune tissues are pharmacological; others such as those of the heart, kidneys, reproductive organs, adrenals and pancreas are toxicities unrelated to the intended pharmacologic activity, occur at all doses.

**Study title:** 52-week oral (gavage) toxicity study in the cynomolgus monkey

**Note:** the study was reviewed by Dr. S. Kunder for NDA   Modifications have been made to the review. b(4)

**Key study findings:**

- The SDZ RAD (RAD001) related toxicities, included: mortality (3/8 at 0.9 mg/kg), GI clinical signs (soft feces, diarrhea), decreased body weight /food consumption (mainly in pre-scheduled deaths), hematological effects (↓ erythroid parameters and lymphocytes, ↑ % band cells and fibrinogen), organ weights (↓ testes and epididymis).
- The main target organs on microscopic examinations were GI (acute inflammation) and male reproductive organs.

**Study no:** 1463-045

**Volume # and page #:** Module 4

**Conducting laboratory and location:** \_\_\_\_\_ b(4)

**Date of study initiation:** Aug 10 1997

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** SDZ RAD, X011 0937/X176 1297, purity: not reported; control (placebo): hydroxypropylmethylcellulose (HPMC) 3 cps and \_\_\_\_\_ Mesh (supplied by the sponsor, Batch # 96818/988 000 21 and #97800006, respectively). b(4)

**Formulation/vehicle:** aqueous solution, water

**Methods (unique aspects):** none

**Dosing:**

Species/strain: cynomolgus monkeys

#/sex/group or time point (main study): 4

Satellite groups used for toxicokinetics or recovery: No separate groups

Age: 2-9 yrs

Weight: 3.0-5.1 kg (males), 2.7-5.3 kg (females)

Doses in administered units: SDZ RAD: 0.1, 0.3, 0.9 mg/kg; placebo: HPMC 8.1 mg and lactose 0.9 mg

Route, form, volume, and infusion rate: oral gavage, 5 mL/kg

**Observations and times:**

Clinical signs: daily

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: prior to study, study weeks 26, 39, 52

EKG: prior to study, study weeks 13, 26, 39, 52

Hematology: prior to study, study weeks 13, 26, 39, 52

Clinical chemistry: prior to study, study weeks 13, 26, 39, 52

Urinalysis: prior to study, study weeks 26, 39, 52 for 16 h

Gross pathology: at necropsy

Organs weighed: at necropsy, see table

Histopathology: at necropsy, see table

Toxicokinetics: day 1, weeks 26, 39, 52 at 0, 1, 2, 4, 7, and 24 h post-dose

Other: blood pressure, prior to study, study weeks 26, 39, 52

**Results:**

Dose (mg/kg)	0	0.1	0.3	0.9 (terminated after 39 weeks)
N (/sex/group)	4	4	4	4
Mortality		1 male (#7179), sacrificed moribund day 225 (not drug related, due to severe glomerulonephritis)		1 male, sacrificed moribund, day 142 (chronic diarrhea, hunched posture, poor physical condition) 2 females, sacrificed day 183, day 258 (chronic diarrhea, emaciation, poor physical condition)
Clinical signs			Males (4/4): soft feces and diarrhea (starting 4-6 weeks post dosing)	All animals: diarrhea, soft feces (starting 4-6 weeks post dosing)
Body weights ↓		Not remarkable	↓ weight: 2 M	↓ weights: mainly in animals subjected to moribund sacrifice
Food consumption		↓ in #7179 prior to sacrifice	↓ in two males (#7607 and 7779)	Decreases in animal sacrificed for morbidity prior to sac
Ophthalmoscopy	No treatment-related effect observed			
Electrocardiography and blood pressure	No treatment-related effect observed			
Hematology		Week 13: F: ↑ % band cells (100%, not statistically significant)  Week 13: F: ↑ % band cells (100%, not statistically significant)  Week 39: F: ↑ % band cells (200%)	Week 13: F > ↓ HGB (10%), > ↑ % band cells (200%, not statistically significant)  Week 26: F: ↑ % band cells (300%, not statistically significant)  Week 39: F: ↑ % band cells (500%)  Week 52 M: ↓ lymphocyte (%): 42%	Week 13: F > ↓ HGB (16%), Hct (15%), MCV (10%), MCH (11%) > ↑ % band cells (600%) > ↑ fibrinogen (47%) Week 26: F > ↓ HGB (19%), Hct (17%), MCV (11%), MCH (14%) > ↑ % band cells (1400%) > ↑ fibrinogen (131%) Week 39: > F: ↑ % band cells (1100%, not statistically significant) > ↑ % band cells and ↓ lymphocyte (lymphocytopenia) were noted in pre-schedule deaths.
Clinical chemistry*		#7179M: ↑ blood urea, creatinine, cholesterol/HDL, K <sup>+</sup> , α-amylase ↓ total protein/albumin,	Not remarkable	#4064/A (M): ↑ blood urea, creatinine, glucose, K <sup>+</sup> , α-amylase, phosphorus, lipase ↓ Na <sup>+</sup> , Ca <sup>2+</sup> , Cl <sup>-</sup> , cholesterol/HDL #8249F: ↑ blood urea, AST, AP,

	Na <sup>+</sup> , phosphorus		triglycerides, Ca <sup>2+</sup> , K <sup>+</sup> , α-amylase ↓ Na <sup>+</sup> , Cl <sup>-</sup> , lipase #8251F: ↑ bilirubin, AST, K <sup>+</sup> , triglycerides ↓ phosphorus
Urinalysis	#7179M: + protein and glucose	+ glucose (1 F, through the study); +protein (1 M, Week 26)	+ glucose (1 F, Week 39)
Organ weights ↓ testes: ab§ relative to BW epididymis:ab§ relative to BW	<b>Note:</b> individual findings (foot note)	(% decrease from control) 57% (not significant) 44% (not significant) 57% 40%	73% (not significant) 73% (not significant) 53% (not significant) 50% <b>Note:</b> individual findings (foot note)
Gross pathology	#7179M (moribund) ascites, hydropericardium, renal and hepatic enlargement	3 M: congested large intestine, mesenteric lymph nodes: enlargement	1M, 2F (moribund): mesenteric lymph node enlargement, congestion of intestinal vessels 1M lung, white-yellow focus 1F, cecal ulceration
Histopathology		3M, 2F: acute inflammation large intestine 2M: immaturity of testes (1M immaturity of prostate, seminal vesicles)	2F inflammation of colon /cecum  2M, 2F: stomach inflammation/erosion  all M: testicular immaturity
Toxicokinetics AUC (ng·h/mL) Males Day 1 Week 26 Week 39 Week 52 Females Day 1 Week 26 Week 39 Week 52 C <sub>max</sub> (ng/mL) Males Week 1 Week 26 Week 39 Week 52 Females Week 1 Week 26 Week 39 Week 52	1.740 57.739 87.809 98.028 26.056 78.013 80.465 59.561 0.696 5.389 6.476 8.532 8.759 15.795 11.984 10.135	135.857 270.747 349.909 275.571 161.315 144.396 246.685 176.222 13.814 24.776 36.393 24.092 31.214 15.816 35.445 20.541	362.596 690.809 941.299 NA 732.490 395.736 471.777 NA 27.900 67.599 84.921 NA 86.079 37.157 47.824 NA

\* Large inter individual differences were noted, mainly due to differences between the survivals and the pre-schedule deaths. Changes in the latter reflected the consequences of fluid loss (due to diarrhea) and malnutrition (due to GI lesions). Findings in the sacrificed animals are described.

§: absolute organ weights

Organ weight findings:

- #7179M: ↑ kidney, liver; ↓ pancreas, thymus, testes, epididymis and prostate
- #4064/A (M): ↑ adrenal; ↓ thymus, testes, epididymis and seminal vesicles
- #8249F: ↓ thymus, uterus
- #8251F: ↑ kidney, liver and uterus; ↓ pituitary and thymus,

NA: not applicable

**Summary of individual study findings:**

Study findings included diarrhea, decreased body weights and food consumption, increased fibrinogen, increased % band cells, glucose and protein in urine, decreased testes and epididymis weights, congested large intestine, enlarged mesenteric lymph nodes, inflammation of intestines and stomach, and testicular/seminal vesicle/prostate immaturity. High dose (0.9 mg/kg) animals were sacrificed in moribund condition due to poor health; the group was terminated at 39 weeks.

**Special repeat-dose toxicology studies:**

## ■ Formulation comparison:

**Study title:** A comparative 2-week oral (gavage) toxicity study in the rat with a micro-emulsion and a solid dispersion [Study no: — project 617951 (203-078)] **b(4)**

Note: this study was reviewed by Dr. S Kunder for NDA — Formulation comparison was made between SDZ RAD micro-emulsion (batch no. Y182 0895, purity: 99.6%) and SDZ RAD solid dispersion (batch no. 95701, purity: 100.1%). The result indicated that there are no major toxicologic differences between the two forms of SDZ RAD used in this rat study. The “study summary and conclusions” was excerpted from Dr. Kunder’s review.

**Study summary and conclusions (with modifications):**

This comparative study of SDZ RAD microemulsion and SDZ RAD solid dispersion in rats resulted in no deaths but demonstrated the toxicities typically seen in other rat toxicity studies. Decreased food consumption and weight loss were seen in both groups at the high dose. Decreased organ weights included ↓ brain, spleen, testes, and prostate weights at the 15 mg/kg dose (both formulations) and ↓ pituitary, pancreas, thymus and uterus weights (only on solid dispersion form) at 1.5 and 15. mg/kg. Seminal vesicles were reduced in size in males at 15 mg/kg (9/10). Histopathologic findings at 1.5 and 15 mg/kg included atrophy of lymphoid tissues (thymus, spleen, lymph nodes), male (testes: tubular degeneration, spermatid symplasts, vacuolation; prostate and seminal vesicle: atrophy) and female (uterus: atrophy) reproductive organs, myocardial degeneration, swelling of brain ventricles, increased incidence of alveolar histiocytosis in the lung and vacuolation of the adrenal cortex. Changes observed mainly at 15 mg/kg included: bone marrow and sternum (atrophy and hypocellularity), eye (swelling of the cortical lens fibers), male (epididymis: oligospermia; prostate and seminal vesicle: hyposecretion) and female (mammary gland and vagina: atrophy) reproductive organs, and spleen (↓ hemopoiesis). Changes such as myocardial degeneration and swelling of the brain ventricles may be related to underlying infection exacerbated by immunosuppression. No major toxicologic differences between the two forms of RAD used in this rat study were demonstrated.

## ■ Investigation of impurities: batch control studies

Reviewer's note: The following request was submitted to the sponsor by the CMC reviewer, Dr. Ravi Kasliwal (Document # 3745429\_ANSW\_MP\_840\_3):

“Based on the proposed limits for each of the following specified impurity, the maximum per day exposure, for 10 mg daily dose, would be as noted below:

- Specified impurity ✓
- Specified impurity
- Specified impurity
- Specified impurity
- Specified unidentified

b(4)

Provide the toxicological qualification level for each impurity and the study number in which the level was qualified. Also, if the proposed limits are higher than the levels qualified, reduce the limit for the impurity and provide correspondingly amended specifications.”

The sponsor responded on October 30, 2008 and provided the toxicological qualification levels for each impurity in question and the study number in which the level was qualified. The following tables were provided by the sponsor.

**Table 4-1 Toxicological level of specific impurity**

Drug Product			Qualification Animal Toxicology Studies				
Impurities	Release (%)	Control (%)	Estimated total daily intake <sup>1</sup> (mg/day)	Concentration (%)	Dose (mg/kg/day)	Total daily intake (mg/kg/day)	Safety margins <sup>2</sup>
							222
							60
							45
							69
							Below qualification threshold based on ICHQ3B(R2) for 10 mg/day dose

b(4)

<sup>1</sup> Based on daily dose of 10 mg/day

<sup>2</sup> Safety margin = estimated total daily intake in animals/estimated total daily intake in human (Assuming 60 kg body weight)

<sup>3</sup> Study no. 96/SPM091/0532: 2-week oral toxicology study in rats (Batch Y254 1295, doses up to 5 mg/kg/day)

<sup>4</sup> Study 991094: 4-week oral toxicity study in rats (Batch X033 0199, doses up to 5 mg/kg/day)

<sup>5</sup> Study RCC 634678: 2-week oral toxicity study in rats (Batch X096 0796, doses up to 15 mg/kg/day)

**Table 4-2 Toxicological qualification level of specific impurity**

Specified Impurity	Max daily intake based on 10 mg dose levels (µg)	Study no. and description
		Studies with Batch no. X096 0796 (levels of _____) Study 634678: 2-week oral study in rats (0, 1.5 and 15 mg/kg) Study Mut.Bakt 66/96: in vitro Ames assay
		Study Z63: in vitro chromosome aberration study in V79 Chinese hamster cells Studies with Batch no. Y254 1295 (levels of _____) Study 96/SPM091/0532: 2-week oral toxicity study in rats (0, 0.25, 5 mg/kg/daily)
		Studies with Batch no. X096 0796 (levels of _____) Study 634678: 2-week oral study in rats (0, 1.5 and 15 mg/kg) Study Mut.Bakt 66/96: in vitro Ames assay Study Z63: in vitro chromosome aberration study in V79 Chinese hamster cells
		Studies with Batch no. X033 0199 (levels of _____) Study 991094: 4-week oral study in rats 0, 1.5 and 5 mg/kg Study 001801: in vitro Ames assay Study 001831: in vitro chromosome aberration assay with V79 Chinese hamster cells
		Studies with Batch no. X033 0199 (levels of _____) Study 991094: 4-week oral study in rats (0, 1.5 and 5 mg/kg) Study 001801: in vitro Ames assay Study 001831: in vitro chromosome aberration assay with V79 Chinese hamster cells
		Below the qualification threshold ( _____ ) based on ICHQ3B(R2) for a 10 mg/day dose
		Below the qualification threshold ( _____ ) based on ICHQ3B(R2) for a 10 mg/day dose

b(4)

Of note, the margin of safety provided by the sponsor seems to be based on a mg/kg analysis. A calculation based on body surface area also shows acceptable safety margins. Therefore, based on the toxicology studies, the sponsor's justification of the proposed control limit (%) is acceptable. The genotoxicity potential of the impurities is addressed in Section 2.6.6.4.

The following section is a summary review of pertinent repeat dose toxicology studies for the impurities of concern. The batch number of RAD001 containing these impurities are in bolded prints. These studies were reviewed by Dr. S. Kunder for NDA \_\_\_\_\_

b(4)

**Study title:** Comparative toxicity study in HanIbm Wistar rats with batches differing in by-product content [**Study no:** 96/SPM091/0532 (203-076)]

Note: this study was reviewed by Dr. S Kunder for NDA \_\_\_\_\_ Batch comparison was made between Batch no. Y003 0196 (stabilized, purity: 90.5%) and **Y254 1295** (stressed\*, purity: 82.2%). The result indicated that there were no significant differences in toxicology findings between these batches. The key findings were excerpted from Dr. Kunder's review.

b(4)

\*: The stressed batch contained \_\_\_\_\_ a degradation product, while the stabilized batch was stabilized with: \_\_\_\_\_ The toxicity profiles of these two batches were compared in this study. b(4)

**Key study findings:** The pharmacologic action of RAD was evident in the thymus, spleen, and lymph nodes. Myocarditis may be at least partially related to immunosuppressive properties of RAD001. Effects on the reproductive organs in both males (testes, prostate, epididymides, seminal vesicles) and females (uterus) appear to be partially a toxic effect on endocrine hormones. The toxic effect in eyes, i.e., swelling/disruption of fibers in anterior cortex of the lens, is similar to the previously seen lens toxicity. The stressed RAD (Batch # Y254 1295), presumed to contain the impurity \_\_\_\_\_ (not quantified), did not appear to cause additional toxicities. At the stressed 0.25 mg/kg dose, uterine atrophy was seen but not in the unstressed RAD females. Otherwise existing toxicities were not exacerbated by the administration of stressed RAD. b(4)

**Study title:** 4-week oral toxicity study in rats (batch comparison)

[Study no: 991094]

Note: this study was reviewed by Dr. S Kunder for NDA \_\_\_\_\_ Batch comparison was made between Batch no. X033 0199 (batch 1, purity 92.9%) and Batch no. X153 1098 (batch 2, purity 96.9%). The result indicated that there were no significant differences in toxicology findings between these two batches. The key findings were excerpted from Dr. Kunder's review. b(4)

**Key study findings:** Both batches of RAD exerted expected effects on thymus, spleen and lymph nodes. Other toxicities included those in male reproductive (testes, prostate gland, seminal vesicles, epididymides; mammary gland) and female reproductive (pituitary, ovaries, uterus, vagina) organs, which showed effects in the reproductive/endocrine axis. Other toxicities included myocardial degeneration and swelling of cortical lens fibers of the eye, seen in other RAD studies as well as adrenal cortex vacuolation. Batch 1 also had renal vasculopathy, seen in other rat studies. Otherwise, the toxicity profiles of each of these batches are nearly identical.

**Study title:** A comparative 2-week oral (gavage) toxicity study in the rat with two different batches [Study no: \_\_\_\_\_ project 634678 (203-077)] b(4)

Note: this study was reviewed by Dr. S Kunder for NDA \_\_\_\_\_ Batch comparison was made between batch nos. X081 0596 and X096 0796. The result indicated that there were no significant differences in toxicology findings between these two batches. The findings were also similar to other toxicology studies in rats.

**Histopathology inventory (optional):** Summary of pivotal toxicity studies only.

Study (Duration)	96/SPM083/1130 (26-wk)	96/SPM078/1067 (26-wk)	1463-045 (52-wk)
Species	Rat	Monkey	Monkey
Adrenals	x*, §	x, §	x, §
Aorta	x*	x	x
Bone Marrow smear	x*	x	x
Bone (femur)	x*	x	x
Brain	x*, §	x, §	x, §
Cecum	x*	x	x
Cervix	x, §	x, §	x
Colon	x*	x	x
Diaphragm		x	
Duodenum	x*	x	x
Epididymis	x**, §	x, §	x, §
Esophagus	x*	x	x
Eye	x	x	x
Fallopian tube			
Gall bladder		x	x
Gross lesions			
Harderian gland	x*		
Heart	x**, §	x, §	x, §
Ileum	x*	x	x
Injection site			
Jejunum	x*	x	x
Kidneys	x**, §	x, §	x, §
Lacrimal gland	x*	x	x
Larynx			
Liver	x, §	x, §	x, §
Lungs	x**, §	x, §	x
Lymph nodes, axillary		x	
Lymph nodes, bronchial			x
Lymph nodes (sub)mandibular	x*	x	x
Lymph nodes, mesenteric	x	x	x
Mammary Gland	x*	x	
Nasal cavity			
Optic nerves			
Ovaries	x*, §	x, §	x, §

Study (Duration)	96/SPM083/1130 (26-wk)	96/SPM078/1067 (26-wk)	1463-045 (52-wk)
Species	Rat	Monkey	Monkey
Pancreas	x*	x, §	x, §
Parathyroid	x*	x, §	x
Peripheral nerve			
Pharynx			
Pituitary	x*, §	x, §	x, §
Prostate	x*, §	x, §	x, §
Rectum	x*	x	x
Salivary gland	x**, §	x, §	x
Sciatic nerve	x*	x	x
Seminal vesicles	x*, §	x, §	x, §
Skeletal muscle	x*	x	x
Skin	x*	x	x
Spinal cord	x*	x	x
Spleen	x, §	x, §	x, §
Sternum	x*	x	x
Stomach	x**	x	x
Testes	x**, §	x, §	x, §
Thymus	x**, §	x, §	x, §
Thyroid	x*, §	x, §	x, §
Tongue	x*	x	x
Trachea	x*	x	x
Urinary bladder	x*	x	x
Uterus	x, * §	x, §	x, §
Vagina	x*	x	x
Zymbal gland			

x, histopathology performed (all groups, but not recovery animals)

\* performed only in Groups 1 and 4

\*\* performed in all groups and recovery animals

§ organ weight obtained

#### 2.6.6.4 Genetic toxicology

**Study title:** Mutagenicity test using *Salmonella typhimurium*

##### **Key study findings:**

- SZD RAD 666 at concentrations of 8-5000 µg/plate, with or without S-9 mix, was not mutagenic in *Salmonella typhimurium* TA98, TA97a, TA100, TA102 and TA1535 strains, under the conditions tested.

**Study no.:** #Mut.Bakt. 27/95

**Volume #, and page #:** Electronic submission, Module 4 (Mut.Bukt.27/95.pdf)

**Conducting laboratory and location:** Genetic Toxicology, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland.

**Date of study initiation:** June 16, 1995

**GLP compliance:** Yes (OEDC)

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** RAD001 (SDZ RAD 666), Batch # 94902, Purity: 96.7 %

**Formulation/vehicle:** Distilled water

**Methods:**

Strains: *Salmonella typhimurium* TA97a, TA98, TA100, TA1535 and TA102

Concentration selection criteria

Basis of concentration selection: based on previous experiments. The sponsor employed 5 mg/plate as the highest concentration in the study. RAD001 precipitated on the test plates at  $\geq 2500$   $\mu\text{g}/\text{plate}$ . RAD001 was not bacteriotoxic up to 5000  $\mu\text{g}/\text{plate}$ , i.e., the highest concentration tested.

Test agent stability: Stable

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Vehicle: DMSO (100  $\mu\text{L}/\text{plate}$  for plate incorporation test)

Negative controls: vehicle control

Positive controls: all dissolved in DMSO

With S-9: TA97a, TA98, TA100, TA102 and TA1535: 2-aminoanthracene (3  $\mu\text{g}/\text{plate}$ ),  
TA98: Benzo[a]pyrene (3  $\mu\text{g}/\text{plate}$ )

Without S-9: TA98: 2-nitrofluorene (2  $\mu\text{g}/\text{plate}$ ), TA 97a: 9-aminoacridine (100  $\mu\text{g}/\text{plate}$ ),  
TA100 and TA1535: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (3  $\mu\text{g}/\text{plate}$ ), TA102: mitomycin C (0.5  $\mu\text{g}/\text{plate}$ ).

Exposure conditions:

Incubation and sampling times:

➤ Plate incorporation: 4 days

Concentrations used:

➤ Experiment 1: 8, 40, 200, 1000 and 5000  $\mu\text{g}/\text{plate}$ .

➤ Experiments 2: 156.25, 312.5, 625, 1250, and 2500  $\mu\text{g}/\text{plate}$

Study design: Plate incorporation for both Experiment 1 and Experiment 2.

Analysis:

No. of replicates: 3 plates for each test compound concentration

Counting method: automated colony counter (image analyzer). Due to precipitations on the tester plates at 2500 and 5000  $\mu\text{g}/\text{plate}$ , manual counting of the corresponding tester plates was necessary in most cases.

Criteria for positive results:

The test compound was considered mutagenic if it produced, in  $\geq$  one concentration and one strain, a response  $\geq$  twice the control incidence (see also "positive control" in Study validity, below).

**Result:**

Study validity:

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- Both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value ( $\pm$  S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain, except for TA102 (1.5 fold is acceptable).
- There was a minimum of three nontoxic concentrations ( $\leq$  50% reduction in mean number of revertants/plate relative to the mean vehicle control value) in each tester strain, both in the absence and presence of S9-mix.

All tester strain culture titers ( $10^8$  cells/mL) were less than conventionally recommended titers ( $3 \times 10^8$  cells/mL).

#### Study outcome:

##### ● Experiment 1:

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration ( $\mu$ g/plate)	Revertant colonies/plate (mean $\pm$ SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	100 $\mu$ L/plate	16 $\pm$ 6	160 $\pm$ 6	33 $\pm$ 9	116 $\pm$ 13	213 $\pm$ 25
RAD001	8	15 $\pm$ 4	165 $\pm$ 12	30 $\pm$ 4	117 $\pm$ 5	268 $\pm$ 6
	40	15 $\pm$ 4	162 $\pm$ 3	35 $\pm$ 3	100 $\pm$ 7	274 $\pm$ 13
	200	17 $\pm$ 2	155 $\pm$ 8	34 $\pm$ 5	105 $\pm$ 9	265 $\pm$ 27
	1000	13 $\pm$ 2	159 $\pm$ 13	36 $\pm$ 6	106 $\pm$ 14	274 $\pm$ 14
	5000*	19 $\pm$ 8	149 $\pm$ 11	NA**	136 $\pm$ 17	202 $\pm$ 28
Positive control	(see above for + controls)	479 $\pm$ 52	1545 $\pm$ 164	286 $\pm$ 19	1612 $\pm$ 97	1210 $\pm$ 38
With S-9						
Dist. H <sub>2</sub> O	100 $\mu$ L/plate	18 $\pm$ 3	172 $\pm$ 9	43 $\pm$ 4	118 $\pm$ 11	324 $\pm$ 15
RAD001	8	16 $\pm$ 8	169 $\pm$ 18	38 $\pm$ 4	123 $\pm$ 7	347 $\pm$ 27
	40	16 $\pm$ 1	170 $\pm$ 12	43 $\pm$ 4	114 $\pm$ 2	361 $\pm$ 16
	200	15 $\pm$ 2	167 $\pm$ 3	39 $\pm$ 8	121 $\pm$ 5	353 $\pm$ 5
	1000	16 $\pm$ 2	172 $\pm$ 11	41 $\pm$ 6	107 $\pm$ 11	393 $\pm$ 16
	5000*	17 $\pm$ 2	154 $\pm$ 9	46 $\pm$ 4	142 $\pm$ 6	316 $\pm$ 38
Positive control	(see above for + controls)	280 $\pm$ 41	832 $\pm$ 130	2071 $\pm$ 23§ 426 $\pm$ 9	1245 $\pm$ 72	912 $\pm$ 36

\*: precipitation

\*\* NA (not available): the data were printed partially in the report and the numbers were not legible.

§: Two positive controls for TA98 (with S9): the upper value is 2-aminoanthracene (3  $\mu$ g/plate) and lower value is benzo[a]pyrene (3  $\mu$ g/plate). This was similar to Experiment 2.

##### Experiment 2:

Treatment	Concentration ( $\mu$ g/plate)	Revertant colonies/plate (mean $\pm$ SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
DMSO	100 $\mu$ L/plate	18 $\pm$ 5	166 $\pm$ 3	35 $\pm$ 3	118 $\pm$ 5	290 $\pm$ 26

RAD001	156.25	15 ± 2	157 ± 6	26 ± 2	99 ± 13	206 ± 37
	312.5	16 ± 1	155 ± 8	33 ± 4	109 ± 6	240 ± 45
	625	16 ± 2	148 ± 9	33 ± 7	102 ± 24	307 ± 2
	1250	17 ± 5	154 ± 8	32 ± 6	121 ± 9	313 ± 28
	2500*	23 ± 2	148 ± 15	30 ± 5	125 ± 17	274 ± 40
Positive control	(see above for + controls)	1134 ± 68	1218 ± 227	304 ± 4	952 ± 32	996 ± 69
With S-9						
DMSO	100 µL/plate	17 ± 1	174 ± 7	41 ± 3	133 ± 11	353 ± 22
RAD001	156.25	12 ± 5	166 ± 13	37 ± 7	116 ± 8	351 ± 15
	312.5	13 ± 6	156 ± 11	43 ± 4	112 ± 5	376 ± 19
	625	14 ± 3	167 ± 10	47 ± 6	113 ± 21	390 ± 8
	1250	13 ± 4	170 ± 15	47 ± 8	106 ± 2	412 ± 29
	2500*	18 ± 7	179 ± 5	52 ± 4	127 ± 15	186 ± 24
Positive control	(see above for + controls)	369 ± 60	693 ± 45	1927 ± 30§ 345 ± 8	1084 ± 38	931 ± 89

\*: precipitation; §: see footnote above.

**Study title:** Chromosome aberration test with V79 Chinese hamster cells

**Key study findings:**

- SDZ RAD 666 (RAD001) did not show clastogenic potential with or without S9-mix under the conditions of the study.

**Study no.:** #Z59

**Volume #, and page #:** Electronic submission, Module 4 (z59.pdf)

**Conducting laboratory and location:** Genetic Toxicology, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland.

**Date of study initiation:** July 11, 1995

**GLP compliance:** Yes (OECD)

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SDZ RAD 666 (RAD001), Batch # 94902, Purity: 96.7 %

**Formulation/vehicle:** Dimethyl sulphoxide (DMSO)

**Methods:**

A total of two experiments, namely CA1 and CA2, were performed:

- CA1: 3 hr exposure to SZD RAD666, without or with S9.
- CA2: 20 hr exposure to SZD RAD666 without S9, and 3 hr exposure with S9.

Cells: V79 Chinese hamster cells

Concentration selection criteria

Based on reduced cell growth (cytotoxicity studies) and on the depression of mitotic index during the chromosomal aberration test.

Test agent stability: Stable

Test agent solubility in test medium:

Precipitations of SDZ RAD 666 were observed at concentrations  $\geq$  313 µg/mL.

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Negative (vehicle) controls: MEM + 1% DMSO.

Positive controls:

With S-9: Cyclophosphamide (CP: 15.0  $\mu$ M)

Without S-9: Ethyl methanesulfonate (EMS, 12.5 mM)

Historical control data: 1.91% abnormal cells (0.75%-4.25%) in the medium control and 2.97% (0.75%-6%) in 10% S9 control.

Assessment of cell growth: cell density estimated relative to the control:

+++ : normal cell density, ++ : 75-100% cell density from the control, + : 50-75%,

- : 25-50%, and -- : <25%.

Exposure conditions:

Incubation and sampling times:

➤ Pulse treatment 3 hr and recovery time 17-20 hr without (CA1 and CA2) or with S9 (CA1 and CA2)

➤ Continuous treatment 20 hr without S9 (CA2)

Concentrations used in the Experiments:

➤ Cytotoxicity test (for both CA1 and CA2): 6, 8.58, 12.26, 17.53, 25.06, 35.83, 51.22, 73.22, 104.67, 149.64, 213.92, 305.82, 437.19, and 625  $\mu$ g/mL.

➤ Cell count assessment from the cytotoxicity test (Data not shown):

✧ SDZ RAD 666 related cytotoxicity was concentration-dependent.

✧ Treatment for 3 hr without S9-mix: 72-35% of negative control at SDZ RAD 666 concentrations of 17.53-625  $\mu$ g/mL, except 35.83  $\mu$ g/mL (80.7%).

✧ Treatment for 20 hr without S9-mix: 120-11% of the negative control at SDZ RAD 666 concentrations 149.64-625  $\mu$ g/mL. Cell counts at concentrations less than 149.64  $\mu$ g/mL were not determined.

✧ Treatment for 3 hr with S9-mix: 66-8% of MEM control at SDZ RAD 666 concentrations 35.83- 625  $\mu$ g/mL.

➤ Cell morphology assessment from the cytotoxicity test (Data not shown):

✧ Treatment for 3 hr without S9-mix: no effects on cell morphology up to 104.67  $\mu$ g/mL. Rounded cells were noted at concentrations  $\geq$  149.64  $\mu$ g/mL.

✧ Treatment for 20 hr without S9-mix: treatment-related morphological changes (e.g., rounded and floating cells, with increasing severity) at concentrations  $\geq$  35.85  $\mu$ g/mL.

✧ Treatment for 3 hr with S9-mix: treatment-related morphological changes (e.g., rounded and floating cells, with increasing severity) at concentrations  $\geq$  213.92  $\mu$ g/mL.

✧ In general, SDZ RAD 666 produced a concentration-dependent decrease in the mitotic index.

➤ Based on reduction of cell growth and depression of mitotic index, the following concentrations were analyzed in the chromosomal aberration assay designated as Experiment CA1 and CA2 (SDZ RAD 666 concentrations as  $\mu$ g/mL):

	First Experiment (CA1) µg/mL	Second Experiment (CA2) µg/mL
Without S9 Treatment time Cell count (% of control)	35, 53, 81 3 h ~ 80-55%	15, 23 20 h Not determined (normal morphology at < 25 µg/mL)
With S9 Treatment time Cell count (% of control)	57, 87, 131 3 h ~65-30%	61, 87, 123 3 h ~65-30%

**Note: for % changes in mitotic index, see tables for study outcomes of CA1 and CA2 in the following 3 pages.**

Study design: Counting the % cells with chromosome aberration in metaphase

- Only structural aberrations were counted. Numerical aberrations were not determined by this protocol.
- Cytotoxicity was based on mitotic index (% of mitotic cells within the total population of mitotic and non-mitotic cells), cell morphology as well as cell growth/count.
- Statistics: A statistical analysis was not performed, because aberration values did not exceed the historical control range.

Analysis:

No. of replicates: Duplicate cultures for each test compound concentration, vehicle and positive controls.

Counting method:

- Observation under the microscope. The following structural aberrations were recorded: chromatid breaks (deletions), isolocus breaks, chromosome breaks, all forms of chromatid exchanges, decentric, tracentric, ring chromosomes and interstitial deletions; but did not include cells with only gaps (i.e., chromatid gaps and isolocus gaps).
- Cells with more than five aberrations were recorded as multiple aberrant cells.
- The mitotic index was determined by counting 1000 cells originally from one Petri dish (2000-4000 cells per concentration) and recording the numbers of metaphases among them. The mitotic index was expressed as a percentage of the associated control value.

Criteria for positive results:

The criteria were not included in the protocol; however, since results were within the historical range of the laboratory, results as presented are acceptable.

**Result:**

Study validity:

The study is considered valid, because:

- There was an apparent increase (no statistical analysis) in percent aberrant cells in the positive control relative to the solvent control in each assay, with or without S9.
- The vehicle control data were within the laboratory historical range.

Study outcome (chromosome aberration assay): Tables from the sponsor

- Experiment CA1 (without S9): 3 hour- (and recovery for 17 hours)

<b>Experiment CA1</b>				
	<b>Cell Growth</b>	<b>Mitotic Index %</b>	<b>% Abn. Cells</b>	<b>% Cells Exch.</b>
<b>Control</b>				
MEM +1% DMSO	+++	8.0*	1.0	0.0
<b>SDZ RAD 666 (µg/ml)</b>				
440	++	30.0**	ND	ND
289	++	35.0	ND	ND
189	++	38.8	ND	ND
124	++	36.3	ND	ND
81	++	36.3	0.5	0.0
53	+++	33.8	0.5	0.0
35	+++	58.8	0.5	0.0
<b>Positive Control, EMS (mM)</b>				
12.5	ND	ND	6.0	0.0

ND not determined

\* Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.

\*\* Mitotic indices as % of the controls.

% cells Exch: % cells with exchanges

● Experiment CA2 (without S9): 20 hour

<b>Experiment CA2</b>				
	<b>Cell Growth</b>	<b>Mitotic Index %</b>	<b>% Abn. Cells</b>	<b>% Cells Exch.</b>
<b>Control</b>				
MEM +1% DMSO	+++	17.5*	1.0	0.0
<b>SDZ RAD 666 (µg/ml)</b>				
200	+	0.0**	ND	ND
130	+	0.0	ND	ND
84	+	0.0	ND	ND
55	+	0.0	ND	ND
36	++	4.6	ND	ND
23	+++	44.1	1.0	0.0
15	+++	58.5	1.5	0.0
<b>Positive Control, EMS (mM)</b>				
12.5	ND	ND	10.0	0.0

- Experiment CA1 and CA2 (with S9): 3 hour-incubation and recovery for 17 hr.

	Experiment CA1				Experiment CA2				
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.		Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.
<b>Control</b>									
S9+1% DMSO	+++	11.0*	1.0	0.0		+++	11.8*	1.5	0.0
<b>SDZ RAD 666 (µg/ml)</b>									
300	++	30.0**	ND	ND	250	++	37.4	ND	ND
198	++	40.0	ND	ND	176	++	35.7	ND	ND
131	++	41.8	2.0	0.0	123	++	49.4	2.5	0.0
87	+++	57.3	0.5	0.0	87	++	67.2	1.5	0.0
57	+++	80.9	1.0	0.0	61	+++	86.0	0.5	0.0
38	+++	92.7	ND	ND	43	+++	116.6	ND	ND
25	+++	88.2	ND	ND	30	+++	148.9	ND	ND
<b>Positive Control, CP (µM)</b>									
15.0	ND	ND	25.0	4.0	15.0	ND	ND	14.0	0.0

**Study title:** Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells using the Microtitre® fluctuation technique

**Key findings:**

- SDZ RAD 666 was not mutagenic in the TK mutation test under the experimental conditions.

**Study no.:** 1463-4-1052

**Volume #, and page #:** Electronic submission, Module 4 (1463-4-1052.pdf)

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** January 17, 1996

**GLP compliance:** Yes (OECD)

**QA reports:** yes (x) no ( )

**Drug, lot #, and % purity:** RAD001 (SDZ RAD 666), Batch # 95905, Purity: 93.4 %

**Formulation/vehicle:** Dimethyl sulphoxide (DMSO)

b(4)

**Methods***In vitro* TK mutation test in L5178Y mouse lymphoma cellsStrains/species/cell line:

L5178Y mouse lymphoma cells

Doses used in definitive study:

## Experiment 1:

Without S9-mix: 7.5, 15, 30, 45, 60, and 90\* µg/mL

With S9-mix: 15, 30, 60, 90\* and 120\* µg/mL

## Experiment 2:

Without S9-mix: 20, 30, 40, 50\*, 55\*, 60\*, and 65\* µg/mL

With S9-mix: 30, 40, 50, 60, 65, 70\*, and 75\* µg/mL

*Note: \*Treatment at these concentrations was excluded from test statistics due to excessive toxicity. Plates at some of these concentrations were not tested for viability/5-TFT resistance; see table of the mutagenicity testing result (below) for details.*

Basis of concentration selection:

The selection of a range of concentrations used in the final mutagenicity testing was based on a preliminary cytotoxicity range-finder experiment (with and without S9-mix), in which SZD RAD 666 at concentrations of 15.625 to 500 µg/mL (limited by solubility) was used. The highest concentration was determined by the solubility in the culture medium.

Negative control:

The vehicle of the test article (DMSO): diluted 100 fold in the treatment medium.

Positive controls:

Chemical	Source	Stock* concentration (µg/mL)	Final concentration (µg/mL)	S-9
4-nitroquinoline 1-oxide (NQO)	_____	5	0.05	-
		10	0.10	-
benzo(a)pyrene (BP)	_____	200	2.00	+
		300	3.00	+

b(4)

\* All solutions were prepared in anhydrous analytical grade dimethyl sulphoxide (DMSO). BP and NQO stock solutions were stored as frozen aliquots at - 80°C.

Incubation and sampling times:

- Exposure to SZD RAD666 (and controls): 3 h.
- Expression period for 5-trifluorothymidine (TFT)-resistant mutation: days (the sponsor did not specify).
- Cell survival and viability: assessed 9-12 days after plating an average of 1.6 cell/well of 96-well microtiter plate. Cells were exposed to SZD RAD 666 at various concentrations.
  - Plating efficiency (PE) for viable cells =  $P(\text{viable})/\text{number of cells plated per well}$  (i.e., 1.6), where P represents probable number of clones/well ( $= -\ln(\text{empty wells}/\text{total wells})$ )
  - Percentage relative survival (%RS) =  $[\text{PE}(\text{test})/\text{PE}(\text{control})] \times 100$
- Mutant selection: TK-deficient mutants formed microcolonies in 12 days, which were counted with the naked eye. The cell concentration was  $2 \times 10^3$  cells per well (384 well plate, totally 4 plates for TFT resistance for each culture)
  - Plating efficiency (PE) for mutant =  $P(\text{mutant})/2 \times 10^3$ , where P represents probable number of clones/well ( $= -\ln(\text{empty wells}/\text{total wells})$ )
- Mutation frequency (MF): the number of mutant per  $10^6$  viable cells.
  - $\text{MF} = [P(\text{mutant})/2 \times 10^3] \times [1.6/P(\text{viable})] \times 10^6$

**Results**Study validity (comment on replicates, counting method, criteria for positive results, etc.):

The study is considered valid, because:

- The plating efficiency (i.e., survival) in the solvent controls was  $\geq 50\%$  (i.e., 66% and 71% survival in the absence and in the presence of S9, respectively, see Table 1).
- At least three of the five concentrations of the test substance had an acceptable number of surviving cells ( $10^6$ ) analyzed for expression of the TK mutation.
- The mutant frequency of the positive control (4-nitroquinoline 1-oxide and benzo(a)pyrene) was at least three times (3 to 8-fold for NQD and 4 to 9-fold for BP, respectively) that of the solvent controls.
- The spontaneous mutant frequencies were within historical control data range.

The historical means of solvent and positive control (data from Appendix 4-7)

Experiment 1 (without S9):

Treatment	Current		Historical		Ratio C/H
	MF	MFi-MFc	MF	MFi-MFc	
Negative control	154.14		170.19		0.906
0.05 NQO	508.92	354.77	445.52	275.34	1.289
0.1 NQO	718.87	564.73	662.50	492.31	1.147

Experiment 1 (with S9):

Treatment	Current		Historical		Ratio C/H
	MF	MFi-MFc	MF	MFi-MFc	
Negative control	178.07		161.72		1.101
2 BP	1281.96	1103.89	798.38	636.66	1.734
3 BP	744.61	566.54	1087.17	925.44	0.612

Experiment 2 (without S9):

Treatment	Current		Historical		Ratio C/H
	MF	MFi-MFc	MF	MFi-MFc	
Negative control	174.39		174.41		1.000
0.05 NQO	1228.29	1053.90	464.09	289.69	3.638
0.1 NQO	1371.68	1197.29	691.34	516.93	2.316

Experiment 2 (with S9):

Treatment	Current		Historical		Ratio C/H
	MF	MFi-MFc	MF	MFi-MFc	
Negative control	180.05		153.76		1.171
2 BP	976.22	796.17	933.67	779.90	1.021
3 BP	1693.77	1513.72	1249.42	1095.66	1.382

Study outcome:

Cytotoxicity test and range-finder experiment:

- Precipitation occurred at 250 and 500 µg/mL after 3 hr treatment incubation in the presence and absence of S9.
- Complete toxicity was observed at higher concentrations. The highest surviving concentrations were 62.5 and 125 µg/mL in the absence and presence of S9, which yielded 10.5% and 0.8% survival, respectively.

The following table summarizes the mutagenicity testing result:

Experiment 1

Treatment (µg/mL)	%RS	-S-9		Treatment (µg/mL)	%RS	+S-9	
		Mutant frequency#				Mutant frequency#	
0	100.0	154.14		0	100.0	178.07	
7.5	96.3	132.11 NS		15	102.1	133.73 NS	
15	132.0	144.96 NS		30	90.6	140.85 NS	
30	144.8	138.62 NS		60	21.7	91.80 NS	
45	34.2	126.43 NS		90 X	0.0		
60	3.9	76.76 NS		120 X	0.2		
90 \$	1.6						
Linear trend		NS		Linear trend		NS	
NQO				BP			
0.05	126.5	508.92		2	54.2	1281.96	
0.1	107.8	718.87		3	70.4	744.61	

## Experiment 2

Treatment (µg/mL)	-S-9		Treatment (µg/mL)	+S-9	
	%RS	Mutant frequency#		%RS	Mutant frequency#
0	100.0	174.39	0	100.0	180.05
20	118.6	98.01 NS	30	97.1	119.93 NS
30	111.3	117.29 NS	40	88.5	100.58 NS
40	44.4	117.26 NS	50	67.1	114.99 NS
50 X	1.2		60	21.1	139.48 NS
55 \$	0.6		65	15.1	121.45 NS
60 \$	0.0		70 \$	2.5	
65 \$	0.0		75 \$	0.4	
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	99.3	1228.29	2	65.2	976.22
0.1	89.7	1371.68	3	46.8	1693.77

# Per 10<sup>6</sup> viable cells  
 \$ Not plated for viability / 5-TFT resistance  
 X Treatment excluded from test statistics due to excessive toxicity  
 NS Not significant  
 RS Relative Survival

Comment:

In the absence or presence of S9-mix, SDZ RAD 666 induced a concentration-related increase in toxicity (i.e., decreased % relative survival/RS). At concentration ranges where % RS were measurable (i.e., ≥ 44.4% in the absence of S9 and ≥ 15.1% in the presence of S9), there was no concentration-dependent mutant frequency.

Conclusion:

SDZ RAD 666 was not mutagenic in the *in vitro* TK mutation test in L5178Y mouse lymphoma cells, in the absence or presence of S9-mix.

**Study title:** Mouse bone marrow micronucleus test by the oral route

**Key study findings:**

- SDZ RAD 666 was negative in the mouse bone marrow micronucleus assay when given orally at up to 500 mg/kg/day (2 doses 24 hr apart).

**Study no.:** MK 36 (Sandoz 203-041)

**Volume #, and page #:** Electronic module (pharmtox\tox\MK36.pdf)

**Conducting laboratory and location:** Genetic Toxicology, Sandoz Pharma Ltd., CH-4002 Basle, Switzerland.

**Date of study initiation:** August 16, 1995

**GLP compliance:** Yes (OECD)

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** SDZ RAD 666 (SDZ 222-666; as a microemulsion as a concentration of 2% w/w), Batch #Y182 0895, Purity: 99.2% by HPLC analysis.

**Formulation/vehicle:** 5% glucose solution (batch #Y154 1291)

**Methods:**

Species: CD-1 mice (— CD1(ICR)BR) b(4)

Dose selection criteria:

Due to limited solubility of the test compound, the maximum technically feasible dose was 500 mg/kg. Also, because of the limitation of the administration volume (maximum 25 mL/kg for oral gavage), 500 mg/kg was the maximum feasible dose (MFD). According to a dose-range finding study, SDZ RAD 666 at the dose of 500 mg/kg was tolerated and only moderate clinical signs (such as sedation) were noted.

Test agent stability: Stable

Metabolic activation system: not applicable

Controls:

Vehicle: 5% glucose solution

Negative controls: placebo (non-vehicle) from the sponsor (Batch #Y174 0895)

Positive controls (Group 5):

Triethylenemelamine (TEM): intravenous injection at 0.15 mg/kg.

Study design:

➤ Mice:

~9-10 weeks, weight: 26-37 g. Micronucleus analysis: n=5/sex/dose; recovery animals (500 mg/kg only) n=2/sex; positive control: n=5/sex/dose.

➤ Dose schedule: twice orally at 0, 50, 160 and 500 mg/kg (as Groups 1, 2, 3 and 4), two doses 24 hour apart (i.e., at 0 and 24 hr). Dosing volume: 25 mL/kg.

➤ Micronucleus assay:

✧ Bone marrow harvest time points: Once in all groups at 48 hours after the first dose.

✧ Slide analysis: The bone marrow cells collected from both femurs of each mouse were used to prepare smears on slides (cytopsin slides). Two smears were prepared from each animal. Cells on slides were stained, and scored for micronuclei and the micronucleated polychromatic erythrocytes (MPE) and micronucleated normochromatic erythrocytes (MNE) via a Image Analyzer. Also, the normochromatic erythrocytes (NE) on the slides were analyzed separately for micronuclei. b(4)

✧ Micronucleus frequency (% micronucleated cells) was determined by analyzing 4000 polychromatic erythrocytes (PE) per animals (2000 PE per slide). The number of micronucleated polychromatic erythrocytes observed, i.e., MPE, was the mean value expressed per 4000 PE examined.

✧ Information of historical background frequency of micronuclei was not available in this report.

Assay acceptance criteria:

➤ The data were analyzed using test for significance of the differences in the mean values of micronucleated cells, using the RS/I procedure "Test Means Unpooled" for the comparison of two independent samples and the Mann-Whitney test (RS/I-software package). The significance level was  $p \leq 0.05$ .

➤ A positive response (mutagenic) in the test was if the compound induced a micronucleus frequency that was statistically significantly above the control level.

- Bone marrow toxicity was indicated by a substantial and statistically significant decrease in the proportion of PE, i.e., the compound did not affect the ratio of PE to NE.

**Result:**Study validity:

The study is considered valid, because of:

- Acceptable controls: the positive control, administered via the same route as the test article, had a statistically significantly higher mean percentage of MPE than the vehicle control ( $p \leq 0.05$ ).
- Acceptable high dose: the high dose used 500 mg/kg, was the MFD of dose range-finding study.

Study outcome:

- ✧ There was no treatment related bone marrow toxicity.
- ✧ Micronucleus analysis (mean  $\pm$  SD) at 48 hr sampling time: table from the sponsor. Data are expressed as mean  $\pm$  SD.

Group	Dose (mg/kg)	Sex	No. of animals	Frequency (%)		
				MPE	MNE	PE
Control	Vehicle	m+f	5+5	0.17 $\pm$ 0.05	0.20 $\pm$ 0.11	43.7 $\pm$ 8.5
SDZ RAD 666	50	m+f	5+5	0.15 $\pm$ 0.07	0.16 $\pm$ 0.07	46.7 $\pm$ 3.9
SDZ RAD 666	160	m+f	5+5	0.13 $\pm$ 0.05	0.14 $\pm$ 0.06	37.2 $\pm$ 15.1
SDZ RAD 666	500	m+f	5+5	0.10 $\pm$ 0.06	0.14 $\pm$ 0.09	43.8 $\pm$ 11.8
TEM	0.15	m+f	5+5	2.08 $\pm$ 0.60	0.45 $\pm$ 0.14	49.5 $\pm$ 11.6

- ✧ Treatment with SDZ RAD 666 did not increase the incidence of micronucleated polychromatic erythrocytes (MPE) at 48 hr sampling times.
- ✧ The incidence of micronucleated normochromatic erythrocytes (MNE) for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.

**Genotoxicity of Impurities:**

The degradant impurities \_\_\_\_\_ have genotoxicity structural alerts. In batch X096 0796 and batch X033 0199 of SDZRAD, the content of \_\_\_\_\_ was \_\_\_\_\_ respectively. The genotoxicity potential of batch X096 0796 and batch X033 0199 was assessed. The study results are reviewed and summarized in the following table:

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Batch X096 0796			
Study #	System	Concentrations	Results
Mut. Bakt. 66/96	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98, TA97a, TA100, TA102 and TA1535	Experiment #1: invalid, due to an experimental error Experiment #2: 1.45-909 µg/plate Experiment #3: 28.4-454.5 µg/plate	Negative in mutagenicity. With or without S9-mix <i>Note</i> : SDZ RAD was not bacteriotoxic up to 909 µg/plate, and precipitated at ≥ 454.5 µg/plate.
Z63	Chromosome aberration: V79 Chinese hamster cells	– S9: 136-224 µg/mL (3 hr, Expt. #1), or 50-200 µg/mL (20 hr, Expt. #2) + S9: 100-1000 µg/mL (3 hr, Expt. #5), or 292-500 µg/mL (3 hr, Expt. #6)	Negative in clastogenicity <i>Note</i> : ↓ cell growth at: – S9: 119 µg/mL (3 hr), 170 µg/mL (20 hr) + S9: 702 µg/mL
Batch 033 0199			
#001801	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98, TA97a, TA100, TA102 and TA1535	Experiment #1: 12.5-200 µg/plate	Negative in mutagenicity. With or without S9-mix
#001831	Chromosome aberration: V79 Chinese hamster cells	– S9: 500-2000 µg/mL (3 hr, Expt. B), 500-2000 µg/mL (20 hr, Expt. C), 630- 1000 µg/mL (20 hr, Expt. D), or 500- 1587.4 µg/mL (20 hr, Expt. E) + S9: 793.7-2000 µg/mL (3 hr, Expt. C & E) or 630-1000 µg/mL (3 hr, Expt. D)	Negative in clastogenicity <i>Note</i> : no concentration dependent ↓ cell growth within concentrations tested

**2.6.6.5 Carcinogenicity**

*Reviewer's note:*

- This section was reviewed by Dr. S. Kunder for NDA            (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is re-formatted to fit the current NDA template.
- The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the findings reported in this review. See below the recommendations made by the ECAC (Dec 2, 2003):  
“Executive CAC Recommendations and Conclusions:

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**Mouse Study:**

This study was acceptable to the committee, based on a >10% suppression of weight gain in the high dose groups relative to the control groups. The committee concurred that there were no drug-related tumor findings in this study.

**Rat Study:**

The committee concurred that the study reached an MTD, based on a >10% suppression of weight gain in the high dose groups relative to the control groups as well as a weight decrement of >10% in the high-dose groups compared to the mean weight of the control groups, that the study was therefore adequate and that there were no drug-related tumor findings in this study.”

- Everolimus was referred to as RAD (for SDZ RAD, RAD001).

**Study title:** Oncogenicity study by oral gavage administration to CD-1 mice for 104 weeks

**Key study findings:** RAD administration for 104 weeks caused pharmacology-related findings and previously seen reproductive effects. Only one neoplastic finding was observed, i.e. osteoma of the femur in females. The incidence of this finding was within the historical control rates.

Adequacy of the carcinogenicity study and appropriateness of the test model: The study achieved an MTD as the high dose group (males and females) had weight loss in excess of 10% at final necropsy. Sufficient numbers lived to the terminal sacrifice to produce a valid study for statistical analysis. The study was conducted in accordance with the protocol and provided sufficient histopathological data from the designated organs and tissues to evaluate both the non-neoplastic and neoplastic effects of everolimus at all dose levels including the effects in control animals.

Evaluation of tumor findings: The statistical review of this study showed no positive linear trends for male mice and one positive trend for female mice. Femur (including joint) osteoma, a rare tumor, was significant in the females ( $p=0.021$ ) compared with the combined control group but within the range of historical controls. Pairwise comparisons for this tumor were not significant for any of the dose groups compared to the combined control. None of the other observed neoplastic tumors were statistically significant by trend analysis or exact tests

**Study no.:** SPM118/973229 (BS-418)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 10 Dec 1996

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** RAD, batch no. X081 0596, X011 0397, X176 1297; 100.7, 94.1, 92.5%

#### Methods

Doses: 0, 0, 0.1, 0.3, 0.9 mg/kg

Basis of dose selection: MTD, based on results of a 13-week oral toxicity study in mice.

This 13-week study used doses of 0.15, 0.5, 1.5, 5, and 15 mg/kg/day. Toxicity findings of the 13-week study included reduced body weight (13.6%, males, 15 mg/kg/day), microvesiculation of the zona fasciculata/glomerulosa, adrenals (males in 0.5-15 mg/kg/day groups), and glandular atrophy of the uterus (females, 1.5-15 mg/kg/day).

Species/strain: CD-1 mice, \_\_\_\_\_

Number/sex/group (main study): 60

Route, formulation, volume: oral gavage, suspension in HPMC, 10 mL/kg

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Frequency of dosing: once daily  
 Satellite groups used for toxicokinetics or special groups: 15  
 Age: 35-42 days  
 Animal housing: individual  
 Restriction paradigm for dietary restriction studies: not applicable  
 Drug stability/homogeneity: not provided  
 Dual controls employed: yes  
 Interim sacrifices: none scheduled  
 Deviations from original study protocol: none

### Observation times

Mortality: daily  
 Clinical signs: daily  
 Body weights: weekly  
 Food consumption: weekly  
 Histopathology: Peer review: yes ( ), no ( x )-not specified in protocol  
 Toxicokinetics: weeks 4, 13, 48, 70, before termination of treatment

### Results

#### Mortality:

##### Animal Survival table

Dose (mg/kg) week	Males (60/group)					Females (60 /group)				
	Control 1	Control 2	0.1	0.3	0.9	Control 1	Control 2	0.1	0.3	0.9
51	57	59	58	58	57	57	57	56	54	56
60	54	59	57	56	55	54	52	52	53	52
70	52	52	55	53	53	50	46	48	49	48
80	49	51	52	48	48	46	42	44	44	45
90	42	45	43	43	43	41	34	36	37	39
101	36	31	35	37	39	31	29	27	30	37
104	33	29	33	32						

All surviving females were sacrificed after 101 weeks when the number of survivors in the 0.1 mg/kg group reached 25; surviving males were sacrificed after 104 weeks of treatment.

#### Deaths during study

Mode of death	Group and sex/number of animals									
	Cm	Cm	0.1 mg/kg m	0.3 mg/kg m	0.9 mg/kg m	Cf	Cf	0.1 mg/kg f	0.3 mg/kg f	0.9 mg/kg f
Sacrificed in extremis	16	19	22	23	17	18	16	28	17	17
Found dead	8	9	4	1	1	4	6	1	2	0
Sacrificed for humane reasons	3	3	1	4	4	8	9	7	11	6
total	27	31	27	28	22	30	31	36	30	23

Cm: control male; Cf: control female.

Clinical signs: No treatment-related signs were seen.

Ophthalmology and hematology: No treatment-related effects.

Body weights:

Difference in body weight compared to controls

	Percent change from controls					
	Males			Females		
	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
Week 52	+2.5	+3.5	-10.5	+1.0	+6.5	-8.5
Week 78	+2.5	+4.5	-7.5	+3.0	-2.5	-14.5
Week 101	-3.0	+6.0	-9.5	-8.0	-10.5	-17.0
Week 104	+0.5	+6.0	-12.0	-	-	-

Food consumption: There were no treatment-related differences in food consumption

Gross pathology:

Swellings/masses

Group/sex	Multiplicity					# of animals	Total # with swelling	Mean week of onset
	0	1	2	3	4 or more			
Cm	47	7	5	0	1	13	21	47
Cm	45	6	7	0	2	15	29	52
0.1 mg/kg m	41	11	5	2	1	19	33	44
0.3 mg/kg m	48	6	1	5	0	12	23	53
0.9 mg/kg m	37	14	8	1	0	23	33	50
Cf	42	5	3	0	0	8	11	62
Cf	42	6	2	0	0	8	10	65
0.1 mg/kg f	45	4	0	0	1	5	8	50
0.3 mg/kg f	43	4	1	1	1	7	14	46
0.9 mg/kg f	60	0	0	0	0	0	0	---

Includes swellings which regressed or were not positively identified at necropsy.

Cm: control males; Cf: control females.

Histopathology:

Non-neoplastic:

Males	Control 1	Control 2	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
<b>Testes</b>					
Flaccid	12/60	13/60	18/60	20/60	26/60
Tubular vacuolation	15	12	20	15	27
Degeneration of germinal epithelium	27	21	27	25	35
<b>Epididymides</b>					
Hyospermia	13	11	15	8	24
Sciatic nerve					
Axonal degeneration	17	13	19	20	25
Females	Control 1	Control 2	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
<b>Thymus</b>					
involution	11	10	7	11	19
<b>Sciatic nerve</b>					
Axonal degeneration	17	16	18	17	26

**Neoplastic:**

1<sup>st</sup>=Incidence of neoplastic tumors-scheduled and unscheduled sacrifices (including animals found dead)  
 2<sup>nd</sup>=Incidence of neoplastic tumors –animals sacrificed at 104 weeks  
 3<sup>rd</sup>=Total incidence of neoplastic tumors

	Male control 1	Male Control 2	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg	Female Control 1	Female Control 2	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
<b>Adrenal cortex</b>										
Cortical adenoma	0/26 4/33 4/59	3/31 3/29 6/60	3/25 0/33 3/58	4/27 2/32 6/59	0/21 3/38 3/59	0/30 1/30 1/60	0/31 0/29 0/60	0/36 0/24 0/60	1/30 0/30 1/60	0/23 0/37 0/60
Neuroblastoma	0/26 0/33 0/59	1/31 0/29 1/60	0/25 0/33 0/58	0/26 0/33 0/59	0/21 0/38 0/59	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Cortical adenocarcinoma	0/26 0/33 0/59	0/31 0/29 0/60	0/25 0/33 0/58	0/26 0/33 0/59	0/21 0/38 0/59	1/30 0/30 1/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
<b>Adrenal medulla</b>										
Pheochroma cytoma	0/26 1/33 1/59	1/31 0/29 1/60	0/25 0/33 0/58	0/27 0/32 0/59	0/21 0/38 0/59	0/30 1/30 1/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
<b>Brain</b>										
astrocytoma	0/27 0/23 0/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	1/22 0/38 1/60	0/30 0/30 0/60	0/31 0/29 0/60	1/36 0/24 1/60	0/30 0/30 0/60	0/23 0/37 0/60
<b>Cecum</b>										
Adenoma	0/27 0/33 0/60	0/31 0/29 0/60	0/26 0/33 0/59	0/28 0/32 0/60	0/22 0/38 0/60	0/30 1/30 0/60	0/31 0/29 0/60	0/35 0/24 0/59	0/30 0/30 0/60	0/23 0/37 0/60
<b>Femur</b>										
osteoma	0/27 0/33 0/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	1/30 0/30 1/60	1/23 1/37 2/60
<b>Hardarian gland</b>										
Adenoma	0/27 3/33 3/60	3/31 2/29 5/60	5/27 3/32 8/59	0/28 3/32 3/60	2/22 3/38 5/60	2/30 1/30 3/60	4/31 4/29 8/60	1/36 2/24 3/60	2/30 2/30 4/60	2/23 1/37 3/60
Carcinoma	2/27 1/33 3/60	0/31 0/29 0/60	1/27 0/32 1/59	1/28 1/32 2/60	0/22 0/38 0/60	1/30 1/30 2/60	0/31 2/29 2/60	1/36 1/24 2/60	1/30 2/30 3/60	0/23 3/37 3/60
<b>Jejunum</b>										
adenoma	0/25 0/33 0/58	0/30 0/29 0/59	0/24 0/33 0/57	0/28 0/32 0/60	0/21 0/38 0/59	0/30 0/30 0/60	0/31 0/29 0/60	0/25 0/24 0/59	0/30 0/30 0/60	0/23 1/37 1/60
carcinoma	0/25 1/33 1/58	0/30 0/29 0/59	0/24 0/33 0/57	0/28 0/32 0/60	0/21 0/38 0/59	0/30 0/30 0/60	0/31 0/29 0/60	0/25 0/24 0/59	0/30 0/30 0/60	0/23 0/37 0/60
<b>Kidneys</b>										
Cortical adenoma	0/27 1/33 1/60	0/31 0/29 0/60	0/27 1/33 1/60	2/28 0/32 2/60	0/22 2/38 2/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Cortical carcinoma	0/27 0/33 0/60	0/31 0/29 0/60	0/27 0/33 0/60	1/28 1/32 2/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
<b>Liver</b>										
Hepatocellular Adenoma	3/27 12/33 15/60	4/31 4/29 8/60	0/27 4/33 4/60	3/28 9/32 12/60	2/22 8/38 10/60	0/30 1/30 1/60	0/31 1/29 1/60	0/36 2/24 2/60	1/30 1/30 2/60	0/23 1/37 1/60

Hemangioma	0/27 0/33 0/60	0/31 1/29 1/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Hemangiosarcoma	1/27 0/33 1/60	0/31 1/29 1/60	0/27 0/33 0/60	1/28 0/32 1/60	0/22 0/38 0/60	0/30 1/30 1/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 1/30 1/60	0/23 0/37 0/60
Hepatocellular carcinoma	3/27 3/33 6/60	3/31 2/29 5/60	5/27 4/33 9/60	3/28 2/32 5/60	2/22 3/38 5/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Cholangiocellular carcinoma	0/27 0/33 0/60	1/31 0/29 1/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Lungs										
Pulmonary adenoma	1/27 6/33 7/60	5/31 9/29 14/60	3/27 11/33 14/60	7/28 7/32 14/60	3/22 11/38 14/60	4/30 6/30 10/60	3/31 3/29 6/60	3/36 3/24 6/60	2/30 6/30 8/60	1/23 6/37 7/60
Pulmonary carcinoma	3/27 4/33 7/60	7/31 2/29 9/60	5/27 0/33 5/60	3/28 0/32 3/60	2/22 0/38 2/60	0/30 0/30 0/60	0/31 0/29 0/60	2/36 0/24 2/60	1/30 1/30 2/60	1/23 0/37 1/60
Mammary area Cranial/caudal carcinoma	0/27 0/33 0/60	1/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	1/30 2/30 3/60	3/31 0/29 3/60	1/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Ovaries										
adenoma						0/30 2/30 2/60	0/31 1/29 1/60	0/36 1/24 1/60	0/30 1/30 1/60	0/23 0/37 0/60
luteoma						0/30 0/30 0/60	0/31 0/29 0/60	0/36 1/24 1/60	0/30 1/30 1/60	0/23 2/37 2/60
Granulosa cell tumor-B						0/30 0/30 0/60	0/31 1/29 1/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 1/37 1/60
hemangioma						0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	1/23 0/37 1/60
Granulosa cell tumor-M						0/30 0/30 0/60	0/31 0/29 0/60	0/36 1/24 1/60	0/30 0/30 0/60	0/23 0/37 0/60
Pancreas										
Islet cell adenoma	0/27 0/33 0/60	0/31 0/29 0/60	0/26 0/33 0/59	0/28 1/32 1/60	0/22 0/38 0/60	0/30 0/30 0/60	0/30 0/29 0/59	0/26 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Pituitary										
adenoma	0/27 1/33 1/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 1/30 1/60	0/31 1/29 1/60	0/36 0/24 0/60	0/28 0/30 0/58	0/23 2/37 2/60
Seminal vesicles										
adenoma	0/33 0/27 0/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 2/38 2/60					
Spleen										
hemangiosarcoma	0/27 0/33 0/60	0/31 1/29 1/60	0/26 0/33 0/59	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	0/30 0/29 0/59	0/26 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Stomach										
Squamous cell papilloma	0/27 1/33 1/60	0/31 0/29 0/60	0/27 0/33 0/60	1/28 0/32 1/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60

Submandibular salivary glands										
Myoepithelial tumor	0/27 0/33 0/60	0/31 0/29 0/60	0/27 0/32 0/59	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	1/30 0/29 1/59	0/35 0/24 0/59	0/29 0/30 0/59	0/23 0/37 0/60
Testes										
Interstitial cell tumor	1/27 2/33 3/60	1/31 0/29 1/60	0/27 0/33 0/60	0/28 1/32 1/60	1/22 0/38 1/60					
hemangiosarcoma	0/27 1/33 1/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60					
Thymus										
thymoma	0/27 1/31 1/58	0/24 0/27 0/57	0/26 0/32 0/58	0/26 0/32 0/58	0/23 0/37 0/58	0/30 0/30 0/60	0/30 0/29 0/59	0/35 0/24 0/59	0/30 0/30 0/60	0/23 0/36 0/59
Thyroid										
Follicular adenoma	0/26 0/33 0/59	1/31 1/29 2/60	0/27 0/33 0/60	1/28 1/32 1/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 1/29 1/60	0/36 0/24 0/60	0/29 0/30 0/59	0/23 0/37 0/60
Uterine cervix										
Stromal polyp						0/29 0/30 0/59	0/31 0/29 0/60	0/36 2/24 2/60	0/30 0/30 0/60	0/23 0/37 0/60
fibroma						0/29 0/30 0/59	0/31 0/29 0/60	1/36 0/24 1/60	0/30 0/30 0/60	0/23 0/37 0/60
leiomyoma						1/29 3/30 4/59	0/31 1/29 1/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 1/37 0/60
leiomyosarcoma						0/29 1/30 1/59	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	1/23 0/37 1/60
Stromal sarcoma						0/29 0/30 0/59	0/31 1/29 1/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Uterus										
Stromal polyp						0/30 4/30 4/60	3/31 2/29 5/60	0/36 3/24 3/60	0/30 1/30 1/60	1/23 3/37 4/60
fibroma						0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 1/37 1/60
leiomyoma						0/30 3/30 3/60	0/31 1/29 1/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 1/37 1/60
leiomyosarcoma						0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
adenocarcinoma						0/30 1/30 1/60	1/31 1/29 2/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Endometrial sarcoma						2/30 0/30 2/60	0/31 0/29 0/60	0/36 0/24 0/60	0/30 0/30 0/60	0/23 0/37 0/60
Abdomen										
Leimysarcoma	0/1 0/0 0/1	0/3 0/0 0/3	0/2 0/0 0/2	1/1 0/0 1/1	0/2 0/0 0/2	0/1 0/0 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/0 0/1	0/2 0/0 0/0
Buccal cavity										

papilloma	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 1/1 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0
Hemopoietic tumor										
Malignant lymphoma (unclassified)	0/27 1/33 1/60	1/31 0/29 1/60	1/27 0/33 1/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	1/31 0/29 1/60	0/36 0/24 0/60	0/30 0/30 0/60	1/23 0/37 1/60
Histiocytic sarcoma	1/27 0/33 1/60	1/31 0/29 1/60	0/27 0/33 0/60	0/28 0/32 0/60	3/22 0/38 3/60	5/30 0/30 5/60	1/31 3/29 4/60	3/36 1/24 4/60	3/30 1/30 4/60	1/23 1/37 2/60
Follicular center cell lymphoma	4/27 3/33 7/60	2/31 2/29 4/60	2/27 1/33 3/60	2/28 0/32 2/60	1/22 1/38 1/60	2/30 3/30 5/60	6/31 0/29 6/60	1/36 0/24 1/60	4/30 1/30 5/60	2/23 0/37 2/60
Granulocytic leukemia	1/27 0/33 1/60	0/31 0/29 0/60	0/27 0/33 0/60	1/28 0/32 1/60	1/22 1/38 1/60	1/30 0/30 1/60	1/31 0/29 1/60	1/36 0/24 1/60	2/30 0/30 2/60	3/23 0/37 3/60
Lymphocytic/lymphoblastic lymphoma	3/27 1/33 4/60	0/31 0/29 0/60	0/27 0/33 0/60	1/28 0/32 1/60	3/22 0/38 3/60	2/30 0/30 2/60	3/31 2/29 5/60	3/36 1/24 4/60	5/30 0/30 5/60	3/23 0/37 3/60
Pleomorphic lymphoid lymphoma	0/27 0/33 0/60	0/31 0/29 0/60	0/27 0/33 0/60	0/28 0/32 0/60	0/22 0/38 0/60	0/30 0/30 0/60	0/31 0/29 0/60	0/36 0/24 0/60	1/30 0/30 1/60	0/23 0/37 0/60
Musculo-skeletal										
Sarcoma	0/3 0/1 0/4	0/6 0/1 0/7	1/5 0/2 1/7	0/2 0/0 0/2	0/1 0/1 0/2	1/1 0/0 1/1	0/3 0/2 0/5	0/3 0/2 0/5	0/2 0/0 0/2	0/1 1/2 1/3
osteosarcoma	0/0 0/0 0/0	1/6 0/0 1/6	0/5 0/0 0/5	0/2 0/0 0/2	0/1 0/0 0/1	0/1 0/0 0/1	1/3 0/0 1/3	0/3 0/0 0/3	1/2 0/0 1/2	0/1 0/0 0/1
osteoma	0/0 0/1 0/1	0/0 0/1 0/1	0/0 0/2 0/2	0/0 0/0 0/0	0/0 0/1 0/1	0/0 0/0 0/0	0/0 0/2 0/2	0/0 0/2 0/2	0/0 0/0 0/0	0/0 1/2 1/2
Peritoneum										
mesothelioma	0/0 0/0 0/0	1/3 0/0 1/3	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/0 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0
Skin										
Hemangiosarcoma # Examined at Necropsy not provided	1/6 ? 1/12	0/5 ? 0/13	0/9 ? 0/14	0/7 ? 0/11	0/10 ? 0/21	0/16 ? 0/17	0/6 ? 0/6	0/9 ? 0/9	0/11 ? 0/11	0/4 ? 0/4
Sarcoma # Examined at Necropsy not provided	0/6 ? 0/12	0/5 ? 0/13	0/9 ? 0/14	0/7 ? 0/11	0/10 ? 0/21	1/16 ? 1/17	0/6 ? 0/6	0/9 ? 0/9	0/11 ? 0/11	0/4 ? 0/4
Thorax										
mesothelioma	0/2 0/0 0/2	1/3 0/0 1/3	0/1 0/0 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/0 0/1	0/1 0/0 0/1	0/2 0/0 0/2	0/0 0/0 0/0	0/0 0/0 0/0

**Toxicokinetics:**

Dose (mg/kg)	week	sex	AUC (0-24) ng•h/mL
0.1	4	m	434.5
		f	333.0
	101	m	169.5
		f	150.5
0.3	4	m	756.0
		f	358.2

Dose (mg/kg)	week	sex	AUC (0-24) ng•h/mL
	101	m	412.8
		f	346.4
0.9	4	m	1633.3
		f	2210.9
	101	m	1377.8
		f	3084.2

**Conclusions:**

The statistical review of this study showed no dose mortality trends for the male and female mice. Exposure to drug can be considered adequate as an MTD was achieved and more than 50% of mice were still alive at 89-90 weeks for both males and females. No positive linear trends were found for male mice. The one positive trend for female mice, osteoma of femur (including joint), a rare tumor, was significant in the females ( $p=0.021$ ) compared with the combined control group. The incidence rate in this study was 1.64% in the middle dose group and 3.05% in the high dose group. However, when analyzed with historical control incidence rates this lesion was not statistically significant. Historical incidence rates for osteomas in female CD-1 mice ranged from 1.43 to 3.08%. Pairwise comparisons for this tumor were not significant for any of the dose groups compared to the combined control. None of the other observed neoplastic tumors were statistically significant by trend analysis or exact tests.

Survival was approximately 55% in males at 104 weeks and 42% in females after 101 weeks. Survival was similar in all treated groups, although greater decreases in group mean body weights were seen in the high dose group. Food consumption was unaffected by treatment. Histopathology findings included treatment-related changes in the thymus, testes, and epididymides. High dose females had thymic involution. Reproductive effects were observed in the testes and epididymides of high dose males.

**Study title:** Oncogenicity study by oral gavage administration to Hanibm rats for 104 weeks

**Key study findings:** There were no neoplastic findings. RAD administration for 104 weeks caused pharmacology-related findings in the immune tissues and lungs. In addition, treatment-related effects were seen in the lens, liver and adrenal gland and reproductive organs.

**Adequacy of the carcinogenicity study and appropriateness of the test model:** The study achieved MTD as the high dose group (males and females) had weight loss in excess of 10% at final necropsy. Sufficient numbers lived to the terminal sacrifice to produce a valid study for statistical analysis. The study was conducted in accordance with the protocol and provided sufficient histopathological data from the designated organs and tissues to evaluate both the non-neoplastic and neoplastic effects of everolimus at all dose levels including effects in control animals.

**Study no.:** SPM113/973228 (BS-468)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 19 Sept 1996

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** RAD, batch no. X081 0596, X011 0397, X176 1297; 100.7, 94.1, 92.5%

**Methods**

Doses: 0, 0, 0.1, 0.3, 0.9 mg/kg

Basis of dose selection (MTD, MFD, AUC etc.): MTD

Species/strain: Hanibm Wistar rats, b(4)

Number/sex/group (main study): 30

Route, formulation, volume: oral gavage, RAD solid dispersion in 0.16 % HPMC, 5 ml/kg

Frequency of dosing: daily

Satellite groups used for toxicokinetics or special groups: 0, 0.1, 0.3, 0.9 mg/kg, 6/sex/dose

Age: 35-42 days

Animal housing: 2/cage

Restriction paradigm for dietary restriction studies: none

Drug stability/homogeneity: not provided

Dual controls employed: yes

Interim sacrifices: none scheduled

Deviations from original study protocol: none

**Observation times**

Mortality: daily

Clinical signs: daily

Body weights: prior to treatment, start of treatment, weekly

Food consumption: prior to treatment, start of treatment, weekly

Histopathology: Peer review: yes ( x ), no ( )

Toxicokinetics: weeks 4, 13, 26, and 48, 0.5, 1, 2, 4, 7, and 24 h after dosing

**Results**

Mortality:

Animal Survival table

Dose (mg/kg)	Males (60/group)					Females (60 /group)				
	Control 1	Control 2	0.1	0.3	0.9	Control 1	Control 2	0.1	0.3	0.9
week										
51	59	60	59	60	59	59	60	59	58	60
61	57	60	59	60	57	59	57	56	56	59
70	57	59	58	60	50	58	56	55	56	57
80	55	55	55	58	45	55	53	53	52	57
90	47	45	49	55	43	48	49	50	51	56
101	37	40	38	51	40	44	44	42	45	53
104	36	36	35	48	40	37	37	38	42	51

Deaths during study

Mode of death	Group and sex/number of animals									
	Control 1 (M)	Control 2 (M)	0.1 mg/kg m	0.3 mg/kg m	0.9 mg/kg m	Control 1 (F)	Control 2 (F)	0.1 mg/kg f	0.3 mg/kg f	0.9 mg/kg f
Sac'd in extremis	9	9	8	5	11	12	15	9	13	3
Found dead	0	3	1	0	0	2	0	3	1	1
Sac'd for humane reasons	15	12	16	7	9	9	8	10	4	5
total	24	24	25	12	20	23	23	22	18	9

Sac'd: sacrificed.

Clinical signs: No treatment-related signs were seen.

Ophthalmology: Ophthalmic examinations during weeks 53, 78 and 104 showed increased incidence of anterior suture line opacity in the lens for the 0.9 mg/kg group and during week 104 for the 0.3 mg/kg group. Increased incidence of capsular opacity was seen during week 104 in the 0.9 mg/kg group and in males in the 0.3 mg/kg group. Increased incidence of anterior polar opacity of the lens was seen during weeks 52 and 78 for males of the 0.9 mg/kg group but was not observed during week 104.

Hematology: During week 104, males (0.3, 0.9 mg/kg) had slightly elevated packed cell volume, hemoglobin, red cell count and mean cell volume. Leukocyte and neutrophil counts were elevated for males receiving 0.1 mg/kg. Platelet counts were decreased for males and females receiving 0.9 mg/kg and males receiving 0.3 mg/kg.

Body weights:

Difference in body weight compared to controls

	Percent change from controls					
	Males			Females		
	0.1	0.3	0.9	0.1	0.3	0.9
Week 52						
Week 78						
Week 101						
Week 104	-6.0	-11.0	-32.5	-10.5	-13.5	-34.0

Food consumption: Food consumption was slightly decreased in the high dose group.

Gross pathology:

Swellings/masses

Group/sex	Multiplicity					Number of animals with swellings	Total number of swellings	Mean weeks of onset
	0	1	2	3	4 or more			
Cm	30	14	11	4	1	30	53	69
Cm	26	15	12	3	4	34	64	64
0.1 mg/kg m	27	12	13	6	2	33	65	54
0.3 mg/kg m	29	12	13	4	2	31	59	64
0.9 mg/kg m	41	12	5	2	0	19	28	66

Cf	36	19	4	1	0	24	30	85
Cf	33	19	7	1	0	27	36	89
0.1 mg/kg f	41	18	0	0	1	19	22	83
0.3 mg/kg f	47	7	5	0	1	13	21	79
0.9 mg/kg f	53	5	1	1	0	7	10	91

Includes swellings which regressed or were not positively identified at necropsy.

Cm: control males; Cf: control females.

Histopathology:

Non-neoplastic:

	Males					Females				
	Control 1	Control 2	0.1	0.3	0.9	Control 1	Control 2	0.1	0.3	0.9
<b>Testes</b>	60	60	60	60	60					
Tubular vacuolation	0	0	0	0	17					
Spermatid degeneration	0	0	0	1	28					
Partial Spermatid depletion	0	0	0	0	19					
Atrophic tubules	2	3	3	4	18					
Inc degeneration of meiotic spermatocytes	0	0	0	0	8					
Spermatid retention	0	0	0	0	3					
<b>Epididymides</b>	60	60	60	60	60					
Reduced sperm count	3	6	5	8	12					
<b>Kidneys</b>	60	60	60	60	60	60	60	60	60	60
Pigmented cortical tubules (lipofuscin)	0	0	2	16	44	8	11	17	34	51
<b>Liver</b>	60	60	60	60	60	60	60	60	60	60
Eosinophilic foci hepatocellular alteration	20	11	22	27	34	6	1	4	10	12
Clear cell foci	37	39	42	52	40	23	23	29	34	43
Portal tract senile change	5	1	5	12	15	5	7	8	19	6
Periacinar hepatocytic fatty vacuolation	0	2	2	6	6	2	0	0	1	2
<b>Lungs</b>	60	60	60	60	60	60	60	60	60	60
Alveolar macrophages	0	9	6	10	31	6	4	4	4	15
Alveolar pneumonitis	7	0	6	21	32	6	6	1	3	5
Alveolar necrosis	0	0	0	0	9	0	0	0	0	1
Eosinophilic deposition	1	0	3	8	24	0	1	0	6	15
Pigment laden macrophages	0	0	0	0	0	0	1	1	2	10
Peribronchiolar lymphocytes	0	0	0	0	5	0	0	0	0	0
<b>Mesenteric lymph nodes</b>	60	60	60	60	60	60	59	60	60	60
Macrophage aggregation	26	25	44	45	44	10	10	22	23	13

Mast cells in sinuses	1	2	8	21	10	2	1	6	10	9
stomach	59	60	59	60	60	60	60	60	59	60
Proliferation of mucous neck cells	0	2	8	11	14	0	0	3	4	5
Hyperkeratosis/ Acanthosis of keratinized region	3	8	16	28	28	3	0	7	9	9
<b>Thymus</b>	60	60	60	60	60	59	59	60	60	60
Atrophy	6	4	5	11	18	5	3	4	2	8
<b>Ovaries</b>						60	60	60	60	60
Ovarian cyst						5	4	7	6	21
<b>Uterus</b>						60	60	60	60	60
Squamous metaplasia						2	2	3	1	21
<b>Hardarian glands</b>	60	59	60	60	60	60	60	60	60	60
Chronic inflammation	3	2	3	10	14	6	2	6	4	11
Lachrymal glands	60	60	60	60	60	59	60	60	60	60
Inflammatory infiltration	17	18	25	39	43	7	4	7	3	6
Dilated acini	22	17	31	32	31	2	2	2	2	0
<b>Pancreas</b>	60	60	60	60	60	59	60	60	60	60
Foci of lymphocytes	0	0	1	4	4	0	0	0	2	5
Skeletal muscle	60	60	60	60	60	60	60	60	60	60
Atrophy	2	0	0	11	14	2	2	1	3	4
Focal degeneration and necrosis	0	0	1	0	3	0	0	0	1	0
Chronic inflammation	3	1	2	23	24	0	0	1	3	10
Submandibular salivary glands	60	60	60	60	60	59	60	60	60	60
Lymphocytic infiltration	0	0	0	4	6	1	2	0	1	2
<b>Eyes</b>	60	60	60	60	60	60	60	60	60	60
Lenticular degeneration	9	7	10	11	18	2	1	3	3	2
adrenals	60	60	60	60	60	60	60	60	60	60
Focal cortical hypertrophy	15	9	9	3	1	12	12	3	6	1
Focal cortical hyperplasia	6	3	4	0	0	6	8	5	3	0
Cortical fatty vacuolation	29	18	9	3	1	11	12	2	5	2
<b>Kidney</b>	60	60	60	60	60	60	60	60	60	60
Progressive senile nephropathy	46	50	38	27	12	43	45	33	38	33
Papillary hyperplasia	4	6	2	1	0	8	10	8	1	1
Mineralization of pelvic epithelium	25	27	22	10	4	45	50	49	42	39
<b>Mammary gland</b>	59	60	60	60	60	60	60	60	60	60
Acinar hyperplasia	0	0	0	0	0	23	18	15	13	6
Ductular dilation	1	0	1	0	0	25	30	23	27	10

**Neoplastic:**

1 <sup>st</sup> = Incidence of neoplastic tumors-scheduled and unscheduled sacrifices (including animals found dead)										
2 <sup>nd</sup> =Incidence of neoplastic tumors –animals sacrificed at 104 weeks										
3 <sup>rd</sup> =Total incidence of neoplastic tumors										
Dose (mg/kg)	Males					Females				
	Control 1	Control 2	0.1	0.3	0.9	Control 1	Control 2	0.1	0.3	0.9
<b>Adrenal cortex</b>										
Cortical adenoma	0/24	1/24	0/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	1/35	1/48	0/40	0/37	0/37	0/38	0/42	0/51
	0/60	1/60	1/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60
<b>Adrenal medulla</b>										
Pheochroma cytoma	2/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	1/18	0/9
	2/36	0/36	0/35	0/48	0/40	0/37	1/37	1/38	0/42	1/50
	4/60	0/60	0/60	0/60	0/60	0/60	1/60	1/60	1/60	1/59
ganglioneuroma	0/24	0/24	1/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	1/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/42	0/50
	1/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
<b>Brain</b>										
Granular cell tumor-b	1/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	1/36	1/36	0/35	1/48	0/40	0/37	1/37	0/38	2/42	1/51
	2/60	1/60	0/60	1/60	0/60	0/60	1/60	0/60	2/60	1/60
Oligodendroglioma	1/24	1/24	0/25	0/12	1/20	0/23	0/23	0/22	0/18	0/9
Malignant reticulosis	0/24	0/24	0/25	0/12	1/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60
Astrocytoma	1/24	0/24	3/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	1/38	0/42	0/51
	0/60	0/60	3/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60
Mixed glioma	1/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	1/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
Granular cell tumor-m	0/24	1/24	0/25	0/12	0/20	0/23	0/23	0/22	1/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	1/38	0/32	0/51
	0/60	1/60	0/60	0/60	0/60	0/60	0/60	1/60	1/60	0/60
Meningial sarcoma	0/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	1/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/32	0/51
	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60
<b>Colon</b>										
carcinoma	0/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	1/37	0/38	0/42	0/51
	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60
<b>Duodenum</b>										
carcinoma	0/23	0/24	0/24	0/12	0/20	1/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/36	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	0/59	0/60	0/60	0/60	0/60	1/60	0/60	0/60	0/60	0/60
<b>Eyes</b>										
Amelanotic melanoma	0/24	0/23	0/25	1/12	0/20	0/23	0/23	0/22	0/18	0/9
	0/36	0/36	0/36	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	0/60	0/59	0/60	1/60	0/60	0/60	0/60	0/60	0/60	0/60
<b>Jejunum</b>										
leiomyoma	0/24	0/23	0/25	0/12	0/20	0/23	0/23	0/22	0/18	0/9
	1/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	1/60	0/59	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60
<b>Kidneys</b>										
Liposarcoma	0/24	0/24	0/25	0/12	0/20	0/23	0/23	0/22	1/18	0/9
	0/36	0/36	0/35	0/48	0/40	0/37	0/37	0/38	0/42	0/51
	0/60	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60	0/60

Tubular sarcoma	1/24 0/36 1/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Lymph node mesenteric										
hemangioma	3/24 11/36 14/60	3/24 10/36 13/60	0/25 1/35 1/60	0/12 0/48 0/60	1/20 2/40 3/60	3/23 3/37 6/60	1/22 0/37 1/59	0/22 1/38 1/60	0/18 0/42 0/60	0/9 0/51 0/60
Liver										
Hepatocellular Adenoma	0/24 1/36 1/60	0/24 0/36 0/60	0/25 1/35 1/60	0/12 2/48 2/60	0/20 0/40 0/60	0/23 0/37 0/60	1/23 2/37 3/60	0/22 2/38 2/60	0/18 0/42 0/60	0/9 1/51 1/60
Lungs										
Pulmonary adenoma	0/24 0/36 0/60	0/24 2/36 2/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 1/40 1/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 1/42 1/60	0/9 0/51 0/60
Mammary										
Fibroadenoma	0/23 0/36 0/59	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	9/23 2/37 9/60	9/23 11/37 20/60	4/22 1/38 5/60	1/18 2/42 3/60	0/9 0/51 0/60
Adenoma	0/23 0/36 0/59	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	1/22 1/38 2/60	0/18 0/42 0/60	0/9 0/51 0/60
Carcinoma	0/23 0/36 0/59	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	1/23 2/37 3/60	0/23 1/37 1/60	0/22 1/38 1/60	1/18 1/42 2/60	1/9 1/51 2/60
Ovaries										
Granulosa cell tumor-b						0/23 1/37 1/60	0/23 0/37 0/60	0/22 1/38 1/60	0/18 0/42 0/60	0/9 0/51 0/60
Undifferentiated gonadal stromal tumor						0/23 0/37 0/60	0/23 2/37 2/60	0/22 0/38 0/60	0/18 0/42 0/60	1/9 0/51 1/60
Sertoli cell tumor						0/23 1/37 1/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Tubulostromal tumor						0/23 1/37 1/60	0/23 1/37 1/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 1/51 1/60
Pancreas										
Islet cell adenoma	0/24 3/36 3/60	2/24 0/36 2/60	1/25 0/35 1/60	0/12 0/48 0/60	0/20 0/40 0/60	1/22 0/37 1/60	1/23 0/37 1/60	0/22 1/38 1/60	0/18 0/42 0/60	0/9 0/51 0/60
Islet cell carcinoma	1/24	1/24	0/25	0/12	0/20	0/22	0/23	0/22	0/18	0/9
Parathyroid										
adenoma	0/23 2/36 2/59	1/24 0/34 1/58	0/25 0/35 0/60	0/12 0/48 0/60	0/19 0/35 0/54	0/20 0/34 0/54	0/22 0/36 0/58	0/20 0/38 0/58	0/18 0/42 0/60	0/9 1/48 1/57
Pituitary										
Adenoma	9/24 8/36 17/60	14/24 8/36 22/60	9/25 12/34 21/59	7/12 7/48 14/60	0/20 5/40 5/60	16/23 16/37 32/60	14/23 17/37 31/60	15/22 20/38 35/60	11/18 20/42 31/60	3/9 11/51 12/60
ganglioneuroma	0/24 0/36 0/60	0/24 0/36 0/60	0/25 0/34 0/59	0/12 0/48 0/60	0/20 1/40 1/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
carcinoma	0/24 0/36 0/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	1/22 0/38 1/60	0/18 0/42 0/60	0/9 0/51 0/60
Prostate										

adenocarcinoma	0/24 0/36 0/60	1/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60					
Rectum										
carcinoma	0/24 1/36 1/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Spleen										
Hemangiosarcoma	0/24 0/36 0/60	0/24 1/36 1/60	1/25 0/35 1/60	0/12 0/48 0/60	0/20 0/40 0/60	1/23 0/37 1/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Submandibular gland										
adenoma	0/24 1/36 1/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	0/22 0/37 0/59	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
fibrosarcoma	0/24 0/36 0/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 1/40 1/60	0/22 0/37 0/59	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Testes										
Interstitial cell adenoma	0/24 1/36 1/60	0/24 1/36 1/60	0/25 1/35 1/60	0/12 0/48 0/60	0/20 0/40 0/60					
Thymus										
Thymoma	0/24 1/36 1/60	1/24 4/36 5/60	2/25 1/35 3/60	0/12 1/48 1/60	0/20 0/40 0/60	2/23 6/36 8/59	3/22 8/37 11/59	2/22 6/36 8/59	0/18 4/42 4/60	0/9 3/51 3/60
Thymoma (epithelial)	0/24 0/36 0/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/48 0/60	0/20 0/40 0/60	1/23 0/36 1/59	0/22 0/37 0/59	0/22 0/36 0/58	0/18 0/42 0/60	0/9 1/51 1/60
Thyroids										
Follicular adenoma	2/24 3/36 5/60	0/24 7/35 7/59	1/25 4/35 5/60	0/12 3/48 3/60	0/20 1/40 1/60	1/22 1/37 2/59	0/23 1/37 1/59	0/22 0/38 0/60	0/18 0/42 0/60	0/9 2/51 2/60
Parafollicular cell adenoma	0/24 2/36 2/60	0/24 5/35 5/59	0/25 0/35 0/60	1/12 1/48 2/60	0/20 0/40 0/60	2/22 6/37 8/59	1/23 3/37 4/59	1/22 3/38 4/60	0/18 1/42 1/60	0/9 0/51 0/60
Parafollicular cell carcinoma	0/24 1/36 1/60	2/24 1/35 1/59	0/25 1/35 1/60	0/12 1/48 1/60	0/20 0/40 0/60	0/22 0/37 0/59	0/23 1/37 1/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Follicular cell carcinoma	1/24 1/36 2/60	1/24 1/35 2/59	0/25 1/35 1/60	0/12 1/48 1/60	0/20 2/40 2/60	0/22 0/37 0/59	0/23 0/37 0/60	0/22 0/38 0/60	0/18 1/42 1/60	0/9 0/51 0/60
Urinary bladder										
Transitional cell carcinoma	1/24 0/36 1/60	0/24 0/36 0/60	0/25 0/35 0/60	0/12 0/47 0/59	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Uterine cervix										
polyp						0/23 1/37 1/60	0/23 1/37 1/60	0/22 0/38 0/60	1/17 0/42 1/59	1/9 1/51 2/60
Uterus										
Adenoma						0/23 1/37 1/60	0/23 0/37 0/60	0/22 0/38 0/60	0/18 0/42 0/60	0/9 0/51 0/60
Adenocarcinoma						2/23 3/37 5/60	2/23 1/37 3/60	0/22 0/38 0/60	1/18 0/42 1/60	0/9 0/51 0/60
Endometrial sarcoma						1/23	0/23	0/22	0/18	0/9

schwannoma						0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	1/18 0/42 1/60	0/9 0/51 0/60
polyp						3/23 8/37 11/60	1/23 3/37 4/60	1/22 7/38 8/60	1/18 6/42 7/60	0/9 0/51 0/60
Fallopian tubes										
Granular cell tumor						0/0 0/0 0/0	0/0 1/1 1/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/1 0/1
Vagina										
sarcoma						0/23 0/37 0/60	0/23 0/37 0/60	0/21 0/38 0/59	1/18 0/42 1/60	0/9 0/51 0/60
Abdomen										
Mesothelioma	0/1 0/0 0/1	0/0 0/0 0/0	0/1 0/0 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/0 0/1	0/2 0/0 0/2	0/0 0/0 0/0	0/3 0/0 0/3	1/1 0/0 1/1
schwannoma	0/1 0/0 0/1	0/0 0/0 0/0	1/1 0/0 1/1	0/0 0/0 0/0	0/0 0/0 0/0	0/1 0/0 0/1	0/2 0/0 0/2	0/0 0/0 0/0	0/3 0/0 0/3	0/1 0/0 0/1
Hemopoietic tumor										
Malignant lymphoma (unclassified)	0/24 0/36 0/60	0/24 0/36 0/60	1/25 0/35 1/60	0/12 0/48 0/60	0/20 0/40 0/60	0/23 0/37 0/60	0/23 0/37 0/60	0/22 0/38 0/60	1/18 0/42 1/60	0/9 0/51 0/60
Large granular cell leukemia	0/24 0/36 0/60	1/24 0/36 1/60	1/25 0/35 1/60	0/12 0/48 0/60	0/20 1/40 1/60	0/23 0/37 0/59	0/23 0/37 0/60	0/22 0/38 0/60	1/18 0/42 1/60	0/9 0/51 0/60
Lymph node renal										
hemangioma	0/0 0/0 0/0	0/0 0/1 0/1	0/0 0/0 0/0	0/0 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/2 0/2	0/0 1/1 1/1	0/0 0/0 0/0	0/0 0/2 0/2
Lymph node inguinal										
hemangioma	0/3 0/0 0/3	0/6 0/0 0/6	0/6 0/0 0/6	0/10 0/0 0/10	0/4 0/0 0/4	0/10 0/0 0/10	0/11 0/0 0/11	1/7 0/0 1/7	0/5 0/0 0/0	0/2 0/0 0/2
Musculo-skeletal										
ameloblastoma	½ 0/0 1/2	0/1 0/0 0/1	0/2 0/0 0/2	0/3 0/0 0/3	0/1 0/0 0/1	0/1 0/0 0/1	0/4 0/0 0/4	0/2 0/0 0/2	0/0 0/0 0/0	0/1 0/0 0/1
Osteosarcoma	½ 0/0 1/2	0/1 0/0 0/1	0/2 0/0 0/2	0/3 0/0 0/3	1/1 0/0 1/1	0/1 0/0 0/1	0/4 0/0 0/4	0/2 0/0 0/2	0/0 0/0 0/0	0/1 0/0 0/1
Skin										
fibroma	0/9 0/20 0/29	0/14 0/19 0/33	0/10 0/23 0/33	0/5 0/25 0/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 1/5 1/9	0/5 0/5 0/10	0/3 0/7 0/10	0/1 0/4 0/5
Keratocanthoma	1/9 2/20 3/29	0/14 2/19 2/33	0/10 0/23 0/33	0/5 0/25 0/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 0/5 0/9	0/5 0/5 0/10	0/3 0/7 0/10	0/1 0/4 0/5
Papilloma	0/9 2/20 2/29	0/14 1/19 1/33	0/10 0/23 0/33	0/5 2/25 2/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 0/5 0/9	0/5 0/5 0/10	1/3 0/7 1/10	0/1 0/4 0/5
Schwannoma	0/9 1/20 1/29	1/14 0/19 1/33	0/10 0/23 0/33	0/5 0/25 0/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 0/5 0/9	0/5 0/5 0/10	1/3 0/7 1/10	0/1 0/4 0/5
Fibrosarcoma	0/9 0/20 0/29	1/14 0/19 1/33	1/10 0/23 1/33	0/5 0/25 0/30	0/8 0/13 0/21	0/7 1/7 1/14	¼ 0/5 1/9	1/5 0/5 1/10	1/3 0/7 1/10	0/1 0/4 0/5

Basal cell carcinoma	0/9 0/20 0/29	0/14 0/19 0/33	0/14 0/23 0/33	1/5 0/25 1/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 0/5 0/9	0/5 1/5 1/10	0/3 0/7 0/10	0/1 0/4 0/5
Squamous carcinoma	0/9 0/20 0/29	1/14 0/19 1/33	0/10 0/23 0/33	0/5 0/25 0/30	0/8 0/13 0/21	1/7 0/7 1/14	0/4 0/5 0/9	1/5 0/5 1/10	0/3 0/7 0/10	0/1 0/4 0/5
Hemangioma	0/9 0/20 0/29	0/9 0/20 0/29	0/14 0/23 0/33	0/5 0/25 0/30	0/8 0/13 0/21	0/7 1/7 1/14	0/4 0/5 0/9	0/5 0/5 0/10	0/3 0/7 0/10	0/1 0/4 0/5
Sarcoma	0/9 0/20 0/29	0/9 0/20 0/29	0/14 0/23 0/33	0/5 1/25 1/30	0/8 0/13 0/21	0/7 0/7 0/14	0/4 0/5 0/9	0/5 0/5 0/10	0/3 0/7 0/10	0/1 0/4 0/5
uretor										
papilloma	0/0 0/0 0/0	0/0 0/0 0/0	0/0 1/1 1/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/1 0/1	0/0 0/0 0/0	0/0 0/0 0/0	0/0 0/0 0/0

### Toxicokinetics:

Dose (mg/kg)	week	Sex	AUC (0-24) ng•h/mL
0.1	4	M	9.4
		F	5.2
	101	M	2.5
		F	1.0
0.3	4	M	45.7
		F	34.2
	101	M	25.7
		F	11.4
0.9	4	M	103.4
		F	53.4
	101	M	138.2
		F	42.9

### Conclusions:

None of the observed neoplastic tumors were statistically significant by trend analysis or exact tests. No dose mortality trends were found for the male rats. A significant negative trend across treatment groups for mortality was found for female rats.

Survival was approximately 58% in males and 62% of females after 104 weeks. Survival was comparable among all groups. Food consumption was slightly decreased in the high dose group; the rest of the groups were unaffected by treatment. Histopathology findings included treatment-related changes in the testes, epididymides, ovaries and uterus in the 0.9 mg/kg group. Pharmacology-related changes included thymic atrophy, inflammatory changes in the Harderian glands, mesenteric lymph nodes, lachrymal glands, lungs, pancreas, skeletal muscle and submandibular gland. In the lung, increased incidence of alveolar macrophages was found, with eosinophilic deposition and pigment-laden macrophages. In the liver, effects such as increased incidence of senile portal liver tract changes in males receiving 0.3 and 0.9 mg/kg appear treatment-related. Axonal degeneration of the sciatic nerve in females receiving 0.9 mg/kg also was treatment-related. Lens changes included anterior suture line opacity and increased incidence of lenticular degeneration in males at 0.9 mg/kg. Age-related effects of the adrenal cortex, focal hypertrophy, hyperplasia and fatty vacuolation, were reduced in treatment groups.

**2.6.6.6 Reproductive and developmental toxicology***Reviewer's note:*

- This section was reviewed by Dr. S. Kunder for NDA [redacted] (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is re-formatted to fit the current NDA template. b(4)
- Study #3074R (An oral fertility and embryo-fetal development study in female rats) is reviewed by Dr. Todd Palmby (DDOP).
- Everolimus was referred to as RAD (for SDZ RAD, RAD001).

**Fertility and early embryonic development**

**Study title:** An oral 13-week investigative fertility study in male rats with 13 weeks recovery

Note: the study was reviewed by Dr. S. Kunder for NDA [redacted] Modifications have been made to the review. b(4)

**Key study findings:**

- Fertility of male rats was significantly decreased by RAD at 5.0 mg/kg: the male fertility index was zero at the end of treatment period and 60-65% during the 10-13 week recovery period, indicating incomplete recovery.
- Reduced organ weights (i.e. testes, epididymides, and prostate) and histopathologic findings in the testes (germ cell degeneration and vacuolation of seminiferous epithelium) and epididymides (oligospermia/aspermia, segmental degeneration and spermatic debris in the lumen, and epithelial cell vacuolation) were evident at 5 mg/kg.
- The sperm count and testosterone levels were decreased at 5 mg/kg, without full reversibility after 13-weeks of recovery.

**Study no.:** 7073R

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** Novartis Pharma AG,  
Basel, Switzerland

**Date of study initiation:** May 13, 1996

**GLP compliance:** yes

**QA reports:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** RAD, batch no. X035 0396, na, 83%

**Formulation/vehicle:** solid dispersion in water/ hydroxymethyl cellulose solution

**Methods:**

Species/strain: rat/ HanIbm:WIST, [redacted]  
Doses employed: 0, 0.1, 0.5, 5.0 mg/kg  
Route of administration: oral gavage  
Study design:

b(4)

**Part 1:** RAD administered orally to males daily for 10 weeks prior to mating, for a 3-week mating period, until day 92 of study

**Part 2:** RAD administered orally to males daily for 92 days, followed by a 13-week recovery period, including a 3-week mating period with sacrifice on day 183 of study (the mating occurred after ~10 weeks of a treatment-free period). Females in both parts of study were untreated, paired with treated males and necropsied on day 16 post coitus and examined with fetuses

Number/sex/group: 20

Parameters and endpoints evaluated: male mating index, female mating index, female fecundity index, preimplantation loss, post implantation loss, sperm analysis, LH, FSH and testosterone determinations (blood and pituitary).

### Results:

- Mortality: 1M (#47), 0.1 mg/kg, day 92 during blood sampling; 1M (#20), 5.0 mg/kg, sacrificed moribund day 18 (piloerection, rapid breathing, prostration; reduced food consumption, loss of body weight days 11-15; at necropsy, lung discoloration may be due to misdosing)
- Clinical signs: skin lesions (due to bacterial infection), 1M, 0.1 mg/kg, 1M, 0.5 mg/kg, 11M, 5.0 mg/kg
- Body weight: significantly reduced mean body weight at 5 mg/kg, and weight gains at  $\geq 0.5$  mg/kg. At 5 mg/kg, increased weight gains were observed at the end of recovery period, although the mean body weight was still significantly less than the control. The % changes from the control are summarized:

	Part 1 (D1-D92)	Part 2 (D1-D183; dosing D1-D92)
Mean body weight		
5 mg/kg	↓ 6-21% (starting on D4)	D1-92: ↓6-19% (starting D8); D95-D183: ↓18-9%; D1-D183: ↓ 9%
Body weight gains		
0.5 mg/kg	↓ 17%	
5 mg/kg	↓ 76%	D1-92: ↓ 76%; D95-D183: ↑ 83%

- Food consumption: decreased at 5.0 mg/kg throughout 13-week treatment (↓19% and 17% of the control in part 1 and part 2, respectively), reversible during recovery period.
- Toxicokinetics: on Day 92

	0.1 mg/kg	0.5 mg/kg	5.0 mg/kg
$C_{max}$ (ng/mL)	2.8	8.4	40.4
$AUC_{0-6h}$ (ng•h/mL)	7.1	23.3	143.6
$AUC_{0-24h}$ (ng•h/mL)	9.9	52.0	414.8
$t_{max}$ (hr)	3.6	1.6	0.6

**LOQ: 0.1 ng/mL**

- Reproductive data:

Part 1 (male data):	Control	0.1 mg/kg	0.5 mg/kg	5.0 mg/kg
Males mating	20/20	20/20	20/20	19/20
Males with pregnancy confirmed in female	19/20	20/20	19/20	0/19
Male fertility index	95	100	95	0
Part 2 (male data):				
Males mating	20	20	20	18
Males with pregnancy confirmed in female	20	19	18	*13
Male fertility index	100	95	90	*60-65

Part 1 (female data):	Control	0.1 mg/kg	0.5 mg/kg	5.0 mg/kg
Females with viable fetuses	18/19	19/20	19/20	No pregnancy
Females with fetal death	1/19	1/20	0	NA
Corpora lutea/dam	13.89	12.80	13.89	NA
Implantation /dam	11.42	10.80	12.74	NA
Preimplantation loss/sites	47/217	40/216	22/242	NA
Live fetuses/dam	10.79	9.85	11.74	NA
Dead fetuses/dam	0.11	0	0	NA
Resorptions-early/dam	0.53	0.95	1.00	NA
Resorption -late/dam	0	0	0	NA

Part 2 (female data):	control	0.1 mg/kg	0.5 mg/kg	5.0 mg/kg
Females with viable fetuses	19/19	19/19	18/18	*12/12
Females with fetal death	00	0	0	0
Implantion /dam	12.37	11.84	12.39	12.92
Preimplantation loss/dam	6.78	10.31	9.43	6.04
Live fetuses/dam	11.74	11.05	11.44	12.08
Dead fetuses/dam	0.16	0	00	0
Resorptions-early/dam	0.47	0.79	0.94	0.83
Resorption -late/dam	0	0	0	0

NA: not applicable.

\* Note: 18/20 females were sperm positive; however, 12/20 were pregnant. One out of 20 could not be identified as pregnant and was eliminated (this results in a discrepancy between the # of animals reported pregnant under "part 2: male data", indicating 13 females being pregnant, and "part 2: female data", indicating 12 females being pregnant).

➤ Organ weights: 5 mg/kg, % reductions from the control are summarized:

	Absolute weights		Relative weights to BW	
	Part 1 (D92)	Part 2 (D183)	Part 1 (D92)	Part 2 (D183)
Testis, left	56%	20%	45%	NR
right	56%	16%	46%	
Epididymis, left	48%	17%	30%	NR
right	48%	14%	35%	
Prostate	39%	7%	24%	NR

NR: Not remarkable

➤ Histopathology:

Part 1: Day 92

0.1 mg/kg:

Kidney: hydronephrosis (2/20)

0.5 mg/kg:

Testes:

➤ ↑ adluminal positioned irregular and misshapen spermatid nuclei (slight)

kidney : hydronephrosis (2/20), tubular hyaline droplets (3/20)

5.0 mg/kg:

Testes: testicular atrophy 19/19 males,

➤ Slight effect on testicular morphology

➤ Depletion of round and elongate spermatid and spermatocytes (slight to severe)

- Degeneration: germ cells (minimal to moderate)
- Vacuolation: seminiferous epithelium (minimal to marked)

Epididymides: 19/19 males

- Oligospermia to aspermia,
- Segmental degeneration (minimal to slight) and spermatic debris in the lumen (minimal to moderate) of duct epithelium
- Vacuolation (caput) and droplet formation/desquamation (cauda) of epithelial cells

Seminal vesicles/coagulation glands: colloid reduced;

Kidney: hydronephrosis (2/20), tubular hyaline droplets (2/20)

Part 2: Day 183, mainly at 5.0 mg/kg

Testes: testicular atrophy, 6/20 males that showed loss of fertility and ↓ organ weights

- Depletion of round and elongate spermatid and spermatocytes (marked to massive)
- Degeneration: germ cells (minimal to slight)
- Vacuolation: seminiferous epithelium (slight to marked)

Epididymides:

- Oligospermia to aspermia (6/20 males)
- Segmental degeneration (slight to moderate, 13/20 males) and spermatic debris in the lumen (minimal to moderate) of duct epithelium
- Vacuolation (caput) and segmental droplet formation/desquamation (cauda) of epithelial cells

Kidney: hydronephrosis, tubular hyaline droplets, all doses (1/20)

- Sperm analysis: statistically significant % changes from the control are summarized below.

	Part 1		Part 2
Dose (mg/kg)	0.5	5	5
Sperm motility (% motile) ↓	11%	89%	a 25-60%
Sperm head count (count/g) ↓		86%	22%

a: extremely low motility (% motile <25%, compared with control of ~74%) in 4 males and low motility (<60%) in 3 males.

- LH, FSH and testosterone determinations (blood and pituitary):

Part 1: Decreased testosterone (56% from control), 5.0 mg/kg; all other values unaffected;

Part 2: Decreased testosterone (58% from control, not statistically significant), 5.0 mg/kg, after recovery; all other values unaffected

There were no histopathological or immunohistochemical changes in the pituitary glands in treated animals.

### Conclusions:

Fertility of male rats was significantly decreased by RAD at 5.0 mg/kg, with histopathologic effects of the testes and epididymides. The sperm head count and testosterone levels were decreased, without full reversibility after 13-weeks of recovery. The male fertility index was zero at the end of treatment period and 60-65 during the 10-13 week recovery period, indicating incomplete recovery of fertility after the treatment had stopped. Testicular atrophy

was seen in all animals treated at 5.0 mg/kg, and was still seen in 6/20 rats following recovery. Drug levels were not determined at the end of recovery.

## Embryofetal development

**Study title:** An oral fertility and embryo-fetal development study in female rats

**Note:** this study is reviewed by Dr. Todd Palmby (DDOP, OODP)

### Key study findings:

- Reproductive and embryo-fetal toxicities observed at  $\geq 0.1$  mg/kg:
  - $\uparrow$  pre- and post-implantation losses
  - $\uparrow$  early resorptions (reached statistical significance at 0.9 mg/kg)
  - $\uparrow$  incidence of malformations of thoracic vertebrae, ribs and sternbrae only as measured by number of fetuses affected, not by litters affected
- No independent maternal toxicities observed at doses used, as weight loss and food consumption changes were due to  $\uparrow$  resorption rates
- Dose-dependent increases in early resorption (at  $\geq 0.1$  mg/kg) suggesting the drug effect on female fertility.

**Study no.:** 3074R

**Volume #, and page #:** Electronic module 4 (toxicology\reproductive and developmental toxicity\embryo-fetal development\3074R.pdf)

**Conducting laboratory and location:** Novartis Pharma AG, Basle, Switzerland;

b(4)

**Date of study initiation:** May 14, 1996

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** SDZ RAD (SDZ 222-666)(RAD001), X035 0396, 86.8%

### Methods

Doses: 0 (control), 0.1, 0.3, 0.9 mg/kg/day (0.6, 1.8 and 5.4 mg/m<sup>2</sup>/day)

Species/strain: Wistar (HanIbm:WIST).

b(4)

Number/sex/group: 20/group

Route: oral gavage

Formulation: solid dispersion (9.1%) with hydroxypropylmethyl cellulose 3cps (HPMC 3 cps) and \_\_\_\_\_ mesh as carriers

b(4)

Volume: 2 mL/kg/day

Satellite groups used for toxicokinetics: 6 females/group

Study design: Female rats were acclimated for at least one week before use in this study. Study animals were treated 2 weeks prior to pairing and continued until gestational day (gd) 16. Main study females were sacrificed at gd 21 and examined with their fetuses. Toxicokinetic study satellite females were

necropsied and examined with their fetuses at gd 16 after the last blood sampling.

*Dose Justification:* Dose levels were chosen based on the results of a dose-range-finding (DRF) study in female rats (#1060R). A NOAEL for both maternal- and embryo-toxicity was determined to be 0.15 mg/kg/day, and upper doses of 0.5 and 1.5 mg/kg/day were toxic to the embryos. However, differences in the RAD001 formulation between DRF study and the study under review (#3074R) may account for differences in TK data between these studies (see table below), i.e. the main study highest dose of 0.9 mg/kg/day achieved similar or lower mean  $C_{max}$ ,  $AUC_{(0-6h)}$  and embryo tissue concentrations than the NOAEL 0.15 mg/kg dose in the initial DRF study.

**Dose-range-finding study (#1060R) differences in test article batch, formulation and administration:**

Drug, lot #, and % purity: SDZ RAD 666 (SDZ 222-666), Y182 0895, 99.2%

Formulation: microemulsion 2% (20mg/mL) in 5% glucose solution

Volume: 5 mL/kg/day

TK data from dose-range-finding study and main study

	Dose-finding study(#1060R)		Main study(#3074R)		
	0.15 mg/kg	0.5 mg/kg	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
$C_{max}$ (ng/mL)	5	13	8.1	6.5	4.1
$AUC_{(0-6h)}$ (ng•h/mL)	10	45	7.9	11.4	13.2
Embryo (ng/g)	42	45	0.0	0.8	4.7

**Parameters and endpoints evaluated:**

<u>Clinical signs (dams):</u>	twice daily (weekdays) and once daily (weekends and holidays) for mortality, abnormal behavior and clinical signs
<u>Body weight (dams):</u>	measured on days 1, 5, 8, 12 and 15 during mating period and once daily during gestational period (main study and TK satellite animals)
<u>Food consumption (dams):</u>	weighed food remaining from pre-measured amount (from day 1 of treatment and gd 1) on days 5, 8, 12 and 14 of treatment (pre-mating) and on gd 3, 7, 10, 14, 16 and 21
<u>Gross pathology:</u>	(main study animals) macroscopic examination for organ changes and histopathological examination of affected organs if necessary (ovaries, oviducts, uteri, vaginas and mammary glands of all main study females were fixed)
<u>Toxicokinetics:</u>	blood sampling from satellite females on last day of treatment (gd 16) before, and 0.5, 1, 2, 4 and 6 hours after last treatment; blood taken from control animals before treatment to use as reference samples; live embryos and placentae were pooled per litter after females were sacrificed; blood, embryonic and

Terminal evaluations: placental-tissue concentrations of RAD001 measured by liquid chromatography with mass spectrometric detection (limit of quantification = 0.1 ng/mL blood, 1 ng/g tissue)  
sperm-positive females necropsied on gd 21 and number of corpora lutea, implantation sites and resorptions, and number of viable and dead fetuses were counted (main study animals); similar protocol for satellite females done on gd 16

Offspring evaluations:

- body weights and sexes of viable fetuses and placental weights
- external and visceral examinations
- heart and large vessels examined in situ
- examination of kidneys
- head of every third fetus in each litter (at least one per litter) and of fetuses with external brain anomalies cut to detect brain anomalies (Barrow and Taylor method)
- skeletal examination of eviscerated fetuses (Inouye method)
- sponsor's classification of morphological findings:
  - malformations: marked deviations from normal organogenesis and morphogenesis resulting in persistent morphological alterations; originating during organogenesis, early fetal phase of organ differentiation, and during fetal growth
  - retardations: transient delay in growth, morphogenesis and/or ossification which proceeded according to the normal developmental scheme; originating in the late fetal phase of maturation,
  - variations: congenital alterations known to occur in a specific animal strain; persistent structures, e.g. the following findings were also classified as variations:
    - arcus anterior not ossified or retarded,
    - cervical vertebrae bodies nos. 1 and 2 not ossified,
    - sternbrae nos. 2, 5 and 6 not ossified,
    - processus xiphoideus partially fused,
    - hindlimb digits retarded ossification

**Statistical Analysis:** following tests were performed on the in-life and reproduction data:

- Parametric data: ANOVA followed by Dunnett's test (if ANOVA failed, nonparametric test was used)
- Nonparametric data: Krska-Wallis followed by two-tailed, two-by-two comparison without re-ranking the data
- Count data: Chi-square test followed by Fisher's exact test

**Results**

Mortality (dams): no significant mortality

Clinical signs (dams): not remarkable

Body weight (dams):

- ↓ body weight gain during premating period at 0.9 mg/kg/day
- ↓ mean total body weight gain and gravid and empty uterine weights (statistically significant) at the end of the gestation period at ≥ 0.3 mg/kg/day
- Decreased empty uterine weights in treated females supported the notion of toxic effects on female reproductive organs.
- carcass weights and net weight changes were similar in all groups

**Body weight during premating period**

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
N (number of females)	20	20	20	20
Mean body weight on day 1 (g)	214.2	215.8	214.6	215.0
Mean body weight on day 15 (g)	227.2	229.8	228.0	226.5
Weight gain (g)				
Day1-5	2.6	2.1	2.9	0.9
Day5-8	4.5	5.2	3.6	3.9
Day8-12	3.3	3.1	3.3	3.4
Day12-15	2.6	3.7	3.6	3.3
Mean total weight gain (g, day 1-15)	13.0	14.0	13.4	11.6

**Body weight during gestation period**

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
N (number of gravid females day 0)	20	19	19	20
Mean body weight on day 0 (g)	228.0	229.9	229.1	230.0
N (number of gravid females gd 21)	18	18	19	20
Mean body weight on gd 21 (g)	356.8	344.9	336.4	<b>294.8<sup>#</sup></b>
Weight gain (g)				
gd 0-3	12.1	11.5	10.9	9.9
gd 3-7	11.2	11.5	12.2	11.4
gd 7-10	10.9	9.5	9.8	9.0
gd 10-14	17.9	18.1	17.9	<b>12.4<sup>#</sup></b>
gd 14-16	15.8	12.1	<b>9.0<sup>#</sup></b>	<b>5.4<sup>#</sup></b>
gd 16-21	60.6	53.4	<b>47.5<sup>*</sup></b>	<b>16.7<sup>#</sup></b>
Mean total weight gain gd 0-21 (g) <sup>§</sup>	127.8	115.5	<b>107.2<sup>*</sup></b>	<b>64.8<sup>#</sup></b>
Gravid uterine weights (g)	84.5	67.8	<b>58.8<sup>**</sup></b>	<b>23.8<sup>#</sup></b>
Empty uterine weights (g)	7.07	6.66	<b>5.58<sup>*</sup></b>	<b>3.32<sup>#</sup></b>
Carcass weights (g)	272.3	277.1	277.6	271.0
Net body weight changes from day 0 (g)	43.3	47.7	48.4	41.0

\*p<0.05      \*\*p<0.01      #p<0.001

Bolded numbers are statistically significant as compared to the control.

§weight gains without correction for uterine contents

Food consumption (dams):

- no differences between groups during premating period
- decreased food intake in 0.9 mg/kg group during gestation period (statistically significant between gd 16 and 21) [females with total resorptions had lower food consumption]

**Food consumption during pre-mating period (g)**

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
Day1-5	71.2	73.5	78.0	74.4
Day5-8	52.0	53.7	55.8	54.5
Day8-12	71.7	72.8	76.3	73.1
Day12-14	35.8	37.1	37.7	35.3
Day1-14	230.7	237.0	247.9	237.4

**Food consumption during gestation period (g)**

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
gd 0-3	64.2	63.9	65.6	64.0
gd 3-7	93.2	92.7	93.7	90.3
gd 7-10	66.4	65.6	67.3	64.8
gd 10-14	95.6	94.7	97.5	92.6
gd 14-16	48.7	48.5	49.8	46.0
gd 16-21	118.7	117.1	119.4	103.3 <sup>#</sup>
gd 0-21	486.8	482.4	493.2	461.0

<sup>#</sup>p<0.001

Toxicokinetics:

- evidence of placental transfer at 0.3 and 0.9 mg/kg within 6 hours of administration (embryo/maternal blood)
- accumulation in placenta (placenta/maternal blood)

	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /dose	T <sub>max</sub> (h)	AUC <sub>(0-6h)</sub>	AUC <sub>(0-24h)</sub>	AUC <sub>(0-24h)</sub> /dose
0.1 mg/kg	8.1	81	0.5	7.9	20.0	199.9
0.3 mg/kg	6.5	21.5	0.5	11.4	37.5	125.1
0.9 mg/kg	4.1	4.5	1.6	13.2	40.5	45.1

	Embryo(ng/g)	Placenta(ng/g)	Embryo/blood (6h)	Placenta/blood (6h)	Embryo/placenta
0.1 mg/kg	0.0	12.1	0.0	30.1	0.00
0.3 mg/kg	0.8	22.9	0.8	26.2	0.03
0.9 mg/kg	4.7	63.7	2.1	26.4	0.07

limit of quantification : 0.1 ng/ml in blood and 1 ng/g in tissue

Terminal and necroscopic evaluations:

Macroscopic observations:

- no macroscopic findings at 0.1 mg/kg/day
- control – one female with hemopericardium
- 0.3 mg/kg/day – non-pregnant female enlarged and discolored kidneys

Microscopic observations:

- 0.3 mg/kg/day - non-pregnant female had focal, large area of central degeneration/necrosis in corpora lutea, and chronic progressive nephropathy
- 0.9 mg/kg/day – one female with small right eye and negative pupillary reflex revealed microphthalmia

Estrous-cycles: normal in all groups

Reproduction data:

- precoital intervals did not differ significantly among groups
- mean numbers of corpora lutea did not differ significantly among groups
- pre-implantation loss increase in dose-dependent manner (at  $\geq 0.1$  mg/kg, statistically significant at  $\geq 0.3$  mg/kg/day)
  - in 0.9 mg/kg/day group, 23.47% pre-implantation loss was in upper range of historical control range (2.80 to 27.10%)
- mean number of implantations were statistically significantly decreased at  $\geq 0.1$  mg/kg/day (mean value of 0.9 mg/kg/day outside historical control range)
- mean percentage of post-implantation losses were increased in dose-dependent manner at  $\geq 0.1$  mg/kg/day (statistically significant at 0.9 mg/kg)
  - 0.3 and 0.9 mg/kg/day groups above highest historical control value
  - 0.9 mg/kg/day group – 7 of 20 females had total early embryonic resorption of implants
- no statistically significant differences in fetal or placental weight among groups
- female reproductive function (pre- and post-implantations losses, and number of implantation sites) was affected in a dose-dependent and toxicologically relevant manner at all dose groups

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
Females mated	20	20	20	20
Pregnant females	20	19	19	20
Aborted	0	0	0	0
Premature birth	2	1	0	0
Pregnant females at C-section	18	18	19	20
w/ viable fetuses	18	18	19	13
w/ total fetal death	0	0	0	7
Corpora lutea (total)	259	239	256	246
#/animal (mean)	14 .39	13 .28	13 .47	12.30
Implantation sites	244	195	205	191
#/animal (mean)	13.56	<b>10.83*</b>	<b>10.79*</b>	<b>9.55#</b>
Pre-implantation loss (total)	15	44	51	55
%/animal	5.92	19.19	<b>19.60*</b>	<b>23.47**</b>
Live fetuses (total)	230	180	174	69
#/animal (mean)	12.78	<b>10.00*</b>	<b>9.16**</b>	<b>3.45#</b>
% impl./animal (mean)	93.80	90.82	84.96	<b>32.22#</b>
Males (total)	117	102	80	35
#/animal (mean)	6.50	5.67	<b>4.21*</b>	<b>2.69#</b>
Females (total)	113	78	94	34
#/animal (mean)	6.28	<b>4.33*</b>	4.95	<b>2.62#</b>
Post-implantation loss (total)	14	15	31	122
% impl./animal (mean)	6.20	9.18	15.04	<b>67.78#</b>
Dead fetuses (total)	1	0	0	0
Resorptions (early+late) (total)	13	15	31	122
#/animal (mean)	0.72	0.83	1.63	<b>6.10#</b>
% impl./animal (mean)	5.91	9.18	15.04	<b>67.78#</b>
Resorptions (early) (total)	11	13	31	122
#/animal (mean)	0.61	0.72	1.63	<b>6.10#</b>
% impl./animal (mean)	5.15	8.44	15.04	<b>67.78#</b>
Resorptions (late) (total)	2	2	0	0
#/animal (mean)	0.11	0.11	0.00	0.00

% impl./animal (mean)	0.76	0.74	0.00	0.00
	<sup>*</sup> p<0.05	<sup>**</sup> p<0.01	<sup>#</sup> p<0.001	

Bolded numbers are statistically significant as compared to the control

Offspring:

- dose-dependent increased fetal incidence of malformations was found  $\geq 0.1$  mg/kg, and was above historical controls at  $\geq 0.3$  mg/kg/day
  - most were previously found to occur in historical controls
  - $\uparrow$  fetal incidence of spontaneous malformations
- dose-dependent  $\uparrow$  in fetuses with skeletal retardations at  $\geq 0.1$  mg/kg/day
  - the number of affected skeletal locations also increased dose-dependently
  - may be potential effect of secondary pharmacological action of RAD001 inhibiting osteoclast differentiation and activity

	control	0.1 mg/kg	0.3 mg/kg	0.9 mg/kg
Litters evaluated	18	18	19	13
Fetuses evaluated	233	182	174	69
Live fetuses	230	180	174	69
Dead fetuses	1	0	0	0
Late resorption	2	2	0	0
<b>Malformations</b>				
Fetal incidence	4	5	8	5
Litter incidence	3	3	6	4
Affected fetuses/litter (%)	2.00	2.97	5.01	7.50
Cleft sternum (fetuses/litter) (%)	0	0	0	<b>1.73*</b>
Fetal incidence	0	0	0	2
Litter incidence	0	0	0	2
Sternebrae & rib cartilages asymmetrical				
Fetal incidence	0	3	5	1
Litter incidence	0	2	5	1
<b>Retardations</b>				
Fetal incidence	16	19	25	16
Litter incidence	7	14	10	7
Affected fetuses/litter (%)	6.25	11.07	15.46	<b>34.74**</b>
No ossification				
Thoracic vertebral body				
Fetal incidence	4	8	9	3
Litter incidence	2	7	6	3
Forelimb digits				
Fetal incidence	3	8	6	<b>7**</b>
Litter incidence	2	7	3	2
Retarded ossification				
Thoracic vertebral body				
Fetal incidence	9	2	<b>16*</b>	<b>8*</b>
Litter Incidence	4	2	6	4
<b>Variations</b>				
Fetal incidence	215	173	169	63
Litter incidence	18	18	19	13
Affected fetuses/litter (%)	93.97	95.60	97.06	95.19

No ossification				
Cervical vertebral body				
<i>Arcus anterior</i> (fetuses/litter) (%)	17.93	17.64	28.67	46.03
Fetal incidence	43	33	44	21
Litter incidence	13	15	13	10
Retarded ossification				
<i>Arcus anterior</i> (fetuses/litter) (%)	50.86	58.38	46.02	<b>20.32<sup>#</sup></b>
Fetal incidence	118	106	87	<b>20<sup>**</sup></b>
Litter incidence	18	17	17	<b>8<sup>**</sup></b>
sternum (processus xiphoideus)				
Litter incidence	18	15	<b>14<sup>*</sup></b>	<b>8<sup>**</sup></b>
Normal number of ribs				
Fetal incidence	119	83	87	26
Litter incidence	18	18	17	<b>7<sup>**</sup></b>

\*p<0.05      \*\*p<0.01      #p<0.001

Bolded numbers are statistically significant as compared to the control.

⇒ Study was peer reviewed

### Conclusions:

- No maternal toxicity independent of ↑ resorptions
- RAD001 crosses the placenta
- ↑ pre- and post-implantation losses
- ↑ early resorption rates
- ↑ skeletal retardations at ≥ 0.1 mg/kg/day
- ↑ fetal incidence of common spontaneous malformations at ≥ 0.3 mg/kg/day
- ↑ incidence of fetuses with 14 ribs at 0.9 mg/kg/day
- Mean AUC<sub>0-24h</sub> at 0.9 mg/kg/day (5.4 mg/m<sup>2</sup>/day) was 40.5 ng•h/mL which is approximately 10% the mean AUC (373 ng•h/mL) achieved in healthy human males given 1 oral dose of 4 mg (2.5 mg/m<sup>2</sup>) RAD001, or 8% the AUC<sub>0-24h</sub> (514 ng•h/mL) in patients (with solid tumors) who received the recommended dose of 10 mg/day.

**Study title:** An oral embryo-fetal development study in rabbits

Note: the study was reviewed by Dr. S. Kunder for NDA                      Modifications have been made to the review.

b(4)

**Key study findings:** Maternal toxicity, demonstrated by reduced weight gain, food consumption, and mortality, was seen in the 0.2 and 0.8 mg/kg groups. The NOEL for maternal toxicity was 0.05 mg/kg. The maternal toxicity coincides with slightly increased late resorptions in the 0.8 mg/kg group. Fetal malformations were slightly (not statistically significant) increased in treated rabbits over those in controls.

**Study no:** 4070K (203-074)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** Novartis Pharma AG, Basel, Switzerland

**Date of study initiation:** 20 May 1996

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, and % purity:** RAD, batch no. X035 0396, 86.8%

**Formulation/vehicle:** solid dispersion 9.1%/water-HPMC

**Methods:**

Species/strain: female New Zealand hybrid rabbits ( — KBL (NZW) BR), ———

b(4)

Doses employed: 0, 0.05, 0.2, 0.8 mg/kg

Route of administration: oral gavage

Study design: Females were treated from day 6 post insemination to day 18 post insemination (p.i.). Does were sacrificed on day 29 p.i. (post insemination) and examined with fetuses. Satellite females were sacrificed on day 18 post insemination and examined with fetuses after blood sampling (0.5, 1, 2, 4 and 6 hr post dosing).

Number/sex/group: control, 0.05 mg/kg, 20; 0.2, 0.8 mg/kg, 22; satellite for PK, 0.05 mg/kg, 4; 0.2 mg/kg, 2; 0.8 mg/kg, 3

Parameters and endpoints evaluated: see table below.

**Results:**

**Maternal findings**

Dose	0	0.05 mg/kg	0.2 mg/kg	0.8 mg/kg
Mortality			1, day 22 p.i., aborted	1, day 19, p.i.
Clinical signs	Reduced water intake	Reduced water intake 1 dam aborted, day 21 p.i.	Reduced water intake	Reduced water intake
Body weight gain (day 6-18 p.i.) (%)	4.3%	4.3%	2.1% (slightly ↓ weight gain, not statistically significant)	Weight loss, overall ↓ weight gain (-0.8%) during pregnancy
Food consumption				Significantly reduced during treatment
Toxicokinetics D16 p.i. C <sub>max</sub> (ng/mL) AUC <sub>0-6h</sub> / AUC <sub>0-24h</sub> (ng•h/mL)		1.1 5.2/18.4	4.5 16.4/61.2	19.6 85.6/225.6

p.i.: post-insemination

**Fetal findings**

Dose	0	0.05 mg/kg	0.2 mg/kg	0.8 mg/kg
Females with viable fetuses/No. pregnant	19/20	18/20	16/20	19/20
Females died/sacrificed during gestation		1	1	1
Females assessable	19	17	15	18
Females with fetal death	1	0	0	0
Corpora lutea/doe	9.68	9.76	10.67	10.78
Implantation sites/doe	8.21	7.41	9.53	8.17
Preimplantation loss %/doe	16.34	24.29	10.55	24.10
Postimplantation loss %/doe	7.72	4.65	7.00	9.91
Live fetuses/doe	7.89	7.00	9.07	7.17
Dead fetuses/doe	0	0	0	1
Resorptions—early/doe	0.21	0.35	0.27	0.39
Resorptions—late/doe	0.11	0.06	0.20	0.56

Resorptions –early+late/doe	0.32	0.41	0.47	0.94
Placental weights mean (g)	6.43	7.06	6.32	6.95
Fetal weights mean (g)	38.74	42.21	35.63	37.78
<b>Malformations</b>				
fetal incidence*	5/150 (3.3%)	11/119 (9.2%)	9/136 (6.65)	9/129 (7.0%)
litter incidence:	4/18 (22.2%)	9/17 (52.9%)	7/15 (46.7%)	8/18 (44.4%)
*Fetal incidence:				
-aplasia, subclavian artery	1		3	
-subclavian artery doubled			1	
-interrupted aortic arch		1		
-teratology of Fallot			1	
-lung aplasia		1		
-gallbladder aplasia			1	
-kidney, displaced/hypoplasia		1		
-runt		1		1
-external hydrocephalus				2
-internal hydrocephalus		1		
-skull displasia		1		
-additional ossification center cervical vert.	1	1		1
-Hypoplasia of cervical vertebral body		1		1
-rib interrupted ossification		1		1
-ribs fused		1		
-hypoplasia of thoracic vertebral body		1		
-Multiple defects of thoracic vertebral body		1		
-Additional ossification center sternum			2	
-Sternum hyperplastic				2
-Sternebrae fused	1			
-Asymmetrical sternebrae and rib cartilage	2		1	1
<b>Skeletal ossification Retarded/unossified</b>				
Fetal incidence:				
head	87	32	52	75
Cervical vertebral body	18	42	60	14
Thoracic ribs	0	0	0	3
Thoracic vertebral body	11	5	5	7
Sternum	125	98	118	99
Caudal vertebra	0	0	0	1
pelvis	4	2	2	4
hindlimb	2	3	7	8
Ribs	0	0	0	1
cranium	3	0	4	7

Note: malformations in the table represent the sum of external, visceral and skeletal malformations.

**Conclusions:**

Maternal toxicity, demonstrated by reduced weight gain, food consumption, and mortality, was seen in the 0.2 and 0.8 mg/kg groups. The NOEL for maternal toxicity was 0.05 mg/kg. The maternal toxicity coincides with slightly increased late resorptions in the 0.8 mg/kg group. Fetal malformations were slightly (not statistically significant) increased in treated rabbits over those in controls.

## Prenatal and postnatal development

**Study title:** An oral pre- and postnatal development study in rats

**Note:** the study was reviewed by Dr. S. Kunder for NDA   Modifications have been made to the review. b(4)

### Key study findings:

- Oral administration of SDZ RAD to pregnant rats (up to 0.3 mg/kg) from gestation day 6 to lactation day 20 did not induce maternal toxicity or differences in delivery and lactation parameters in F<sub>0</sub> females.
- F<sub>1</sub> offsprings: There were slight reductions in survival and body weights in F<sub>1</sub> pups of dams treated at ≥ 0.1 mg/kg. No drug-related effects on the development of F<sub>1</sub> generation were noted.

**Study no:** 987105 (US-75392)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** Novartis Pharmaceutical Corp.  
Preclinical Safety, Dept of Toxicology/Pathology  
East Hanover, NJ

**Date of study initiation:** 24 October 1998

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** RAD, batch no. X176 1297, na, 92.5%

**Formulation/vehicle:** solid dispersion 9.1%/water-HPMC

### Methods:

**Species/strain:** mated female rats/Wistar Hanover, Hsd Br/Han:WIST, b(4)

**Doses employed:** 0, 0.03, 0.1, 0.3 mg/kg

**Route of administration:** oral gavage (2 mL/kg)

**Study design:** Females were dosed from gestation day 6 to lactation day 20;

**Terminal necropsy** on gestation day 25 for any mated female not delivering; lactation day 21 for mated females; (pups were culled into standard litter sizes on postpartum day 21); pups not selected for F<sub>1</sub> fertility assessment sacrificed after behavioral tests; gestation day 13 for F<sub>1</sub> females selected for fertility assessment; F<sub>1</sub> males selected for fertility assessment sacrificed following completion of F<sub>1</sub> mating period

**Number/sex/group:** 25

**Parameters and endpoints evaluated:** mortality, clinical signs, food consumption, body weight, F<sub>1</sub> in-life examinations and measurements (pre-weaning evaluations: viability, mortality, clinical observations, sex ratio, individual weights, righting reflex, pinna detached, and eye opening and post-weaning evaluations; mortality, clinical observations, acoustic startle, pupillary reflex, vaginal opening, preputial separation, passive

avoidance-learning/acquisition, watermaze-learning and memory, open field motor activity, F<sub>1</sub> mating, individual body weights)

**Results:****Maternal (F<sub>0</sub>)**

Dose mg/kg/day	0	0.03	0.1	0.3
Mortality	All rats survived until terminal sacrifice			
Clinical signs	Not remarkable			
Body weight	Not remarkable			↑; gestation days 6-9, of no toxicological relevance
Food consumption			↑ days 6-9	↑ days 18-21
Necropsy findings			1 female (lost litter) had no milk in mammary glands	
Delivery data	No treatment-related effects; e.g. on duration of gestation, # of viable and stillborn pups.			

**F<sub>1</sub> data**

Dose (mg/kg/day)	0	0.03	0.1	0.3
<b>Pre-weaning evaluation</b>				
Postnatal survival/clinical observations				
Pups died or missing days 1-4 (total numbers/%)	0/0	2/0.9	15*/4.9	5/1.9
Pups surviving 21 days (total numbers/%)	253/99	228/99	289*/95	257/97
Pup body weight/litter (g)				
Day 0	5.9	5.7	5.6*	5.6
Day 7	14.6	14	13.5*	13.5
Day 14	27.8	27.3	25.2*	26.2
Day 21	43.5	42.6	39.5* (↓ 9% from control)	41.2
Morphological, functional, behavioral development	Not remarkable			
Pup necropsy observations (fetuses/litters evaluated)	159/23	145/21	207/25	168/25
Pup found dead: no remarkable observation				
Fetal incidence (N/%)	0/0	0/0	13*/6.3	5/3
Litter incidence (N/%)	0/0	0/0	3/12	3/12
<b>Post-weaning evaluation</b>				
Mortality and clinical signs	Not remarkable			
Pup mean body weights (Days 28-84)				
F <sub>1</sub> males (significant % ↓ from control)			5-7% (D28-49)	
F <sub>1</sub> females (significant % ↓ from control)			4-8% (D28-63)	3-5% (D28-49)
F <sub>1</sub> fertility and general reproductive performance	Not remarkable			
F <sub>1</sub> necropsy observations	Not remarkable			

\*: statistically significant

**Conclusions:**

Oral treatment with SDZ RAD (everolimus) in pregnant female rats from gestation day 6 to lactation day 21 did not induce maternal toxicity or delivery (duration of gestation, number of stillborn or live pups delivered). There were slight reductions in survival and body weights (pre- and post weaning) in F<sub>1</sub> pups of dams treated at 0.1 mg/kg. The post-weaning mean body weights also decreased in F<sub>1</sub> pups of dams treated at 0.3 mg/kg. There were no drug-related effects on the morphological development, motor activity, learning ability or fertility assessment of the F<sub>1</sub> generation.

**2.6.6.7 Local tolerance**

*Reviewer's note:*

- This section was reviewed by Dr. S. Kunder for NDA [redacted] (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is re-formatted to fit the current NDA template.

b(4)

**Study title:** Primary skin irritation/corrosion study with RAD001 in the rabbit (4-hour semi-occlusive application)

**Key study findings:** RAD did not cause skin irritation to rabbits in this irritation assay

**Study no:** [redacted] Project 246781 (BS-811)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** [redacted]

b(4)

**Date of study initiation:** 27-30 October, 1998

**GLP compliance:** no

**QA report:** yes ( ) no ( x )

**Drug, lot #, and % purity:** RAD, batch 98902, 93.5%

**Formulation/vehicle:** solution in water

**Dosing:**

Species/strain: rabbit/albino New Zealand White, [redacted]

b(4)

#/sex/group or time point (main study): 3

Satellite groups used for toxicokinetics or recovery: none

Age: 6 weeks

Weight: <3.5 kg

Doses in administered units: 0.5 g RAD

Route, form, volume, and infusion rate: dermal patch, 4 h

**Observations and times:**

Clinical observations: twice daily

Other: skin irritation/corrosion observation on skin at 1, 24, 48, and 72 h after application

**Results:** No skin irritation was observed after 72h of skin application of RAD.

**Conclusions:** RAD did not cause skin irritation to rabbits in this irritation assay.

**Study title:** Assessment of contact hypersensitivity to RAD001 in the albino guinea pig (maximization test)

**Key study findings:** RAD did not cause contact hypersensitivity

Study no: \_\_\_\_\_ project 246792 (BS-812) **b(4)**  
Volume #, and page #: Module 4  
Conducting laboratory and location: \_\_\_\_\_  
\_\_\_\_\_

**b(4)**

Date of study initiation: 26 Oct-27 Nov 1998  
GLP compliance: no  
QA report: yes ( ) no ( x )  
Drug, lot #, and % purity: RAD, batch 98902, 93.5%  
Formulation/vehicle: solution, 1% carboxymethylcellulose

**Methods** (unique aspects): Experimental animals were intradermally injected with 0.2% RAD and dermally exposed to a 50% concentration. Controls were treated as above with vehicle. At 24h before epidermal induction, all animals were treated with 10% SDS. Two weeks after epidermal application all animals were challenged with 50% RAD and vehicle.

**Dosing:**

Species/strain: guinea pig/albino, females, \_\_\_\_\_ **b(4)**  
#/sex/group or time point (main study): 10 treated, 5 control  
Age: 4 weeks  
Weight: <500g  
Doses in administered units: unable to determine from report  
Route, form, volume, and infusion rate: topical, intradermal, 1% aqueous methylcellulose

**Observations and times:**

Clinical signs: twice daily  
Other: contact dermatitis observation on skin

**Results:** No skin reactions were seen after challenge exposure in treated or control animals.

**Conclusions:**

The results of this study are difficult to interpret as the concentration of RAD is not quantified. Otherwise, RAD did not appear to cause contact hypersensitivity.

**2.6.6.8 Special toxicology studies**

*Reviewer's note:*

- This section was reviewed by Dr. S. Kunder for NDA \_\_\_\_\_ (Special Pathogen and Immunologic Drug Products, October 10, 2003) and is re-formatted to fit the current NDA template. **b(4)**

**Study title:** Comparative study of ophthalmic toxicity by oral gavage administration to CD rats and HanIbm rats for 4 weeks

**Key study findings:** In CD-1 and HanIbm rats, the high dose RAD (5.0 mg/kg) exhibited frank toxicity to the lens of both strains as swelling and disruption of the fibers of the anterior cortex. Younger animals (8 weeks) of both strains were more susceptible than older animals and the CD strain was more susceptible than the HanIbm strain to this toxicity. Myocarditis was exacerbated at both doses and both ages in both strains compared to the incidence in controls.

**Study no:** 96/SPM098/0796 (203-068)

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** \_\_\_\_\_

b(4)

**Date of study initiation:** 1 April 1996

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** SDZ RAD microemulsion 2%, batch no. Y182 0895, na, 92.5%

**Formulation/vehicle:** SDZ RAD2% microemulsion , diluted with 5% glucose solution

**Methods (unique aspects):** none

**Dosing:**

Species/strain: CD-1 rats, \_\_\_\_\_ HanIbm Wistar rats, \_\_\_\_\_

b(4)

#/sex/group or time point (main study): 5/strain

Satellite groups used for toxicokinetics or recovery: none

Age: 8 weeks, 20 weeks (both CD-1 and Han Ibm)

Weight:

- 8 week CD-1: 229-254g (males); 169-187g (females);
- 20 week CD-1: 466-526 g (males), 310-339g (females);
- 8 week HanIbm: 163-183g (males), 114-135g (females);
- 20 week HanIbm: 410-507g (males), 217-237g (females)

Doses in administered units: 0, 0.5, 5.0 mg/kg both strains and age groups

Route, form, volume: oral gavage, 5 mL/kg,

**Observations and times:**

Clinical signs: twice daily

Body weights: pretreatment, weekly

Food consumption: weekly

Ophthalmoscopy:

pretreatment, day 29 of treatment, the eyes of each rat were examined using an indirect ophthalmoscope in the following structures: palpebrae and adjacent structures, conjunctiva, cornea and sclera, anterior chamber and iris, lens and vitreous and ocular fundus.

EKG: not performed

Hematology: not performed

Clinical chemistry: not performed  
 Urinalysis: not performed  
 Gross pathology: at necropsy  
 Organs weighed: at necropsy  
 Histopathology: at necropsy, only eyes examined  
 Toxicokinetics: not performed  
 Other: na

**Results:**

Dose (mg/kg)	CD-1				HanIbm			
	8 week		20 week		8 week		20 week	
	0.5	5.0	0.5	5.0	0.5	5.0	0.5	5.0
Mortality						1 male died week 4 no clinical signs		
Clinical signs	No treatment-related signs were seen							
Body weights				loss				Loss
Food consumption		↓		↓		↓		↓
Ophthalmoscopy Lesions-swelling and disruption, fibers of anterior cortex of lens		5/5 males 5/5 females		3/5 males		4/5 males 4/5 females		
EKG	Not performed							
Hematology	Not performed							
Clinical chemistry	Not performed							
Urinalysis	Not performed							
Organ weights		↑: Lungs, males ↓: Prostate Thymus, all Spleen, all		↑: Lungs, males ↓: Prostate Uterus, Spleen, all		↑: Lungs, all, ↓: Prostate, Thymus, all Testes		↑: Lungs, males ↓: Prostate, Thymus, all Pituitary, females Testes, Uterus, Epididymides
Gross pathology	No treatment-related findings							
Histopathology Heart Myocarditis	4/5 males	5/5 males	5/5 males	5/5 males 4/5 females	1/5 females	2/4 males 2/5 females	5/5 males	5/5 males 3/5 females
Toxicokinetics	Not performed							

**Conclusions:**

In this study to examine primarily ophthalmic effects of RAD in CD-1 and HanIbm rats, drug was administered to 8- or 20-week rats. The high dose (5.0 mg/kg) caused frank toxicity to the lens of both strains, which exhibited as swelling and disruption of the fibers of the anterior cortex. Toxicity to islet cells of the pancreas, seen in other studies, was not observed here. Such a finding, with implications of impaired glucose metabolism, would logically correlate to the lens toxicity. Younger animals of both strains were more susceptible to this toxicity.

Myocarditis was exacerbated at both doses and both ages in both strains compared to the incidence in controls.

**Study title:** A 2-week oral (gavage) mechanistic toxicity study in rats

**Key study findings:** The toxicities observed in this 2-week rat study are similar to those seen in other rat studies. The serologic finding of Coxsackie virus may be related to the myocardial degeneration seen in RAD-treated rats. Toxicity of RAD for male reproductive organs was confirmed and may be related to the decreased testosterone seen in the 15 mg/kg group.

**Study no:** 27EXR

**Volume #, and page #:** Module 4

**Conducting laboratory and location:** Novartis Pharma AG  
Preclinical Safety  
4002 Basel Switzerland

**Date of study initiation:** 12 April 2002

**GLP compliance:** no

**QA report:** yes ( ) no ( x )

**Drug, lot #, radiolabel, and % purity:** RAD 2% microemulsion: not provided

**Formulation/vehicle:** RAD 2% microemulsion diluted with 5% glucose solution

**Dosing:**

Species/strain: Han1bm rats, males

#/sex/group or time point\*: 10 (Study Group 2: 0.15, 1.5 mg/kg) 20 (Study Group 2: controls, 15 mg/kg),

Satellite groups used for toxicokinetics or recovery: none

*\*Note: two study groups, Study group 1 and 2, respectively, were formed to perform the following tests;*

- Study Group 1: CS, BW, FC, CP, ECG, Ser, Path
- Study Group 2: CS, BW, FC, Endo, 2D, Path
- BT = body temperature, ECG = electrocardiography, Ser = serology  
BW = body weight, Endo = endocrinology, 2D = 2-D gel-electrophoresis heart  
CP = clinical pathology, FC = food consumption  
CS = clinical signs, Path = pathology

Age: 12 weeks

Weight: 295-369 g

Doses in administered units: 0, 0.15, 1.5, 15 mg/kg

Route, form, volume: oral gavage, 5 mg/mL

**Observations and times:**

Clinical signs: daily

Body weights: weekly

Food consumption: weekly

Ophthalmoscopy: not performed

EKG: pretest, day 1, day 12

Hematology: days 2, 17  
 Clinical chemistry: days 2, 17  
 Urinalysis: pretest, day 17  
 Gross pathology: at necropsy  
 Organs weighed: at necropsy  
 Histopathology: at necropsy, see table  
 Toxicokinetics: not performed  
 Other: serological assay for pathogens; 2-D gel electrophoresis of heart tissue

**Results:**

Study	Study Group 1				Study Group 2	
	0	0.15	1.5	15	15	0
Mortality	All rats survived until scheduled sacrifice					
Clinical signs	No treatment-related signs were seen					
Body weights				↓	↓	
Food consumption			↓	↓	↓	
Ophthalmoscopy	Not performed					
EKG				Heart rate ↓ 10%	Heart rate ↓ 10%	
Hematology			↑ hematocrit, RBC, HGB Platelets, ↓ Leukocytes, ↓	↑ hematocrit, RBC, HGB Platelets, ↓ Leukocytes, ↓ ↑ Neutrophils	↑ hematocrit, RBC, HGB Platelets, ↓ Leukocytes, ↓ ↑ Neutrophils	
Clinical chemistry			↓ AST ↓ CK ↓ LDH ↑ Cholesterol	↓ AST ↓ CK ↓ LDH ↑ Cholesterol Testosterone ↓	↓ AST ↓ CK ↓ LDH ↑ Cholesterol Testosterone ↓	
Urinalysis	No treatment-related findings reported					
Gross pathology	No treatment-related findings reported					

**Appears This Way  
 On Original**

Histopathology						
Thymus						
Medullary atrophy			10/10	10/10	10/10	
Cortical atrophy			9/10	10/10	8/10	
Heart						
Myocardial degeneration		5/10	10/10	10/10	7/10	3/9
Lungs						
Alveolar macrophages			10/10	10/10	10/10	
Eyes						
Fiber swelling differential or focal		1/10	2/10	10/10	9/10	1/10
Adrenal cortex						
Vacuolation	1/10		9/10	9/10	10/10	2/10
Mammary atrophy				6/10	6/10	
Testes						
Germ cell degeneration			1/10	10/10	10/10	
Vacuoles/seminal epithelium			1/10	7/10	9/10	
Giant multinucleated cells				1/10	3/10	
Tubular atrophy				2/10	3/10	
Prostate						
Atrophy				9/10	7/10	
Seminal vesicles						
Atrophy			5/10	8/10	9/10	1/10
Sternum –bone marrow depletion	1/10	1/10	2/10	5/10	8/10	1/10
2-d gel electrophoresis heart tissue					Tubulin-β, α-1-antiproteinase, apolipoprotein E precursor, transeferrin ↓ (> 50% from control) ↑ Apolipoprotein E	
serology	Antibody against Coxsackie virus B3					
Toxicokinetics	Not performed					

**Conclusions:**

The serologic finding of Coxsackie virus may be related to the myocardial degeneration seen in RAD001-treated rats. Toxicity of RAD001 for male reproductive organs was confirmed and may be related to the decreased testosterone seen in the 15 mg/kg group.

**2.6.6.9 Discussion and Conclusions**

Pharmacology, safety pharmacology, pharmacokinetic/ADME, and toxicology studies supporting the marketing application of everolimus for the proposed indication were conducted in *in vitro* systems as well as in mice, rats, minipigs, and monkeys. The general toxicology studies were conducted in appropriate animal species, using the administration route, dosing schedule/duration that adequately addressed safety concerns for the indicated patient population. The target organs of everolimus are hematopoietic/lymphoid organs (lymph nodes, spleen and thymus), GI tract, heart, lung, skin, pancreas and male and female reproductive organs. There are also rat specific toxicities in lens, as well as minor findings in kidney, liver, adrenal and thyroid.

The rodent and nonrodent species in general did not demonstrate significant differences in everolimus-related toxicity profile; however, the rat appeared to be more susceptible than the monkey to certain toxicities; e.g. dose limiting toxicities were different. Toxicities were mainly attributable to the primary or secondary pharmacological effects of everolimus. In general, toxicities associated with everolimus are comparable to those observed with

temsirolimus (Torsel®), another mTOR inhibitor, approved for treatment of renal cell carcinoma. Below is a summary of effects seen for both everolimus and temsirolimus. Of note, the severity of findings may not be the same.

- Male reproductive organs: e.g. testicular and prostate atrophy; epididymal luminal cellular debris, ↓ sperm count
- Hematopoietic/lymphoid system: e.g. ↓ lymphocytes, ↑ neutrophils, lymphoid atrophy in lymphoid tissues, splenic atrophy and/or hemosiderosis, ↑ RBC
- Coagulation: ↓Platelets, ↑ fibrinogen, ↑ aPTT
- Bone marrow: hypocellularity
- GI tract: inflammation and ulceration
- ↓ serum phosphorus, changes in potassium levels (↓ serum K<sup>+</sup> in temsirolimus-treated animals and ↑urinary K<sup>+</sup> in everolimus-treated animals)
- Lung: accumulation of foamy alveolar macrophages
- Metabolism/endocrine: ↑ cholesterol and triglycerides, pancreatic exocrine/islet cell vacuolation, ↑ amylase
- Heart: myocardial degeneration
- Eyes: lens opacity/cataract-type findings (rats)
- Kidney: ↑ BUN and creatinine, tubular degeneration
- Liver: e.g. ↑ AST and ALT (occasional increases in everolimus-treated animals; in temsirolimus-treated animals, hepatotoxicity was more evident after oral administration of the drug)
- Thyroid: ↓ weight, other effects (↑T4 with temsirolimus and histopathology findings with everolimus)
- Pituitary: ↓ weight

The immunosuppression effects of everolimus were evident in all species tested as lymphoid atrophy in lymph nodes, thymus and/or spleen. Lymphoid atrophy of spleen was more obvious in minipigs and monkeys, while in rats, histopathological changes of spleen, i.e., hemosiderosis, seemed to be mainly secondary to hematological changes. Bone marrow hypocellularity was seen in one animal species (minipig) only. In general, lymphocyte counts decreased while neutrophils, monocytes and % band cells increased in the species studied. Increased RBC, HGB and Hct, accompanied with decreased MCV/MCH were observed in rats. Increased erythroid parameters were also observed in mice (not reviewed in this NDA) and minipigs. Of note, an opposite effects on RBC/HGB/Hct were observed in monkeys. In all species studied, decreased platelet counts and increased fibrinogen were observed. Increased aPTT was only seen in the minipig.

The immunosuppression property of everolimus may lead to inflammation, infection and compromised wound healing in the treated animals. Slower wound healing may also be the result of anti-angiogenesis effects of everolimus. These effects were manifested as findings in stomach (acute inflammation at glandular tissue and mucosal hypertrophy/hyperplasia in rats), intestines (erosion and mucosal atrophy in minipigs, aggregation of macrophages and mucosal inflammation in monkeys), skin (ulceration, scabs, lesions in all species studied), kidney (interstitial inflammation and basophilic tubules in mice), and lung (accumulation of foamy alveolar macrophages in all species). The GI lesions may have contributed to decreased food intake, malnutrition, weight loss and deteriorating conditions in animals, which was the leading cause of mortality in monkeys. Serious skin lesions were associated with moribund sacrifices in the 26 week study in rats. It was noted that everolimus did not cause skin irritation in rabbits or contact hypersensitivity in guinea pigs under the conditions of the study. Finally, infection, based on serologic findings of Coxsackie virus, may have contributed to the myocardial degeneration seen in everolimus-treated rats (see below).

mTOR has been proposed as a homeostatic sensor (Dennis *et al.*, Science 294: 1102-1106, 2001) and is highly sensitive to ATP levels in the cell (Meijer, J Nutr 138: 2057S-20662S, 2003). Furthermore, mTOR may regulate glucose uptake (Majhail *et al.*, J Clin Oncol, 21: 3995-4000, 2003) and glycolysis (reviews by Bjornsti and Houghton, Nature Cancer Review, 4: 334-348, 2004; Abraham and Gibbons, Clin Cancer Res 13: 3109-3114, 2007). In addition, mTOR is involved in proliferation and survival of pancreatic  $\beta$ -cells (Leibowitz *et al.*, Diabetes Obes Metab. 10 Suppl 4:157-69, 2008; Rachdi *et al.*, PNAS 105 (27): 9250-5, 2008). Everolimus did not induce diabetic effects in animals under the conditions tested, although pancreatic toxicity was evident by increased amylase and/or lipase in all species, as well as by histopathological changes in pancreas in multiple species. Hyperglycemia was evident in patients.

The findings of decreased pituitary weights in rats and thyroid weights in monkeys, histopathological findings in thyroid (follicular cell hypertrophy/vacuolation and reduced intrafollicular colloid in male rats) and sex organs (especially lesions in testes and epididymis, also decreased testosterone) may suggest that everolimus may affect the hypothalamic-pituitary axis. The hormonal alterations (i.e.  $\downarrow$  testosterone) may be a direct effect of the drug on the reproductive organs or an indirect effect via the interference with the hypothalamic-pituitary axis.

Under the conditions tested, everolimus did not exert effects on cardiovascular functions (ECG, heart rate, or blood pressure). However, myocarditis and myocardial degeneration and/or fibrosis were found in rats or monkeys. Ocular toxicities, mainly in lens, i.e., swelling and disruption of fibers in the anterior cortex, were unique rat-specific toxicities associated with everolimus. Of note, neither cardiac nor eye toxicities were found in the 26 week study in rats. Considering that systemic exposures ( $C_{max}$  and AUC) were lower in the chronic toxicology study, ocular toxicities may be most affected by the exposure levels, rather than the duration of treatment.

Other targets of everolimus included kidney (increased BUN, creatinine; increased incidence of hydronephrosis and pigment in renal tubular epithelium in rats) and adrenal (microvesiculation in the cortex, may be stress-associated).

Everolimus was not mutagenic in bacterial Ames test and TK mutation test in L5178Y mouse lymphoma cells, and was negative in the *in vitro* chromosomal aberration test in V79 Chinese hamster cells, and *in vivo* micronucleus test in rats. Everolimus induced significant effects on male fertility: atrophy of testes and epididymis, reduced sperm mobility and sperm counts, decreased testosterone levels, infertility (0/19 female pregnant) after 13 week treatment in male rats and 60-65% fertility index (12 or 13 out of 20 females were pregnant) during a 10-13 week treatment-free period. At oral gavage doses  $\geq 0.6 \text{ mg/m}^2$  in female rats (2 weeks prior to mating until gestation day 16), everolimus induced dose-dependent increases in pre- and post-implantation loss and resorption, as well as increased incidence of sternal malformation and retarded skeletal development. The embryofetal toxicities were observed in the absence of maternal toxicity. Since the female rats were treated prior to mating, increased pre-implantation loss may suggest that everolimus may affect female fertility. Embryofetal toxicities, in the presence of maternal toxicities (mortality, decreased body weights and food consumption), were seen in rabbits treated with an oral dose of everolimus at  $9.6 \text{ mg/m}^2$ ; effects included increased resorption. When females ( $F_0$ ) were treated with everolimus at  $\geq 0.6 \text{ mg/m}^2$ , the  $F_1$  pups showed a slight decrease in survival and body weights, but there was no evidence to indicate drug-related effects on the morphological, functional or behavioral development in the  $F_1$  generation.

Unlike other immunosuppressants, including another mTOR inhibitor sirolimus (rapamycin, Rapamune<sup>®</sup>), oral treatment of mice or rats with everolimus up to 104 weeks did not induce carcinogenic effects. The study results were considered acceptable by the Executive Carcinogenic Assessment Committee (December 2, 2003).

Studies were conducted to investigate the general toxicity and genotoxicity of impurities found in the drug substance. There were no remarkable findings.

**2.6.6.10 Tables and Figures**

See the studies reviewed for pertinent tables and figures.

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

General toxicology: all via oral administration

Single Dose Toxicity Studies					
Species	Route	N/sex/dose	mg/kg	mg/m <sup>2</sup>	Significant findings
Mouse	Oral 14 day recovery	5	2000	6000	Clinical signs (2♀: slight dyspnea, slight to moderate ruffled fur) Slightly ↓ body weight: 1 ♀
Rat	Oral 14 day recovery	5	2000	12000	Not remarkable
Repeat Dose Toxicity Studies					
Species	Route	N/sex/	mg/kg/	mg/m <sup>2</sup>	Significant findings

		dose	day	/day	
Rat	Oral Daily 4 week	10 (a)	15	90	<p><u>90 mg/m<sup>2</sup>/d</u>: ↓ BW (up to -30% from control) and weight gains (♂ &gt; ♀, up to -81% from control), not resolved in ♂ (-20% in BW), ↓ food conversion efficiency and food consumption (up to -35% from control), not reversible high incidence of anterior suture line opacities in the lenses, hemoconcentration (↑ RBC, HGB, Hct), ↓ MCH &amp; MCV, ↑ neutrophil &amp; ↓ platelets (both not reversible), ↑ BUN (7-10%), ↑ chole &amp; TG (♀), ↓ albumin (not resolved) &amp; A/G ratio, urinalysis (↓ K<sup>+</sup>), ↓ pituitary (♀), spleen, thymus, testes, epididymes, seminal vesicle, prostate, ovary &amp; uterus weights, ↑ lung weights, organ weight findings in sex organs, lung and pituitary: not resolved, histopathological findings: eye (swelling and disruption of fibers in the anterior cortex), heart (chronic myocarditis), lymphoid organs (mainly lymphoid atrophy in lymph nodes and thymus), lung (foamy alveolar macrophages), stomach (hypertrophy of gastric chief cells), salivary gland (depletion of secretory granules) and male reproductive organs (epididymes and testes: depletion of germ cells, spermatid giant cells, and tubular vacuolation; atrophy in prostate and seminal vesicle), and female reproductive organs (ovary: interstitial cell hypertrophy; uterus: atrophy). Findings in heart, eye and sex organs did not resolve. ↓ Sperm counts (not resolved).</p> <p><u>30 mg/m<sup>2</sup>/d</u>: ↓ BW (up to -24% from control) and weight gains (up to -65% from control), ↓ food conversion efficiency and food consumption (up to -29% from control), ↑ incidence of anterior suture line opacities in the lenses, hemoconcentration (↑ RBC, HGB, Hct), ↓ MCH &amp; MCV, ↑ neutrophils, ↓ platelets, ↑ chole &amp; TG (♀), ↓ albumin &amp; A/G ratio, urinalysis (↓ K<sup>+</sup>), ↓ pituitary (♀), spleen, thymus, epididymes, seminal vesicle, prostate, ovary &amp; uterus weights, ↑ lung weights, histopathological findings: similar to 90 mg/m<sup>2</sup>/d, except epididymes findings.</p> <p><u>9 mg/m<sup>2</sup>/d</u>: ↓ BW (up to -11% from control) and weight gains (up to -33% from control), ↓ food conversion efficiency and food consumption (up to -15% from control), hemoconcentration (↑ RBC, HGB, Hct), ↑ chole (♂) &amp; TG (♀), urinalysis (♂: ↓ K<sup>+</sup>), ↓ pituitary (♀), thymus, epididymes, seminal vesicle &amp; uterus weights, ↑ lung (♂) weights, histopathologic findings: similar to 30 mg/m<sup>2</sup>/d, except findings in eye, prostate, uterus and stomach.</p> <p><u>3 mg/m<sup>2</sup>/d</u>: ↓ BW (♂ -3% from control) and weight gains (♂ -13% from control), hemoconcentration (↑ RBC, HGB, Hct), ↑ Chole (♂), ↓ pituitary (♀), thymus &amp; uterus weights, ↑ lung (♂) weights, histopathological findings: heart (♂) &amp; lung (♂)</p>
		6 (b)	5	30	
		4 (c)	1.5	9	
			0.5	3	
Rat	Oral Daily 4 week	10 (a)	1.5	9	<p><u>9 mg/m<sup>2</sup>/d</u>: ↓ BW (♂, -11% from control) and weight gains, ↓ food conversion efficiency and food consumption (♂, -11% from control), anterior suture line opacities in the lenses (1 ♂, 2 ♀), hematology (↑ RBC, HGB, Hct, ↓ MCH &amp; MCV (♂), ↓ platelets), ↑ amylase, chole &amp; TG (♀), ↓ albumin &amp; A/G ratio, ↓ pituitary (♀), thymus, epididymes, seminal vesicle, ovary &amp; uterus weights, ↑ lung (♂); organ weight findings in pituitary, thymus, sex organs: not resolved; histopathological findings: heart (chronic myocarditis, myocardial fibrosis), thymus</p>
		6 (b)	0.5	3	
		4 (c)	0.25	1.5	
			0.1	0.6	

					<p>(atrophy), lung (diffused alveolar macrophages), salivary gland (depletion of secretory granules) and female reproductive organs (ovary: interstitial cell hypertrophy; uterus &amp; cervix: atrophy). Findings resolved, except heart in 1 ♂.</p> <p><u>3 mg/m<sup>2</sup>/d:</u> hematology (↑ RBC, HGB &amp; Hct (♀), ↓ MCH, MCV &amp; platelets (♂)), ↑chole, ↑amylase &amp; TG (♀), ↓ albumin, ↓ pituitary (♀), thymus, ovary &amp; uterus weights, histopathological findings: thymus (atrophy), lung (♂: diffused alveolar macrophages).</p> <p><u>1.5 mg/m<sup>2</sup>/d:</u> ↓ platelets (♀), ↑ chole, ↑amylase (♀), ↓ pituitary (♀), ovary &amp; uterus weights,</p> <p><u>0.6 mg/m<sup>2</sup>/d:</u> Not remarkable</p>
Rat	Oral Daily 26 week	20 (a) 5 (b) 12 (c)	1.5 0.5 0.15 0.1 0.05	9 3 0.9 0.6 0.3	<p><u>9 mg/m<sup>2</sup>/d:</u> mortality (1 ♂ in WK 23: lesion at foot associated with leukocytosis), ↓ BW (-13% from control) and weight gains (up to -31%), resolved, ↓ food conversion efficiency, hemoconcentration (↑ RBC, HGB, Hct), ↓ MCH (♂) &amp; MCV, ↑ neutrophil, ↓ lymphocytes &amp; platelets (but ♀ in WK 4 only), ↑ amylase &amp; lipase (♀ only, in WK 14), ↑ chole &amp; TG (♀ only: in WK 4), ↓ albumin &amp; A/G ratio, ↓ iron &amp; phosphorus, urinalysis (↑ volume), ↓ kidney, pituitary (♀), thymus, testes, epididymes, seminal vesicle, prostate &amp; uterus weights, ↑relative weight: liver, lung &amp; spleen, organ weight findings in spleen, epididymes and testes (↓) not resolved, histopathological findings: lymphoid organs (mainly lymphoid atrophy in lymph nodes and thymus, and lymphocytolysis in thymus), kidney (hydronephrosis, ↑ pigment within the tubular epithelial cells), lung (accumulation of alveolar macrophages and perivascular lymph infiltration), spleen (hemosiderosis), stomach (acute inflammation at glandular tissue and mucosal hyperplasia/hypertrophy), thyroid (♂: follicular cell hypertrophy and vacuolation and reduced intrafollicular colloid) and male reproductive organs (epididymis and testes: reduced sperm count, depletion of germ cells, partial depletion of one or more generations of germ cells, spermatid giant cells, and tubular vacuolation).</p> <p><u>3 mg/m<sup>2</sup>/d:</u> ↓ BW (♀ -5% from control) and weight gains (♀ -29% from control), ↓ food conversion efficiency, hematology (↓ WBC: ♀ WK 4 &amp; 26, ↑neutrophils, ↓ lymphocytes &amp; platelets), ↑ amylase, ↑ chole &amp; TG (♀ only: in WK 4), ↓ albumin, ↓ iron &amp; phosphorus, urinalysis (↑ volume), ↓ pituitary weights, ↑ relative weight: liver, histopathological findings: lymphoid organs (lymphoid atrophy in lymph nodes and thymus), kidney (hydronephrosis, ↑ pigment within the tubular epithelial cells), lung (accumulation of alveolar macrophages and perivascular lymph infiltration), spleen (hemosiderosis), stomach (acute inflammation at glandular tissue and mucosal hyperplasia /hypertrophy), thyroid (♂: follicular cell hypertrophy and vacuolation and reduced intrafollicular colloid).</p> <p><u>0.9 mg/m<sup>2</sup>/d:</u> ↓ weight gains (♀ -11% from control), ↓ food conversion efficiency, hematology (↓ WBC in WK 4), ↑ amylase (♂), ↑ chole (♂) &amp; TG, ↓ iron &amp; phosphorus, urinalysis (↑ volume), ↓ pituitary weights, histopathological findings: lung (accumulation of alveolar macrophages)</p> <p><u>≤ 0.6 mg/m<sup>2</sup>/d:</u> Not remarkable</p>

Monkey	Oral Daily 4 week	3 (a) 2 (b)	15 5 1.5	180 60 18	<p><u>180 mg/m<sup>2</sup>/d</u>: piloerection (♂) &amp; skin (scabs, lesion), hematology (↓ RBC, HGB &amp; Hct, ↑white counts (WBC, neutrophils and monocytes), ↑fibrinogen), ↑ AST &amp; ALT (♂), ↓ albumin, γ-globulin &amp; phosphorus, ↑ globulin (A-2 and β-), urinalysis (↓ Na<sup>+</sup>), ↓ thymus (not resolved) &amp; pancreas weights, Histopathological findings (resolved): intestine (histiocytosis), spleen (lymphoid atrophy), thymus (lymphoid atrophy), also minor lesions in skin.</p> <p><u>60 mg/m<sup>2</sup>/d</u>: skin (scabs, lesion), hematology (↓ RBC, HGB &amp; Hct, ↑white counts (neutrophils and monocytes), ↑fibrinogen), ↓ thymus weights, similar histopathological findings as 180 mg/m<sup>2</sup>/d</p> <p><u>18 mg/m<sup>2</sup>/d</u>: skin (scabs, lesion), ↑fibrinogen, ↓ thymus weight</p>
Monkey	Oral Daily 26 week	4 (a) 2 (b, not conducted)	5 1.5 0.5 0.1	60 18 6 1.2	<p><u>60 mg/m<sup>2</sup>/d</u>: Mortality (♂1/6, ♀ 1/6), clinical signs (skin lesions, piloerection, hunched posture), ↓ BW &amp; food intake, hematology (↓ HGB, Hct, ↑fibrinogen), clinical chemistry (↑ chol &amp; TG; ↓ albumin, A/G ratio, phosphorus), ↓ thymus, pancreas, spleen (♀) &amp; testes weights, histopathological findings: lymphoid tissues (atrophy: thymus, lymph nodes, spleen), GI (small intestine: aggregation of macrophages, large intestine: mucosal inflammation), skin (epidermal necrosis, inflammation, ulceration), heart (myocardial degeneration), pancreas (degranulation of exocrine cells, degeneration of pancreatic islet cells), adrenal (cytoplasmatic vacuolation) and ovary (reduced follicular development and increase in follicular astresia).</p> <p><u>18 mg/m<sup>2</sup>/d</u>: Mortality (♂2/4), clinical signs (skin lesions, piloerection, hunched posture), ↓ BW (pre-schedule deaths: – 25%), hematology (↓ HGB Hct, ↑fibrinogen), clinical chemistry (↑ chol; ↓ albumin), ↓ thymus, pancreas, spleen (♀) thyroid &amp; testes weights, similar but less severe histopathological findings as 60 mg/m<sup>2</sup>/d</p> <p><u>6 mg/m<sup>2</sup>/d</u>: skin ulceration, ↑ chol, ↓ thymus &amp; spleen (♀) weights,, histopathological findings: pancreas, small intestine, spleen (see 60 mg/m<sup>2</sup>/d)</p> <p><u>1.2 mg/m<sup>2</sup>/d</u>: skin ulceration,</p>
Monkey	Oral Daily 52 week	4 (a)	0.9 (39 weeks) 0.3 0.1	10.8 3.6 1.2	<p><u>10.8 mg/m<sup>2</sup>/d</u>: Mortality (♂1/4, ♀ 2/4), GI clinical signs (soft feces, diarrhea), ↓ BW &amp; food intake (mainly in pre-schedule deaths), hematology (↓ HGB, MCV, MCH &amp; lymphocyte (WK 52), ↑% band cells, fibrinogen), clinical chemistry (mainly in early deaths: ↑ BUN, creatinine, glucose, amylase, lipase, TG, K<sup>+</sup>, phosphorus; ↓ Na<sup>+</sup>, Cl<sup>-</sup>), urinalysis findings (+ glucose and or protein), ↓epididymes &amp; testes weights, histopathologic findings: colon/cecum (inflammation: 2/4 ♀), stomach (erosion /inflammation: 2♂ and 2 ♀), testes (immature: all ♂)</p> <p><u>3.6 mg/m<sup>2</sup>/d</u>: GI clinical signs (♂: soft feces, diarrhea), ↓ BW &amp; food intake (2/4 M), hematology (↓ HGB &amp; lymphocyte (WK 52), ↑% band cells), urinalysis findings (+ glucose and or protein), ↓epididymes &amp; testes weights, histopathological findings: large intestine (inflammation: 3♂ and 2 ♀), testes (immature: 2 ♂), prostaead seminal vesicle (immature: 1 ♂)</p> <p><u>1.2 mg/m<sup>2</sup>/d</u>: ↑% band cells (♀)</p>

Route of administration: SC (subcutaneous), IV (intravenous)

Study groups: main (a), recovery (b, only the control and the high dose groups) and toxicokinetics/TK (c)

Chole: cholesterol, TG: triglycerides

Genetic toxicology:

<i>In Vitro</i> Studies			
Study #	System	Concentrations	Results
Mut. Bakt. 27/95	Bacterial Ames test: <i>Salmonella typhimurium</i> : TA98, TA97a, TA100, TA102 and TA1535	Experiment #1: 8-5000 µg/plate Experiment #2: 156.25-2500 µg/plate	Negative in mutagenicity. With or without S9-mix
Z59	Chromosome aberration: V79 Chinese hamster cells	Experiment #1: – S9 (3 hr): 35, 53, 81 µg/mL + S9 (3 hr): 57, 87, 131 µg/mL Experiment #2: – S9 (3 hr): 15, 23 µg/mL + S9 (20 hr): 61, 87, 123 µg/mL	Negative in clastogenicity
1463-4-1052	TK mutation test Mouse lymphoma L5178Y cells	Experiment #1: – S9: 7.5-60 µg/mL + S9: 15, 30, 60 µg/mL Experiment #2: – S9: 20-50 µg/mL + S9: 30-65 µg/mL	Negative in TK forward mutagenicity
<i>In Vivo</i> Study			
MK 36	Micronucleus assay: Mouse bone marrow	Oral gavage doses at 50, 160 and 500 mg/kg, twice (24 hr apart) ➤ Micronucleus assay: n=5/sex/dose	Negative

Carcinogenicity studies:

Study	Route	Duration	Dose (mg/kg/d)	Results
Mouse (SPM118/ 973229)	Oral gavage	Daily, 104 weeks	0.1, 0.3 and 0.9 (Or, 0.3, 0.9 and 2.7 mg/m <sup>2</sup> ) N=60/sex/dose	<ul style="list-style-type: none"> <li>➤ Adequacy of study: reached the MTD (weight loss &gt;10% at HD) and sufficient survivals at HD up to WK 101. Morality: treated groups comparable to the control.</li> <li>➤ Comparable toxicology findings as reported in other repeat dose studies, i.e., immunosuppression-related non-neoplastic histopathological findings.</li> <li>➤ One neoplastic finding in ♀: osteoma of the femur (1/60 at 0.3 mg/kg, 2/60 at 0.6 mg/kg), within ranges of historical control.</li> </ul>
Rat (SPM113/ 973228)	Oral gavage	Daily, 104 weeks	0.1, 0.3 and 0.9 (Or, 0.6, 1.8 and 5.4 mg/m <sup>2</sup> ) N=60/sex/dose	<ul style="list-style-type: none"> <li>➤ Adequacy of study: reached the MTD (weight loss &gt;30% at HD) and sufficient survivals at HD up to WK 104. Morality: treated groups comparable to the control.</li> <li>➤ Comparable toxicology findings as reported in other repeat dose studies, i.e., lymphoid atrophy, and findings in adrenal, lens, liver, lung and reproductive organs.</li> <li>➤ No drug-related neoplastic findings.</li> </ul>

Reproductive toxicology:

Study	Route	Duration	Dose (mg/kg/d)	Results
Fertility and early embryonic development				
Rat (7073R)	Oral gavage	Daily, 13 weeks with 13 week recovery	0.1, 0.5, 5 mg/kg (Or, 0.6, 3 and	➤ Everolimus at 5 mg/kg affected male fertility: fertility index= 0% (♀:0/19)

			30 mg/m <sup>2</sup> )	pregnant) in WK 13; 60-65% (♀:12/20 confirmed pregnant; one additional female could not be clearly identified as pregnant) ➤ Affected male reproductive systems (main study and recovery): testes & epididymes (↓organ weights, atrophy, ↓ sperm mobility and counts, ↓ testosterone levels.
Embryofetal development				
Rat (3074R)	Oral gavage	Females dosed 2 weeks prior to mating to gestation day (GD) 16 inclusive	0.1, 0.3, and 0.9 (Or, 0.6, 1.8 and 5.4 mg/m <sup>2</sup> )	➤ No treatment related maternal toxicity ➤ Dose-dependent embryonic toxicity (≥ 0.1 mg/kg): ↑ resorption & pre- & post-implantation loss, ↓ numbers of live fetuses. ➤ Dose-dependent fetal toxicity (≥ 0.1 mg/kg/d): ↑ incidence of malformations of thoracic vertebrae, ribs & sternebrae, retarded skeletal development.
Rabbit (4070K)	Oral gavage	Females dosed D6-18 post-insemination (p.i.) inclusive	0.05, 0.2 and 0.8 (Or, 0.6, 2.4 and 9.6 mg/m <sup>2</sup> )	➤ Maternal toxicity: death, clinical signs, weight loss, ↓ food consumption. ➤ Dose-dependent embryonic toxicity (mainly 0.8 mg/kg): ↑ resorption
Prenatal and post natal development				
Rat (987105)	Oral gavage	Females dosed GD 6 to lactation day 20	0.03, 0.1 and 0.3 (Or, 0.18, 0.6, and 1.8 mg/m <sup>2</sup> )	F <sub>0</sub> females: ➤ No treatment related maternal toxicity ➤ No treatment related differences in delivery and lactation parameters. F <sub>1</sub> pups: ➤ A slight ↓ survival and body weights in pups of dams dosed at ≥ 0.1 mg/kg ➤ No drug-related effects on the development of F <sub>1</sub> generation

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Conclusions:

Pharmacology, safety pharmacology, pharmacokinetic/ADME, and toxicology studies supporting the marketing application of everolimus for the proposed indication were conducted in *in vitro* systems as well as in mice, rats, rabbits, and monkeys. The general toxicology studies were conducted in appropriate animal species, using the administration route, dosing schedule and duration that adequately addressed safety concerns for the indicated patient population. The target organs of everolimus are mainly the male and female reproductive organs, hematopoietic/lymphoid organs (lymph nodes, spleen and thymus), GI tract, heart, lung, skin, and pancreas.

Unresolved toxicology issues (if any): None

Recommendations:

There are no pharmacology/toxicology issues which preclude the approval of everolimus (Afinitor) for the intended indication.

Suggested labeling:

Recommendations on labeling have been provided within internal meetings and communicated to the sponsor. A separate review for labeling will be provided if deemed necessary.

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**APPENDIX/ATTACHMENTS**

None.

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/s/

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