# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

**21-560s000**

# PHARMACOLOGY REVIEW(S)

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

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|---|---|
| Application number: | 21-560 |
| Applicant's letter date: | June 30, 2009 |
| CDER stamp date: | June 30, 2009 |
| Product: | Zortress® (everolimus, RAD001) |
| Indication: | Prophylaxis of Organ Rejection in Renal Transplantation |
| Applicant: | Novartis Pharmaceuticals Corporation |
| Review Division: | Division of Special Pathogen and Transplant Products |
| Reviewer: | William H. Taylor, PhD, DABT |
| Supervisor/Team Leader: | William H. Taylor, PhD, DABT |
| Division Director: | Renata Albrecht, MD |
| Project Manager: | Jacquelyn E. Smith, M.A. |

# 1 Executive Summary

## 1.1 Recommendations

No Pharmacology/Toxicology recommendations are currently indicated.

### 1.1.1 Approvability

The original Pharmacology/Toxicology reviewer for this application, Dr. Steven Kunder, indicated approval for this application in his 2003 review:

> *The New Drug Application 21-560 for Certican is approvable based on the preclinical pharmacology and toxicology submission. Toxicities demonstrated in preclinical studies may be monitored or be superseded by the benefit/risk ratio*

*determined by the clinical studies supporting kidney and heart transplant indications*.

This reviewer agrees with Dr. Kunder's assessment and conclusion.

### 1.1.3 Labeling

The purpose of this Pharmacology/Toxicology review is to present an updated label for everolimus (under the new trade name Zortress) for NDA 21-560.

There are two principal differences between the Pharm/Tox portions of the label for Zortress, and the Pharm/Tox portions of the approved label for Afinitor®. The first difference is that the comparison of animal exposures to human exposures (for the transplant indication) is based on human AUC data from kidney transplant trials. The doses (and clinical exposures) for the oncology indication are considerably higher than those for the prophylaxis of kidney rejection. The consequence is that some animal toxicity seen only at higher doses in animals may be appropriate for inclusion in the oncology label, but not in the label for the transplant indication. Additionally, in the oncology Division, human doses are generally expressed in units of $mg/m^2$, which are not appropriate, in this case, for this transplant indication.

Secondly, the Afinitor label Pregnancy Category (Section 8.1) is a "D", whereas the Zortress Pregnancy Category is a "C". John K. Leighton, Ph.D., DABT, Associate Director for Oncology Pharmacology/Toxicology responded to an email request to clarify why the Afinitor label has a "D" Pregnancy Category. Dr. Leighton provided the following explanation in an email on November 17, 2009:

> *Because when ODAC* [Oncology Drug Advisory Committee] *reviewed this topic (pregnancy categories) they determined that the mechanism of action (investigational data) was likely relevant to humans, and thus merited D. We have discussed this numerous times with maternal health, and the clinicians in DDOP wanted to stick with what works (the D).*
>
> *There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.*

Specifically, there are no human data to support the "D" label. After discussing this issue with Abby Jacobs, Ph.D., Associate Director for Pharmacology/ Toxicology, Office of New Drugs, I selected the "C" Pregnancy Category for Zortress based on (1) the data from animal studies, (2) the lack of human pregnancy data supporting a "D" category, and (3) the "C" Pregnancy Category for the same-class drug, Rapamune® (sirolimus/ rapamycin).

The recommended Pharmacology/Toxicology sections for the Zortress label are as follows:

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

**Pregnancy Category C**

There are no adequate and well-controlled studies of Zortress in pregnant women. In rats and rabbits, everolimus crossed the placenta and was toxic to the conceptus. The potential risk for humans is unknown. Zortress should be given to pregnant women only if the potential benefit to the mother justifies the potential risk to the fetus. Women of childbearing potential should be advised to use effective contraception methods while they are receiving Zortress, and if (b) (4), up to 8 weeks after stopping treatment.

Everolimus administered daily to pregnant rats by oral gavage at 0.1 mg/kg from before mating and through organogenesis resulted in increased preimplantation loss and early resorptions of fetal implants. AUCs in rats at this dose were approximately one-third those in humans administered the starting dose (0.75 mg b.i.d.). Everolimus administered daily by oral gavage at 0.8 mg/kg to pregnant rabbits during organogenesis resulted in increased late resorptions of fetal implants. At this dose, AUCs in rabbits were slightly less than AUCs in humans administered the starting clinical dose.

### 8.3 Nursing Mothers

It is not known whether everolimus is excreted in human milk. Everolimus and/or its metabolites readily transferred into the milk of lactating rats at a concentration 3.5 times higher than in maternal serum. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from everolimus, women should avoid breast-feeding during treatment with everolimus.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Everolimus was not carcinogenic in mice or rats when administered daily by oral gavage for 2 years at doses of 0.9 mg/kg. In these studies, AUCs in mice were much higher (at least 20 times) than those in humans receiving 0.75 mg b.i.d., and AUCs in rats were in the same range as those in humans receiving 0.75 mg b.i.d.

Everolimus was not mutagenic in the bacterial reverse mutation assay, the mouse lymphoma thymidine kinase assay, or the chromosome aberration assay usingV79 Chinese hamster cells, or in vivo following two daily doses of 500 mg/kg in the mouse micronucleus assay.

In a 13-week male fertility oral gavage study in rats, testicular morphology was (b) (4) at 0.5 mg/kg and above, and sperm motility, sperm head count and plasma testosterone concentrations were diminished at 5 mg/kg, which caused a decrease in male fertility. There was evidence of reversibility of these findings in animals examined after 13 weeks post-dosing. The 0.5 mg/kg dose in male rats resulted in AUCs in the range of clinical exposures, and the 5 mg/kg dose resulted in AUCs approximately 5 times the AUC in humans receiving 0.75 mg b.i.d. Everolimus did not affect female fertility in nonclinical studies, but everolimus crossed the placenta and was toxic to the conceptus. [see *Pregnancy (8.1)*]

# 2 Drug Information

## 2.1 Drug

### 2.1.2 Generic Name

Everolimus, RAD-001, RAD001

### 2.1.7 Pharmacologic class

Everolimus (RAD001) is a derivative of rapamycin (sirolimus) and is an mTOR (mammalian target of rapamycin) kinase inhibitor and an immunsuppressant.

## 2.2 Relevant IND/s, NDA/s, and DMF/s

IND 52,003, NDA (b) (4) (Afinitor)

## 2.3 Clinical Formulation

Zortress (everolimus) is available as 0.25 mg, 0.5 mg, 0.75 mg and 1 mg tablets.

### 2.3.1 Drug Formulation

Zortress Tablets include the following inactive ingredients: BHT, lactose monohydrate, hypromellose, magnesium stearate, crospovidone, and lactose anhydrous.

## 2.4 Proposed Clinical Population and Dosing Regimen

Adult patients at low-moderate immunologic risk receiving a kidney transplant

## 2.5 Regulatory Background

On December 19, 2002, Novartis Pharmaceuticals Corporation initially filed NDA 21-560 with the FDA for the prophylaxis of organ rejection in allogenic kidney and heart transplant patients. The Office of Drug Evaluation IV issued Approvable letters to Novartis for NDA 21-560 on October 20, 2003 and August 27, 2004. On June 30, 2009 Novartis filed amendments to NDA 21-560 which included new clinical studies to restart the agency's review. In the June 30, 2009 submission, Novartis amended the product label indication to eliminate heart transplant recipients. During the current review cycle, the CDER Division of Medication Error Prevention and Analysis (DMEPA) (b) (4) approved the trade name "Zortress."

Everolimus is approved in Europe as Certican for use as an immunosuppressant to prevent rejection of solid organ transplants. On March 30, 2009, FDA approved everolimus as Afinitor under NDA 22-334 for the treatment of patients with advanced renal cell carcinoma after failure of treatment with sunitinub or sorafenib.

# 3 Studies Submitted

## 3.1 Studies Reviewed

Steven C. Kunder, Ph.D. completed an initial Pharmacology/Toxicology NDA 21-560 review of everolimus (see DARRTS October 21, 2003). Shwu-Luan Lee, Ph.D. completed a Pharmacology/Toxicology review for NDA 22-334 in the Division of Oncology Products for the Afinitor product (DARRTS, March 12, 2009).

Neither the applicant's original study reports nor literature were examined for this review. The source materials for this review are the two previous Pharmacology/ Toxicology reviews in DARRTS for everolimus, the approved label for Afinitor, and the applicant's June 30, 2009 proposed label for Zortress.

William H. Taylor, PhD, DABT
Pharmacology/Toxicology Reviewer

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-21560 | ORIG-1 | NOVARTIS PHARMACEUTICALS CORP | CERTICAN (EVEROLIMUS) TABLETS |

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/s/

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WILLIAM H Taylor
12/18/2009

RENATA ALBRECHT
12/22/2009

***Note:***

***This will be the Standard CDER Coversheet***

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**EXECUTIVE SUMMARY**

**1. Recommendations**

1.1 Recommendation on Approvability

The New Drug Application 21-560 for Certican is approvable based on the preclinical pharmacology and toxicology submission. Toxicities demonstrated in preclinical studies may be monitored or be superceded by the benefit/risk ratio determined by the clinical studies supporting kidney and heart transplant indications.

1.2 Recommendation for nonclinical studies

It is recommended that possible impurities arising from the manufacturer or degradation of everolimus be qualified according to ICH guidelines. It is also recommended that the sponsor elucidate the mechanism of toxicity to the female reproductive organs, attempting to determine hormonal changes caused by everolimus.

1.3 Recommendations on labeling

(b) (4)

(b) (4)

(b) (4)

**2. Summary of nonclinical findings**

2.1 Brief overview of nonclinical findings

Everolimus was studied in mice, rats, minipigs and cynomolgus monkeys to evaluate its toxicities and in rats and rabbits to determine its reproductive toxicity potential. Everolimus causes toxicities in animal studies both by its pharmacologic mechanism of action, immunosuppression, as well as by direct toxicity. Immunosuppression by everolimus resulted in atrophy of the lymphoid organs (thymus, spleen, lymph nodes) as well decreased circulating lymphocytes and total leukocytes.

Toxicities affected by immunosuppression included myocardial degeneration/myocarditis in monkeys and rats at $1.5 mg/kg ($1.7-4.5x human exposure); this is likely related to viral infection emerging under immunosuppression. Other toxicities included reproductive organ toxicity in all species tested including testicular atrophy in monkeys at 0.3 mg/kg (0.9x human exposure) and uterine atrophy and reduced follicular development in mice and monkeys at doses of $1.5 mg/kg ( 4.5-18x human exposure); renal toxicity in rats with tubular degeneration at doses of $5.0 mg/kg (55x human exposure); pancreatic toxicity was seen in monkeys with islet cell degeneration at 5.0 mg/kg (4.5x human exposure) and vacuolation of the exocrine pancreas in minipigs at 5.0 mg/kg (23x human exposure); lung toxicity in mice at $1.5 mg/kg (15x human exposure) and rats at $0.5 mg/kg (0.1x human exposure); and toxicity to the eye as swelling and disruption of cortical fibers of the lens at a dose of 0.9 mg/kg in rats (0.4 x human exposure).

In carcinogenicity studies, no significant tumor findings occurred in rats (0.4x human exposure) and one tumor, osteoma of the femur was significant in mice at 0.9 mg/kg (8.6x human exposure).

Genotoxicity for RAD was not observed in the *in vitro* bacterial reversion (Ames), *in vitro* mammalian mouse lymphoma assay, *in vitro* chromosomal aberration test or *in vivo* mammalian micronucleus assay.

Reproductive toxicity was seen in rat and rabbit studies. Male fertility was decreased in rats at 5.0 mg/kg (0.9x human exposure); sperm were misshapen at 0.5 mg/kg (0.12x human exposure) without effect on fertility. In rat embryo-fetal development studies, skeletal malformations increased at doses $0.3 mg/kg (0.09x human exposure). In rabbit embryo-fetal development studies, no fetal effects were seen until doses were maternally toxic (0.8 mg/kg, 0.14x human exposure).

2.2 Pharmacologic activity

Everolimus is an orally active immunosuppressant drug acting as an inhibitor of intracellular proliferative signaling in activated T lymphocytes at FK binding protein 12 (FKB-12). The complex of RAD/FKB-12 is then believed to bind to and inhibit the kinase mTOR. This arrests activated T lymphocytes in the G1 phase of their cell cycle, preventing their proliferation in response to the response to the foreign antigens of transplanted tissues. Inhibition of mTOR blocks the postreceptor IL-2 signals which mediate T-call proliferation while calcineurin inhibitors (eg. Cyclosporin, Tacrolimus) block IL-2 synthesis and stop the cell cycle as it progresses from G0 to G1 phase.

Nonclinical safety issues relevant to clinical use

-Renal toxicity is of prime importance, especially for renal transplantation. It is well characterized for calcineurin inhibitors.

-Pancreatic toxicity, also well characterized for calcineurin inhibitors, potentially leading to post-transplantation diabetes mellitus.

-Reproductive toxicity/male fertility, counterindicates Certican for pregnant women; however, organ transplantation is typically not conducted in pregnant women. Decreased male fertility may prevent males from impregnating partners after transplantation.

-Eye toxicity seen in rats (disruption of fibers in lens) may cause vision problems.

-Hypercholesterolemia and hypertriglyceridemia, seen in rats and monkeys, and typical of other immunosuppressant drugs used for organ transplantation, may be treated with current antihyperlipodemic therapies following transplantation.

**PHARMACOLOGY/TOXICOLOGY REVIEW**

## 3.1 INTRODUCTION AND DRUG HISTORY

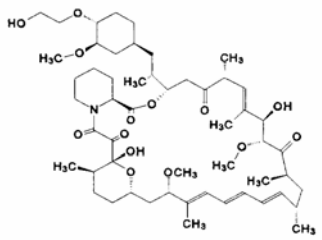
**NDA number**: 21-560
**Review number**: 001
**Sequence number/date/type of submission**: 001
**Information to sponsor**: Yes ( ) No (x )
**Sponsor and/or agent**: Novartis Pharmaceutical Corporation
East Hanover, NJ
**Manufacturer for drug substance**: Novartis Pharmaceutical Corporation

**Reviewer name**: S.Kunder
**Division name**: Special Pathogen and Immunologic Drug Products
**HFD #**: 590
**Review completion date**: 10 Oct 2003

**Drug**:

Trade name: Certican
Generic name: everolimus
Code name: RAD, SDZ RAD, RAD 666, RAD 001
Chemical name: 40-O-(2-hydroxyethyl)-rapamycin; IUPAC= (lR,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(lR)-2[(lS,3R,4R)-4-(2-hydroxyethoxy)-3 methoxycyclohexyll-i-methylethyl)-19,30 dimethoxy-15,17,21,23,29,35-hexamethy1-11,36 dioxa-4-aza-tricyclo[30.3 .1.0$^{4\ 9}$ ]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone

CAS registry number: 159351-69-6
Molecular formula/molecular weight: $C_{53}H_{83}NO_{14}$ / 958.25
Structure:



**Relevant INDs/NDAs/DMFs**: IND 52003

**Drug class**: macrocyclic lactone immunosuppressive

**Indication**: prophylaxis of rejection in allogeneic kidney and heart transplant patients

**Clinical formulation**: 0.25, 0.5, 0.75, and 1.0 mg tablets containing butylated hydroxytoluene, magnesium, stearate, lactose monohydrate, hydroxypropyl-methylcellulose, crospovidone, lactose anhydrous

**Route of administration**: oral

**Proposed use**: kidney and heart transplantation

**Disclaimer**: Tabular and graphical information are constructed by the reviewer unless cited otherwise.
In results where no sex designation is specified, the finding is applied to both sexes.
For study results, increases and decreases are with respect to controls

**INTRODUCTION**

Certican is an immunosuppressant macrolide, structurally related to rapamycin, under development for organ transplantation. Certican is derived from chemical modification of rapamycin, a product of *Streptomyces hygroscopicus.* Certican appears to share the same mechanism of action with rapamycin but its immunosuppressive biochemical mechanism of action is distinct from that of cyclosporin (CsA), tacrolimus (FK506), mycophenolate mofetil, or azathioprine. Certican inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (interleukin [IL]-2, IL-4, IL-7, and IL-15) stimulation by a mechanism different from that of immunosuppressants previous to rapamycin. Certican binds to the immunophilin, FK binding protein 12 (FKBP-12), producing an immunosuppressive complex. Unlike cyclosporin and tacrolimus, the rapamycin FKBP complex appears to have no effect on calcineurin activity. This complex binds to and inhibits the activation of a kinase called the mammalian target of rapamycin (mTOR). Inhibition of mTOR by rapamycin suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.
Certican prolongs allograft survival in animal models of transplantation, including rodents and primates, both for solid organ and for cellular allografts. Nephrotoxicity is a concern with many preceding immunosuppressants, particularly cyclosporin. Certican, possibly due to its differing mechanism of action, appears not to have the severity of nephrotoxicity seen with cyclosporin. Combinations of Certican with other immunosuppressants are problematic. Other toxicities and affects seen in other immunosuppressive drugs including elevation of glucose and hyperlipidemia, are of concern with Certican as well.
**Due to the similarity of chemical structure and toxicologic issues, the reader may wish to compare the Pharmacology/Toxicology review of NDA 21-083 for rapamycin.**

**Studies reviewed within this submission:**
Pharmacology studies are summarized in pharmacology section

Pharmacokinetics and excretion after single intravenous and peroral administration (0.9 mg/kg) of $H^3$-labeled RAD001 to mice
Pharmacokinetics in mice after intravenous bolus administration (0.9 mg/kg) with RAD001
Quantitative determination of rapamycin and SDZ RAD in blood samples after single and multiple administration in human and monkey
In vitro distribution, plasma protein binding and stability of RAD001 in mouse plasma
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)
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)
Distribution and excretion of total radioactivity in rats after peroral administration of 1.5 mg/kg $^{14}$C-labelled SDZ RAD
Whole-body autoradioluminography in albino and pigmented rats after po and iv doses of [$^3$H] RAD001
Embryofetal transfer in pregnant rats on day 13 and day 17 of gestation after po administration of [$^3$H] RAD001
Galactogenic transfer, kinetics and metabolism in milk and blood after single peroral administration (0.9 mg/kg) of $^3$H-labeled RAD001 to lactating rats
Dose-dependent brain penetration in rats
Disposition in rats after single and repeated once daily peroral administration (0.5 mg/kg/day) of $^3$H-labeled RDA001 for 21 consecutive days
Intestinal absorption and presystemic metabolism of SDZ RAD
Permeability study across Caco-2 cell monolayers
Biotransformation in mice following a single oral and intravenous dose (0.9 mg/kg) of $^3$H-RAD
Biotransformation in cynomolgus monkey following a single oral dose of $^3$H-RAD
Inhibition of RAD 001 in vitro metabolism by ketoconazole, itraconazole and fluconazole
An oral dose-escalating study in dogs
A comparative 2-week oral (gavage) toxicity study in the rat with a micro-emulsion and a solid dispersion
A 2-week oral (gavage) dose-range-finding study in minipigs
4-week oral (gavage) toxicity study in minipigs
28-day oral gavage toxicity study in juvenile cynomolgus monkeys with a 2-week reversibility period
SDZ RAD: Toxicity study by oral gavage administration to cynomologus monkeys for 26 weeks followed by a four week reversibility period

52-week oral (gavage) toxicity study in the cynomolgus monkey
Toxicity study by intravenous (bolus) administration to HanIbm Wistar rats for 2 weeks
Two-week intravenous infusion toxicity study in minipigs
4-week toxicity study in cynomolgus monkeys by intravenous infusion followed by a 2-week reversibility period
4-week toxicity study in cynomolgus monkeys by intravenous infusion followed by a 2-week reversibility period
RAD 001, rapamycin and Neoral: 2-week exploratory study in rats
FTY720 in combination with RAD001: 4-week oral (gavage) toxicity in male monkeys with a 4-week recovery period
RAD001 and methotrexate: 4-week oral combination toxicity study in rats
Comparative toxicity study in HanIbm Wistar rats with batches differing in by-product content
A comparative 2-week oral (gavage) toxicity study in the rat with two different batches
4-week oral toxicity study in rats (batch comparison)
An oral 13-week investigative fertility study in male rats with 13 weeks recovery
An oral fertility and embryo-fetal development study in female rats
An oral embryo-fetal development study in rabbits
An oral pre- and postnatal development study in rats
SDZ RAD 666: mutagenicity test using Salmonella typhimurium (batch control)
SDZ RAD 666: mutagenicity test using Salmonella typhimurium (batch control)
SDZ RAD (solid dispersion): mutagenicity test using Salmonella typhimurium (batch control)
RAD 001: mutagenicity test using Salmonella typhimurium (batch control)
SDZ RAD (solid dispersion) Chromosomal aberration test with V79 Chinese hamster cells
RAD001:Chromosomal aberration test with V79 Chinese hamster cells
Oncogenicity study by oral gavage administration to CD-1 mice for 104 weeks
Oncogenicity study by oral gavage administration to Hanibm rats for 104 weeks
Comparative study of ophthalmic toxicity by oral gavage administration to CD rats and HanIbm rats for 4 weeks
A 2-week oral (gavage) mechanistic toxicity study in rats
An oral neonatal and juvenile developmental dose range-finding study in rats
An oral neonatal and juvenile development study in rats with 13- and 26-week recovery periods

**Studies reviewed in IND 52003:**
Acute oral toxicity study in mice.
Acute intravenous toxicity study in mice.
Acute intravenous toxicity study in mice (amendment No. 1 to final report)
Acute oral toxicity study in rats.
Acute intravenous toxicity study in rats.
Acute intravenous toxicity study in rats (amendment No. 1 to final report).
Dose range finding study (intravenous infusion administration) in rats.
A 2-week oral (gavage) dose-range-finding study in rats.
Comparative intravenous infusion study in rats.
Toxicity study by oral gavage administration to HanIbm Wistar rats for 4 weeks followed by a 2 week reversibility period.
A repeat toxicity study by oral gavage administration to HanIbm Wistar rats for 4 weeks followed by a 2 week reversibility period.
Dose escalating study (oral administration) in cynomolgus monkeys.
14-Day dose range finding study (oral route) in cynomolgus monkeys.
Dose finding study in cynomolgus monkeys by intravenous infusion for 2 weeks.
14-Day dose range finding study (intravenous infusion administration) in cynomolgus monkeys. Supplement No. 1: Toxicokinetic report.
Toxicity study by oral (gavage) administration to cynomolgus monkeys for 4 weeks followed by a 2 week reversibility period.
An oral fertility dose-range-finding study in male rats
An oral reproductive toxicity dose-range-finding study in female rats with toxicokinetics and placental transfer.
An oral embryo-fetal development dose-range-finding study in rabbits with toxicokinetics and placental transfer.
Mutagenicity test using *Salmonella typhimurium*
Range-finding toxicity study by oral gavage administration to CD-1 mice for 13 weeks
Toxicity study by oral gavage administration to Hanlbm Wistar rats for 4 weeks followed by a 2 week reversibility period
Toxicity study by oral gavage administration to Hanlbm Wistar rats for 26 weeks followed by a 4 week reversibility period
Combination of Sandimmun-Neoral® and SDZ RAD 4-week oral (gavage) toxicity study in Cynomolgus monkeys

(b) (4)

### 3.2 PHARMACOLOGY

#### 3.2.1 Brief summary

Everolimus had minimal effects in neurological, cardiovascular, pulmonary, renal, and gastrointestinal assays. The primary mechanism of action, immunosuppression, is described below.

#### 3.2.2 Primary pharmacodynamics

Mechanism of action: The main immunosuppressive action of RAD is an inhibition of an intracellular proliferative signal in activated T lymphocytes at FK Binding protein 12 (FKB-12). Growth-factor stimulated phosphorylation of p70 S6 kinase is then inhibited. This kinase is involved in the initiation of protein synthesis. Phosphorylation of p70 S6 kinase is believed to be controlled by FKB-12-rapamycin associated protein (mTOR). The complex of RAD/FKB-12 is then believed to bind to and inhibit mTOR, a regulatory protein controlling cell metabolism, growth and proliferation. This arrests activated T lymphocytes in the G1 phase of their cell cycle, preventing their proliferation in response to the foreign antigens of transplanted tissues. Clonal expansion of antigen specific T cells in response to foreign antigen and IL-2 stimulation is prevented.
For further detail refer to the microbiology review.
Drug activity related to proposed indication: As described above, RAD inhibits the T cell response to the antigens of allogeneic tissue involved in organ transplantation thereby preventing immunologic rejection.

#### 3.2.3 Secondary pharmacodynamics

The antiproliferative action of everolimus inhibits growth factor-stimulated proliferation of vascular smooth muscle cells. In a human umbilical vein, RAD inhibited proliferation stimulated by vascular endothelial growth factor. In the rat carotid artery balloon model, rats subjected to carotid artery angioplasty were treated with RAD and injury –induced neointima formation was inhibited. Similar results were seen in a pig model with a 14-day RAD treatment following coronary balloon injury. In rat aorta transplantation, everolimus inhibits neointimal formation due to damaged endothelial cells. The likely application for these antiproliferative studies is in the drug-coated stent. In rabbits, RAD-coated stents were examined at 28 days after implantation, demonstrating absence of restenosis.

#### 3.2.4 Safety pharmacology

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 with oral doses of 2, 20 and 50 mg/kg. 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 $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 upon blood pressure, heart rate, blood flow, respiratory rate and electrocardiogram. No cardiovascular effects were seen at doses #10 mg/kg i.v. in pigs. In the in vitro assay on HERG currents recorded from stably transfected HEK293 cells, RAD did not inhibit HERG currents.

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.

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.

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.

Abuse liability: none

Other: none

### 3.2.5 Pharmacodynamic drug interactions

The major enzyme of human metabolism of RAD is believed to be CYP3A4. Using CYP3A4 inhibitors ketoconazole and itraconazole as well as a poor inhibitor (fluconazole), the inhibitors were shown to inhibit biotransformation of RAD while fluconazole did not. This supports the role of CYP3A4 in the metabolism of RAD.

## 3.3 PHARMACOKINETICS/TOXICOKINETICS

### 3.3.1 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%), monkey (18%), rat (39-43%) 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, excepting 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. The metabolism of RAD is also the major mechanism of elimination. 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 radiolabeled RAD 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. See summary tables below.

### 3.3.3 Absorption

**Study title:** Pharmacokinetics and excretion after single intravenous and peroral administration (0.9 mg/kg) of $H^3$-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:** [$H^3$]- RAD001, specific activity=49.8 MBq/mg

**Animals:** male CD-1 mice
**Protocol:** Mice were administered $H^3$-labeled RAD001 by intravenous route (2 mg) and oral gavage (1.0 mg). 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 | $H^3$ tmax | $H^3$ Cmax | $H^3$ AUC ( : mol.L) |
|---|---|---|---|---|
| 0.9 | i.v. | 1 | 3.98 | 38.3 |
| 0.9 | p.o. | 0.5 | 0.51 | 4.39 |

**CONCLSIONS**
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%).

**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= 18.7 h. : /ml; $t_{1/2}$ =9.83 h; clearance= 0.67 l/kg

**CONCLUSIONS:** 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 & formulation:** 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.

**Animals:** see above

**Protocol:** 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.

### 3.3.4 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)**

**Dose & formulation:** $H^3$-RAD001 batch 98902, 25.3 MBq/ml

**Animals:** 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 $H^3$-RAD001/RAD001 at a concentration range of 5-5000 ng/ml.

**RESULTS:** blood distribution in plasma: plasma fraction = 98"4% from 5 to 5000 ng/ml
Bound fraction in plasma= 99.9% at 10 ng/ml

**CONCLUSIONS:** RAD was highly bound to mouse blood plasma proteins (99..9%). This is greater than the plasma binding seen in other species ranging from 75 to 84%.

**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)**

**Dose & formulation:** $H^3$-RAD001 batch RA 910-7, 100 ng/ml added to plasma

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

**Protocol:** $H^3$-RAD001 was added to mouse, cynomolgus monkey and human plasma to determine the stability of RAD. Incubation followed at $37^{B}$C. RAD levels in plasma 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 RAD in this study consisted mainly of the ring-opened forms (b) (4). For structures see charts below.

**CONCLUSIONS:** RAD has a short half-life in monkey and human plasma at 37°C. RAD in mouse plasma has a somewhat longer half-life, almost 20 h. This information may be useful in design of in vitro assays involving RAD with plasma. Ring-opened structures 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:** $H^3$-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:** pooled blood and plasma from rats, HAN/WIST
**Protocol:** Erythrocytes were suspended in plasma solutions, incubated with RAD and the resulting supernatant collected. RAD concentration was determined by scintillation counting. Protein binding of RAD added to rat plasma was determined by the erythrocyte partitioning method. A shortened (5 min) RAD incubation time (compared with previous assays) was used due to the findings of the stability study (above).

**RESULTS:** The blood distribution of RAD, 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 peroral administration of 1.5 mg/kg $^{14}C$-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 $^{14}C$-labelled SDZ RAD, batch RSE 009-1, specific activity 4.27 MBq/mg
**Animals:** rats, male HAN Wistar
**Protocol:** Rats were orally administered 1.5 mg/kg $^{14}C$-labelled SDZ RAD. Rats were sacrificed at 2, 8, 24 and 144 h after dosing. Tissue specimens were processed and analyzed by scintillation counting. Other rats were placed in cages to collect urine and feces for determination of $^{14}C$-labelled-RAD elimination.

**RESULTS:** The radiolabeled RAD 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. 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. 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 radiolabeled RAD was demonstrated in the rat, with highest concentrations in liver, gastrointestinal tract and immunologic tissues. Rapid elimination was seen predominantly by the fecal route.

**Study title:** Whole-body autoradioluminography in albino and pigmented rats after po and iv doses of [$^3$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., $H^3$-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. Sagital 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 radiolabeled RAD with greater affinity than non-melanin continuing tissues of the albino rats.

**CONCLUSIONS:** After both oral and intravenous dosing to albino and pigmented rats, radiolabeled RAD distributed similarly among tissues and had no affinity for melanin in tissues of the pigmented rats.

**Study title:** Embryofetal transfer in pregnant rats on day 13 and day 17 of gestation after po administration of [$^3$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)**
**Dose & formulation:** $H^3$-RAD001 batch RA 919-6, specific activity 36.1 MBq/mg in 1:20 RAD placebo/0.9% saline
**Animals:** rats, female HAN:WISTon day 13, 17 of gestation
**Protocol:** Pregnant female rats were administered approximately 0.9 mg/kg [$^3$H] RAD001 by oral gavage. Day 13 and 17 gestation rats were sacrificed at 0.5, 2, 6 and 14 h after dosing with delivery of fetuses and placentas. Also from day 17 rats amnion and amniotic fluid were collected. All day 13 rat tissues and fetuses were processed for liquid scintillation counting. Day 17 fetuses and tissues had radiolabel concentration determined by quantitative whole body radioluminography.

**RESULTS:** Gestation day 13 rats showed highest label concentrations at 0.5 h post-dosing in liver, gastrointestinal tract and lung. At 2 h post-dosing, a similar distribution was seen. Peak tissue levels were seen 6 h post-dosing in the lung, pituitary gland, adrenal cortex spleen and thyroid gland. At 24h post dosing, tissue levels had decreased greatly and no individual tissues predominating. Fetal radioactivity was detected at all timepoints at levels lower than maternal blood. Gestation day 17 rats showed radioactive tissue peaks in the heart, lung, liver, spleen, gastrointestinal tract and placenta at 6 h post-dosing. Radioactivity was detected in the fetus at 6 h post –dose at levels similar to maternal blood. Placenta had detectable radioactivity at all timepoints

**CONCLUSIONS:** Fetal transfer of [$^3$H] RAD001was 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 peroral administration (0.9 mg/kg) of $^3$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, H$^3$-RAD001 batch RA 919-7, specific activity 0.15 MBq/mg
**Animals:** rats, HAN:WIST, lactating day 9 parturition; pups/litter reduced to 5
**Protocol: Female rats were administered** H$^3$-RAD001by oral gavage. Oxytocin was administered i.p. 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 postdosing. H$^3$-RAD001 in the milk and blood samples was measured by liquid scintillation. Blood samples were also subjected to HPLC analysis for metabolites.

**RESULTS:**
The following pharmacokinetic parameters for H$^3$-RAD001 were seen in this study:

| Dose (mg/kg) | | H$^3$ tmax (h) | H$^3$ Cmax (nmol.l) | H$^3$ 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 |

Metabolites found in blood and milk included fatty acid conjugation, hydrolytic and hydroxylated products typical of those seen in other metabolism studies.

**CONCLUSIONS**: Metabolite patterns seen in lactating rats in this study appear to similar to those seen in monkeys and humans. H$^3$-RAD001 was rapidly transferred from blood to milk. Radiolabel was concentrated in milk compared to blood, which may due to the lipid content of milk and the lipophilic nature of RAD.

**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
**Date of study:**
**GLP (no)**
**Dose & formulation:** 0.1, 0.3, 1, 3, 10, 30 mg/kg, i.v.; H$^3$-RAD001 batch RA 910-2, specific activity 58.2mCi/mg
**Animals:** rats, male Wistar
**Protocol:** Rats were administered H$^3$-RAD001by i.v. infusion over 0.5 m. At 2 hours post, rats were sacrificed, blood samples drawn and brains dissected. In a second part of the study, rats received a bolus injection (0.17 min) of 1mg/kg H$^3$-

RAD001. Rats were sacrificed at 0.08, 2, 8, 24 and 168 h after dosing. Blood samples were taken and brains dissected. Radioactivity in tissues was measured by liquid scintillation counting.
**RESULTS:** Brain and blood levels of $H^3$-RAD001 increased linearly up to 1 mg/kg, then (at 2 h post dose) in a nonlinear manner in both tissues. At 168 h concentrations were higher in brain as blood concentrations were barely detectable.

**CONCLUSIONS:** Examination of parent drug and total radioactivity concentrations in the brain appears to indicate that parent and not metabolites enter the brain. The difference in concentration between brain and blood at 168h post dose may indicate brain as a depot for this lipophilic drug.

**Study title:** Disposition in rats after single and repeated once daily peroral administration (0.5 mg/kg/day) of $^3$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,** $H^3$-RAD001 batch RA 919-7, specific activity 51.3 MBq/mg
**Animals:** rats, males, HAN:WIST
**Protocol:** Rats were administered $H^3$-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 | 21 |
| Distribution | Day 1, day 21 autoluminography of carcass | 1, 21 |
| Metabolite profiling | Day 21, 24 h after dose | 21 |

**RESULTS:** Excretion of $H^3$-RAD001 was mostly through the fecal route; after day 8 fecal excretion was $90%. Excretion was nearly complete within 168 h of the last dose. 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 lachrymal gland showing higher concentrations later in the multiple dose samples. Hydroxylated, hydrolytic and fatty acid conjugate metabolites predominated in metabolites recovered from excretions, and tissues as see in other rats metabolism studies. Other complex, unresolved metabolites were seen in feces and urine.

**CONCLUSIONS:** Rats dosed with 0.5 mg/kg $H^3$-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 in the multiple dose rats compared with the single dose rats. Metabolic patterns of fatty acid conjugates and hydroxylated/hydrolytic metabolites were seen both in tissues and excretions.

### 3.3.5 Metabolism

**Study title:** Intestinal absorption and presystemic metabolism of SDZ RAD
**Study no.** DMPK (CH) 1997/417
**Study facility:** Novartis Pharma AG
BT/Drug Metabolism
4002 Basel Switzerland
and
Novartis Pharma AG
CFSS/Drug Metabolism
4002 Basel Switzerland
**Date of study:** 17 Dec 1997
**GLP (no)**
**Dose & formulation:** 0.15, 1.5 mg/kg $^3$H-RAD, in placebo microemulsion: saline
**Animals:** Wistar rats, fasted overnight, ligated mesenteric vein; human intestinal Caco-2 cell monolayers; rat intestinal single pass permeability model
**Protocol:** $^3$H-RAD was injected into the ligated segment of the mesenteric vein. Total mesenteric blood was collected from the ligated rats after 5 min. Blood was analyzed for $^3$H-RAD by liquid scintillation counting and HPLC. Cells from the Caco-2 cell line were plated into diffusion chambers with $^3$H-RAD with either verapamil ( a gp-transport protein inhibitor) or cyclosporin A. Media was collected to measure $^3$H-RAD transport across the cells. In a rat intestinal single pass permeability model the jejunum was cannulated and $^3$H-RADpumped through the segment. Mucosa was scraped from this jejunum segment for liquid scintillation counting.

**RESULTS:** The rats with a ligated segment of the mesenteric vein received either 0.15 mg/kg or 1.5 mg/kg doses. Rats receiving 0.15 mg/kg displayed approximately 47 % biotransformation while rats receiving 1.5 mg/kg had 29 % biotransformation. Caco-2 cell layers showed active pumping of RAD across them which was inhibited by the gr-pump inhibitor verapamil and slightly by cyclosporin A.

**CONCLUSIONS:** These studies show that RAD may be readily absorbed through the intestine as seen in the Caco-2 cell transport. Increasing the dose of $^3$H-RAD appeared to slow transport in the intestinal wall, indicating a saturable mechanism.

**Study title:** Permeability study across Caco-2 cell monolayers
**Study no.** DMPK(CH) R99-2602
**Study facility:** Novartis Pharma AG
Preclinical Safety/Drug Metabolism
ADE Section
4002 Basel Switzerland
**Date of study:** 29 Nov –17 Dec 1999
**GLP (no)**
**Dose & formulation:** (b) (4), seco acid metabolite of RAD, batch LJ2/62613535 ：m
**Animals:** Human intestinal cell line Caco-2 on permeable filter support
**Protocol:** (b) (4) was added to Caco-2 cells on a permeable filter support to measure bidirectional transport. The sponsor is unclear as to the method to determine transport.

**RESULTS:** The sponsor claims that no transport was observed.

**CONCLUSIONS:** If the claim of a lack of transport (b) (4) of across Caco-2 cells is valid, it would appear that this seco acid metabolite would not be absorbed through the intestinal wall into general circulation.

**Study title:** Biotransformation in mice following a single oral and intravenous dose (0.9 mg/kg) of $^3$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, $^3$H-RAD in Sandimmune placebo solution/0.9% sodium chloride
**Animals:** mice, male CD-1
**Protocol:** Rats were administered $^3$H-RAD by either oral or i.v. route. Rats 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 $^3$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 Cmax of 178pmol/ml at a tmax of 1 h. AUC for the oral dosing was 1693 pmol.h/ml. Intravenous dosing did not result in detectable blood levels of $^3$H-RAD. The major component from blood of 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 $^3$H-RAD. Metabolism was through hydrolysis/dehydration and hydroxylation forms seen in human and monkey studies.

**Study title:** Biotransformation in cynomolgus monkey following a single oral dose of $^3$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, $^3$H-RAD, batch RA 919-6, specific activity 1.35 mCi/mg
**Animals:** cynomolgus monkey, males
**Protocol:** Cynomolgus monkeys were administered a single dose of $^3$H-RAD, 5 mg/kg, by orogastric tube. Blood was collected at 1, 2, 4, 7 and 24h post dose. Urine, feces, were collected for the intervals 0-24, 24-48, 48-72 and 72-96h postdose. Radioactivity of blood and excretions were measured by liquid scintillation counting. Blood was also analyzed by HPLPC for metabolite content.

**RESULTS:** The pharmacokinetic parameters determined in this study, cmax= 776, pmol/ml, tmax=1.5 h, AUC=6251 pmol.h/ml are similar to those seen in other monkey studies. Excretion was primarily by feces (~93%). Metabolism was through hydrolysis/dehydration and hydroxylation forms.

**CONCLUSIONS:** Following verification of $^3$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.

### 3.3.6 Excretion

See studies above

### 3.3.7 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
**Animals:** human liver microsomes
**Protocol:** Microsomal preparations were incubated with RAD 1 :l, 0.25 :m alone and with one of either ketoconazole, itraconazole or fluconazole at concentrations of 0.05 to 2 :m for 15 min.
**Analysis:** Supernatants from incubations were measured for RAD by LC-MS analysis

**RESULTS:** Ketoconazole inhibited biotransformation of RAD as is known in CYP3A4 systems. Itraconazole inhibited biotransformation of RAD. Fluconazole did not inhibit RAD biotransformation, an expected result as fluconazole is not a good inhibitor of CYP3A4.

**CONCLSIONS:** CYP3A4 is believed to be the main enzyme of human metabolism of RAD. Using known inhibitors of this enzyme (ketoconazole, itraconazole) and a poor inhibitor (fluconazole), the inhibitors were shown to inhibit biotransformation of RAD. These results indicate that ketoconazole and itraconazole might not be recommended for use with RAD. No clinical drug-drug interaction studies were performed using ketoconazole or itraconazole with Certican.

### 3.3.10 Tables and figures to include comparative TK summary

**Absorption parameters of RAD in animals and human**

| species | Dose Mg/kg | route | formulation | Tmax radioactivity h | Tmax RAD, h | Dose absorbed, % | Bioavailability % |
|---|---|---|---|---|---|---|---|
| Mouse | 0.9 | P.O. | microemulsion | 0.5 | 1 | 12 | 5 |
| Rat | 1.5<br>15.0 | PO | Microemulsion<br>Microemulsion | 4.1"4.6<br>1.8"0.4 | 1.6"1.5<br>2.2"1.1 | 39<br>43 | 14<br>26 |
| Monkey | 5 | P.O. | microemulsion | 2.0"0 | 1.2"0.8 | 18 | 6 |
| human | 0.039 | P.O. | Solid dispersion | 1.7 | 1.5 | 11 | na |

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

| Species | Dose Mg/kg | Route and frequency | Cmax Ng/ml | AUC Ng.h/ml |
|---|---|---|---|---|
| Mouse | 0.9<br>0.9 | P.O., single<br>Iv, single | 108<br>1940 | 1013<br>20283 |
| Rat | 1.5<br>15<br>1<br>10 | P.O., single<br>P.O., single<br>Iv, single<br>Iv, single | 16.7<br>210<br>90.5<br>1347 | 2034<br>818<br>5169<br>43.8 |

| | | | | |
|---|---|---|---|---|
| | 0.5<br>0.5 | P.O., single<br>P.O., 21 days | 3.39<br>3.75 | 79.4 |
| Monkey | 5<br>1 | P.O., single<br>Iv, single | 102<br>415 | 1732<br>5471 |
| patients | 0.39 | P.O., single | 35.1 | 426 |

**RAD blood concentrations after multiple oral dosing in toxicokinetic studies**

| species | Route/duration | Dose<br>Mg/kg | Cmax<br>Ng/ml<br>m | Cmax<br>Ng/ml<br>f | AUC<br>Ng.h/ml<br>m | AUC<br>Ng.h/ml<br>f |
|---|---|---|---|---|---|---|
| mouse | Po/104 wk | 0.1<br>0.3<br>0.9 | 18.5<br>25.4<br>168.5 | 17.7<br>51.4<br>187.9 | 169.5<br>412.8<br>1377.8 | 150.5<br>346.4<br>3084.2 |
| mouse | Po/13 week | 0.15<br>0.5<br>1.5<br>5<br>15 | 55<br>146<br>331<br>4228<br>4188 | 48<br>272<br>639<br>1754<br>5828 | 803<br>1174<br>3472<br>19420<br>45955 | 362<br>1258<br>4877<br>11363<br>43206 |
| rat | Po/104 week | 0.1<br>0.3<br>0.9 | 0.5<br>2.1<br>8.9 | 0.4<br>1.2<br>9.7 | 2.5<br>25.7<br>138.2 | 1.0<br>11.4<br>42.9 |
| rat | Po/2 week<br>Microemulsion<br>Solid<br>dispersion | 0.5<br>1.5<br>5<br>15 | 39<br>891<br>21<br>600 | 30<br>907<br>23<br>591 | 255<br>6890<br>218<br>5770 | 155<br>4693<br>169<br>3743 |
| Rat | Po/4 week | 0.5<br>1.5<br>5<br>15 | 5.9<br>44<br>163<br>400.5 | 11.3<br>38.3<br>117.0<br>644.5 | --<br>117.5<br>1415.6<br>4618.4 | --<br>201.6<br>1005.9<br>4054.0 |
| Rat | Po/4 week | 0.1<br>0.25<br>0.5<br>1.5 | <LOD<br><LOD<br>10<br>52 | <LOD<br><LOD<br>6<br>30 | <LOD<br><LOD<br>102<br>514 | <LOD<br><LOD<br>56<br>237 |
| rat | Po/26 week | 0.15<br>0.5<br>1.5 | 1.5<br>7.1<br>14.4 | 1.1<br>3.7<br>10.6 | 7.1<br>44.1<br>92.7 | 8.1<br>13.1<br>50.8 |
| rat | Po/7week<br>fertility<br>Males | 0.15<br>0.5<br>1.5 | <LOD<br>10<br>41 | | <LOD<br>38<br>118 | |
| rat | Po/13 week<br>Fertility<br>males | 0.1<br>0.5<br>5 | 2.8<br>8.4<br>40.4 | | 9.9<br>52<br>415 | |
| monkey | Po/2 week | 5<br>15<br>45 | nd | | 2460<br>3439<br>5236 | 2156<br>4059<br>3203 |
| monkey | Po/4 week | 1.5<br>5<br>15 | 94.5<br>332.7<br>941.3 | 130.7<br>266.0<br>554.0 | 973.2<br>3130.4<br>8099.3 | 1194.1<br>3259.7<br>5830.8 |
| monkey | Po/4 week<br>juvenile | 0.1<br>0.25<br>0.5 | 10<br>23<br>57 | 15<br>40<br>45 | 156<br>279<br>604 | 195<br>409<br>624 |
| Monkey | Po/26 week | 0.1<br>0.5<br>1 | 20<br>68<br>146 | 33<br>59<br>177 | 145<br>358<br>1106 | 223<br>466<br>1218 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 537 | 383 | 4913 | 3322 |
| monkey | Po/52 week | 0.1<br>0.5<br>0.9 | 8.5<br>24.1<br>84.9 | 10.1<br>20.5<br>47.8 | 98.0<br>275.6<br>941.3 | 59.6<br>176.2<br>471.8 |

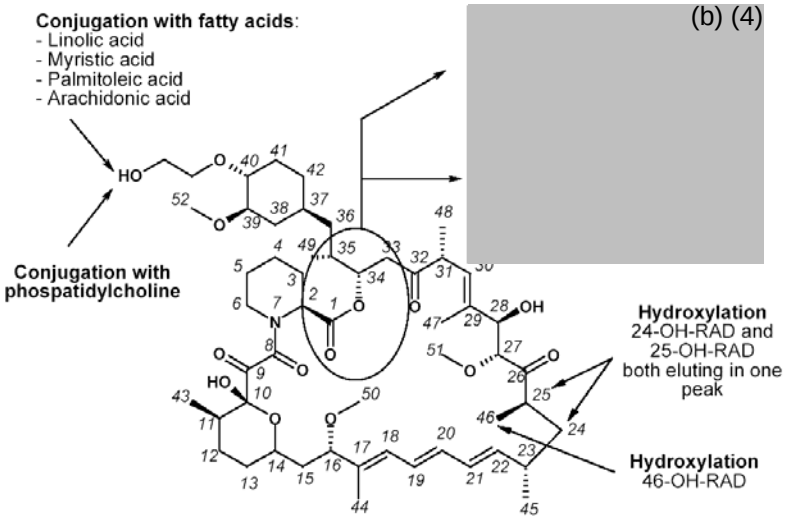
<LOD= less than limit of detection

**Excretion of total radioactivity**

| Species | Dose Mg/kg | route | urine | feces | bile | carcass | Total recovery |
|---|---|---|---|---|---|---|---|
| Mouse | 0.9<br>0.9 | Iv<br>P.O. | 0.64<br>1.90 | 99.5<br>95.2 | | 0.16<br>0.05 | 100.8<br>97.4 |
| Rat | 1<br>1 *<br>10<br>1.5<br>15<br>0.5<br>0.5 | Iv<br>Iv<br>Iv<br>P.O.<br>P.O.<br>P.O.<br>P.O., 21 days | 4.5<br>3.3<br>4.2<br>5.6<br>4.8<br>1.1<br>0.6 | 68.8<br>4.6<br>82.0<br>73.8<br>79.1<br>72.8<br>89.7 | 71.4 | 3.3<br>9.0<br>3.8<br>4.2<br>3.0<br><br><0.1 | 76.8<br>88.6<br>90.1<br>85.2<br>87.0<br>73.9<br>90.3 |
| Monkey | 1<br>5 | Iv<br>P.O. | 7.1<br>7.2 | 66.6<br>75.7 | | | 76.7<br>84.5 |
| Patients | 0.039 | P.O. | 5.1 | 79.5 | | | 84.6 |

*(bile duct cannulated)

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



## 3.4 TOXICOLOGY

### 3.4.1 Overall toxicology summary

General toxicology:
Toxicities observed in animal studies with RAD may be classified as related to pharmacologic effect i.e. immunosuppression and those which appear independently of immunosuppression, direct toxicity. Multiple dose toxicity studies provided the most informative portion of the submission. Multiple dose studies were conducted in mice for up to 13 weeks, rats up to 26 weeks and in cynomolgus monkeys up to 52 weeks. Additional toxicity findings may be gleaned from the 104-week mouse and rat carcinogenicity studies. Due to gastrointestinal intolerability in dogs, cynomolgus monkeys were selected by the sponsor to serve as the non-rodent species for toxicity studies. Toxicity studies with rapamycin also had the same toxicity with dogs. Body weight reduction was seen at the higher doses used for all species studied, at $5.0 mg/kg in mice, at $0.5 mg/kg in rats, at $1.5 mg/kg in minipigs and at $0.5 mg/kg in monkeys. Inflammatory gastrointestinal findings, possibly related to changes in gastrointestinal microflora under immunosuppression at these doses may be responsible for the effect through decreased absorption.
Immunosuppression, the intended action of everolimus, was observed in all species tested, manifesting as thymic atrophy, splenic atrophy and lymph node atrophy associated with a decrease in circulating lymphoid and total white cells.
In studies at the higher doses, other effects secondary to immunosuppression were observed. Effects to skin such as abrasions, ulceration, inflammation and scabs, were observed in monkeys (4-week study, $5.0 mg/kg; 52-week study, 0.3 mg/kg) and mice (13-weeks, $1.5 mg/kg). These effects were seen in previous studies with rapamycin and other immunosuppressive drugs in animal studies. The abrasion by the grating of cages causes sores on feet and ears which are likely entry sites for microorganisms to infect the immunosuppresed animals, despite animal husbandry efforts to maintain a relatively germ-free environment. Heart lesions exemplified by myocardial degeneration were exacerbated by higher doses of RAD in the rat ($ 1.5 mg/kg). In cynomolgus monkeys, myocardial degeneration was observed at $5.0 mg/kg in a 2-week study and at 1.5 and 5.0 mg/kg in a 26-week study. These findings were also seen in rapamycin studies. In the RAD studies, preexisting viral infections may have been exacerbated by immunosuppression. Endogenous viruses are well-characterized in cynomolgus monkey breeding colonies and may only emerge under immunosuppression. Other infections and/or inflammation related to immunosuppression included a 4-week study in minipigs which was compromised by a coccidial infection in the intestine, while the high dose group in the 52-week monkey study was terminated after 39 weeks due to gastrointestinal inflammation.

Reproductive organs were subject to RAD toxicity in all species studied. Decreases in organ weight were accompanied by histopathology findings including testicular atrophy (monkey, 0.3 mg/kg) as well as depletion of germ cells and tubular vacuolation in testes, reduced sperm count in epididymides in males, and in females, uterine atrophy and reduced follicular development. These reproductive effects were seen at doses as low as 1.5 mg/kg in mice and monkeys. Male reproductive toxicity appears related to RAD –related reduction of testosterone as seen in the male rat 13-week fertility study, correlated with impaired fertility. A hormonal basis for the RAD-related toxicity to reproductive organs observed in females remains unclear without further studies.

Toxicity to the lung was observed in both mouse and rat studies. Increased alveolar macrophages were observed in mice at doses $1.5 mg/kg and $0.5 mg/kg in the rat. Eosinophilic deposits in the lung were seen in a rat study comparing batches with different byproduct content. Toxicity to the eye was observed in a rats study in which swelling and disruption of cortical lens fibers at a dose of 5 mg/kg in a one-month study and at 0.9 mg/kg in the 104-wek carcinogenicity study.

Renal toxicity was seen in several rat studies. In CD-1 mice, renal tubular degeneration was observed in a 13-week study at doses of $5.0 mg/kg. Studies in rats demonstrated greater incidence and severity of lipofuscin in the renal tubular epithelial cells after 26 weeks at $0.5 mg/kg and in the 104-week carcinogenicity study at doses $0.3 mg/kg.

Pancreatic toxicity was observed in a 4-week minipigs and 26-week monkey study. The minipigs study showed vacuolation of the exocrine pancreas with necrosis at 15 mg/kg. The monkey study demonstrated pancreatic islet cell degeneration at 5.0 mg/kg and degranulation in the exocrine pancreas. The toxciologic basis of the pancreatic findings is unclear. It is a concern in human organ transplantation which is complicated by so-called post-transplant diabetes mellitus, also observed with immunosuppression by calcineurin inhibitors.

Clinical hematology and biochemistry parameters demonstrated increased neutrophils at $1.5 mg/kg in rats, minipigs and monkeys
Erythrocyte parameters including packed cell volume, hemoglobin and erythrocyte count were increased in rats $0.5 mg/kg and monkeys at $1.5 mg/kg. Platelet counts were decreased in mice, rats, and minipigs at $0..5 mg/kg. fibrinogen was increased in minipigs and monkeys at $1.5 mg/kg. RAD demonstrated effects on lipid metabolism with increased cholesterol and triglycerides in most species tested at doses $0.5 mg/kg. The effect was also manifested in minipigs through increased low density lipoprotein and decreased high density lipoproteins. Decreased serum albumin was seen in all species together with a decreased albumin/globulin ratio at doses $5.0 mg/kg.

Summary table of toxicology findings in multidose oral studies

| **Species** | **Finding** | **LOEL (mg/kg)** | | | **NOAEL (mg/kg)** | | |
|---|---|---|---|---|---|---|---|
| | **duration** | **3 mo** | | **2 yr** | **3 mo** | | **2 yr** |
| Mouse | Thymic atrophy | 0.5 | | 0.9 | 0.15 | | |
| | Skin lesions | 1.5 | | | 0.5 | | |
| | Renal tubular degeneration | 5 | | | 1.5 | | |
| | Pulmonary macrophages | 1.5 | | | 0.5 | | |
| | Testicular germ cells | 0.5 | | 0.9 | 0.15 | | |
| | Uterine atrophy/ Reduced follicular development | 1.5 | | | 0.5 | | |
| | **duration** | **1 mo** | **6 mo** | **2 yr** | **1 mo** | **6 mo** | **2 yr** |
| rat | Thymic/lymphoid atrophy | 0.5 | 0.5 | 0.9 | 0.25 | 0.15 | 0.3 |
| | Chronic myocarditis | 1.5 | | | 0.5 | >1.5 | >0.9 |
| | Pulmonary alveolar macrophages | 1.5 | 0.5 | 0.9 | 0.5 | 0.15 | 0.3 |
| | Testicular germ cell depletion/ tubular vacuolation | 1.5 | 1.5 | 0.9 | 0.5 | 0.5 | 0.3 |
| | Uterine atrophy | 1.5 | | | 0.5 | >1.5 | >0.9 |
| | Lenticular changes, eyes | 5.0 | | 0.9 | 1.5 | >1.5 | 0.3 |
| | Cortical bone depletion | 5.0 | | | 1.5 | | >0.9 |
| | Lipofuscin, Renal cortical tubules | | 0.5 | 0.3 | >15 | 0.15 | 0.1 |
| | **Duration** | **1 mo** | | | | | |
| minipig | Thymic/lymphoid atrophy | 1.5 | | | | | |
| | Parasitic infection w intestinal mucosal erosion | 1.5 | | | | | |
| | Focal acinar vacuolation, pancreatic cells | 5.0 | | | 1.5 | | |
| | Inc testicular | 1.5 | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | tubular atrophy | | | | | | |
| | Uterine/vaginal atrophy | 15.0 | | | 5.0 | | |
| | Necrotic follicles, ovaries | 5.0 | | | 1.5 | | |
| | **Duration** | **1 mo** | **6 mo** | **1 yr** | **1 mo** | **6 mo** | **1 yr** |
| Monkey cynomolgus | Thymic atrophy | 5.0 | 1.5 | | 1.5 | 0.5 | >0.9 |
| | Splenic lymphoid atrophy | 1.5 | 0.5 | 0.9 | <1.5 | <0.5 | 0.3 |
| | Skin lesions | 5.0 | 0.5 | | 1.5 | <0.5 | >0.9 |
| | Intestinal histiocytosis | 5.0 | 0.5 | | 1.5 | <0.5 | >0.9 |
| | GI tract inflammation | | 5.0 | 0.3 | >15 | 1.5 | 0.1 |
| | Myocardial degeneration/ Necrosis | | 1.5 | | >15 | 0.5 | >0.9 |
| | Pancreatic islet cell degeneration | | 5.0 | | >15 | 1.5 | >0.9 |
| | Reduced follicular cell development, ovaries | | 1.5 | | >15 | 0.5 | >0.9 |
| | Tubular atrophy, testes | | | 0.3 | >15 | | 0.1 |

Comparative systemic exposure in oral studies

| species | Dose | Duration Weeks | AUC(0-24) Ng.h/ml | | Multiple of human exposure for NOAEL |
|---|---|---|---|---|---|
| mouse | | | M | F | |
| | 0.15 | 13 | **803** | 362 | 3.1 |
| | 0.5 | | 1174 | **1258** | 4.8 |
| | 1.5 | | 3472 | 4877 | |
| | 5 | | 19420 | 11363 | |
| | 15 | | 45955 | 43206 | |
| mouse | 0.1 | 101 | 160 | | |
| | 0.3 | | 380 | | |
| | 0.9 | | **2231** | | 8.6 |
| rat | 0.5 | 4 | **79** | | 0.3 |
| | 1.5 | | 435 | | |
| | 5 | | 1468 | | |
| | 15 | | 6076 | | |
| rat | 0.15 | 26 | **8** | | 0.03 |
| | 0.5 | | 29 | | |
| | 1.5 | | 72 | | |
| rat | 0.1 | 104 | 2 | | |
| | 0.3 | | 19 | | |
| | 0.9 | | **91** | | 0.4 |
| minipig | 1.5 | 4 | 2670 | | |
| | 5 | | 6057 | | |
| | 15 | | 7856 | | |
| Monkey | 1.5 | 4 | 1086 | | |
| | 5 | | 3204 | | |
| | 15 | | 6978 | | |

| | | | | |
|---|---|---|---|---|
| Monkey | 0.1<br>0.5<br>1.5 | 26 | 189<br>**412**<br>1162 | 1.6 |
| monkey | 0.1<br>0.3<br>0.9 | 52<br><br>(39) | 79<br>**226**<br>706 | 0.86 |

NOAEL exposures in **bold**
These may be compared to the human exposure for a 3 mg/day dose resulting in an AUC(0-24h) of 260 ng.h/ml in a 3-month steady state in clinical study B251

Genetic toxicology:
The requirements of the ICH genotoxicity battery were fulfilled by the sponsor with both *in vivo* and *in vitro* assays.
The following studies were performed:

| Study type | species | route | dose | Outcome |
|---|---|---|---|---|
| In vitro-bacterial reversion (Ames) | S. typhimurium | In vitro | 8-5000 : g/plate, +/- S9 | negative |
| In vitro mammalian Mouse lymphoma L5178Y cells | ---- | In vitro | 7.5-90 : g/ml, +/- S9<br>15-120 : g/ml, +/- S9 | Negative |
| V79 Chinese hamster cells | ---- | In vitro | 15-81 : g/ml,- S9<br>57-131 : g/ml, + S9 | negative |
| In vivo mammalian Micronucleus | mouse | In vivo | 50, 160, 500 mg/kg | negative |

Based on these negative findings, everolimus does not appear to have genotoxic activity.

Carcinogenicity:
In a 104-week carcinogenicity study in mice, statistical review showed no dose mortality trends for the male and female mice. No positive linear trends were found 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 historical control incidence rates. 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% of females after 101 weeks. Survival among treated mice was highest in the high dose groups correlating with lower bodyweight gain. Food consumption was unaffected by treatment. Histopathology findings included treatment-related changes in the thymus, testes, and epididymides. High dose females had thymic involution. Leukocytic infiltration of the renal cortex was reduced in mid- and high dose females and submandibulary salivary gland in treated females, possibly related to immunosuppression. Reproductive effects were observed in the testes and epididymides of high dose males.
The statistical review of the 104-week carcinogenicity study in rats showed no dose mortality trends for the male and female mice. No positive linear trends were found for male or female rats. None of the observed neoplastic tumors were statistically significant by trend analysis or exact tests. No dose mortality trend 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 among treated rats was highest in the mid and high dose groups correlating with lower bodyweight gain. 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. Immunosuppression-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, age-related 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 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

Reproductive toxicology:
The reproductive toxicology studies performed by the sponsor fulfil ICH requirements. These studies included male reproductive function, female fertility and embryofetal and perinatal toxicity. In male fertility studies, treatment for 6 weeks at doses up to 1.5 mg/kg had no effect on reproduction. After 13 weeks of treatment, male fertility was completely inhibited at 5.0 mg/kg, with decreased testosterone, testicular atrophy, germ cell depletion and oligospermia and aspermia of the epididymides. Sperm motility and testicular sperm count were decreased. At 0.5 mg/kg, sperm were misshapen without effect on fertility. Female fertility was unaffected by RAD treatment. RAD crossed the placenta and caused fetotoxicity. In female rat reproductive toxicity studies, pre- and post implantation losses and skeletal retardations resulted at the lowest dose, 0.1 mg/kg and increasing in a dose-dependent manner. In rabbits, embryo-fetal development was observed as an increase of late resorptions at a maternally toxic dose, 0.8 mg/kg. At 0.2 mg/kg, embryotoxicity was not evident although maternal toxicity was seen. The perinatal developmental effect of decreased bodyweight was seen in offspring rats at $0.1 mg/kg. A juvenile study in rats had small reproductive organs at all doses. NOAEL values for reproductive toxicity include: male fertility: 0.5 mg/kg, 6 weeks; 0.1 mg/kg , 13 weeks; female and fetal, 0.15 mg/kg; rabbit female: 1.0 mg/kg; fetal: 0.2 mg/kg.

The following studies were performed:

| Study | species | Doses (mg/kg) | findings |
|---|---|---|---|
| Male fertility | | | |
| Fertility dose-range finding (6 weeks) | Rat | 0, 0.15, 0.5, 1.5 | NOAEL (male): 0.5 mg/kg AUC=38 ng.h/ml<br>1.5 mg/kg: reduced body weight gain, food intake; testicular germ cell degeneration and depletion<br>no male-related effects on reproduction |
| 13-week investigative fertility study w 13-week recovery | Rat | 0, 0.1, 0.5, 5 | NOAEL: 0.1 mg/kg; AUC=9.9 ng.h/ml<br>$0.5 mg/kg: reduced body weight gain<br>0.5 mg/kg: slight effect testicular morphology<br>5 mg/kg; decreased male fertility, decreased testosterone, recovery in 50% after 13 weeks |
| Embryo-fetal studies | | | |
| Reproductive toxicity dose-range finding study with toxicokinetics and placental transfer | rat | 0, 0.15, 0.5, 1.5 | NOAEL (maternal and embryo): 0.15 mg/kg; AUC= 10ng.h/ml<br>$0.5 mg/kg: reduced body weight gain; inc post-implantation loss<br>1.5 mg/kg reduced food intake, resorption of all implants |
| fertility and embryo-fetal development study | rats | 0, 0.1, 0.3, 0.9 | NOAEL (intrauterine development) <0.1 mg/kg, AUC=20.0ng.h/ml<br>NOAEL (maternal) 0.3 mg/kg AUC=37.5 ng.h/ml<br>Embryonic tissue 0.8 ng/g<br>$0.1 mg/kg: increased pre- and postimplantation loss, delay in skeletal development<br>$0.3 mg/kg; impaired body weight development; reduced fetal weight; increased incidence malformations-thoracic vertebrae, ribs, sternebrae<br>0.9 mg/kg: reduced food intake, inc incidence of 14 ribs; 2 fetuses w sternal cleft |
| embryo-fetal development dose range finding study | rabbit | 0, 0.5, 1.0, 1.5, 5 | NOAEL (intrauterine development): 1.0 mg/kg; AUC= 178 ng.h/ml<br>NOAEL (maternal): <0.5 mg/kg<br>$0.5 mg/kg: reduced body weight, food, water intake<br>$1.5 mg/kg: increased postimplantation loss |
| embryo-fetal development study | rabbit | 0, 0.05, 0.2, 0.8 | NOAEL (intrauterine development): 0.2 mg/kg; AUC=61.2 ng.h/ml<br>Embryonic tissue <LOD (1 ng/g)<br>NOAEL (maternal): 0.05 mg/kg AUC=18.4 ng.h/ml |

| | | | |
|---|---|---|---|
| | | | $0.2 mg/kg: one dam died. Body weight loss, reduced food intake, inc late resorptions<br>0.8 mg/kg: one dam died; body weight loss, reduced food intake, inc late resorptions |
| Pre- and postnatal development study | | | |
| Pre- and postnatal development study | rat | 0, 0.03, 0.1, 0.3 | NOAEL (F0 generation): 0.3 mg/kg<br>NOAEL (F1 generation): 0.03<br>$0.1 mg/kg: reduced body weight and survival, F1 generation |
| oral neonatal and juvenile development study with 13- and 26-week recovery periods | rat | 0, 0.15, 0.5, 1.5 | NOAEL < 0.15 mg/kg;<br>sm reproductive organs, males and females, all doses reduced body weight and food consumption |

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**ABBREVIATIONS:**
Na=not applicable
Np= not performed
Sl= slight
Inc= increased
Dec=decreased
p.c.= post coitus
pp= post partum
Others may be noted in text

### 3.4.3 REPEAT-DOSE TOXICITY

**Study title:** An oral dose-escalating study in dogs

**Key study findings:** Ulcerations are seen throughout the gastrointestinal tract, contributing to the diarrhea and dehydration also occurring.

**Study no:** 41DED (BS-674)

**Volume #, and page #:** v.16, p1

**Conducting laboratory and location:** Novartis Pharma AG
Basel, Switzerland
**Date of study initiation:** not provided (approx. 26 Jan 1994)
**GLP compliance:** no
**QA report:** yes ( ) no ( x )
**Drug, lot #, radiolabel, and % purity:** batch BT7/695, na, np
**Formulation/vehicle:** not provided
**Methods** (unique aspects)**:**

**Dosing:**

Species/strain: dog (no breed provided)
#/sex/group or time point (main study): 1
Satellite groups used for toxicokinetics or recovery: none
Age: not provided
Weight: not provided

Doses in administered units: 2 mg/kg (days 1-3), 6 mg/kg (days 4-6), 20 mg/kg (days 7-9)

Route, form, volume, and infusion rate: oral capsule

**Observations and times:**

Clinical signs: daily
Body weights: days 0, 3, 6, 9
Food consumption: days 1-7, 8-9
Ophthalmoscopy: not performed
EKG: not performed
Hematology: day 0, 24 h after last dose
Clinical chemistry: day 0, 24 h after last dose
Urinalysis: not performed
Gross pathology: at necropsy
Organs weighed: not performed
Histopathology: at necropsy
Toxicokinetics: not performed
Other: not performed

**Results:**

Mortality: all dogs survived until necropsy
Clinical signs: tremor 18 mg/kg: sedation, diarrhea, ataxia, salivation, exsiccosis
Body weights: body weight loss at 6, 18 mg/kg
Food consumption: decreased
Ophthalmoscopy: not performed
Electrocardiography: not performed
Hematology: increased partial prothrombin time, increased sedimentation rates;
Clinical chemistry: increased total bilirubin, alkaline phosphatase, cholesterol, triglycerides; decreased glucose
Urinalysis: not performed
Organ weights: not performed
Gross pathology: ulceration of oral and gastrointestinal tract
Histopathology: multifocal ulceration of oral cavity, esophagus, stomach, and large intestine, with neutrophil infiltration,

perivasculitis and lymphoid necrosis; haemorrhagic bronchopneumonia
Toxicokinetics: not performed

**Summary of individual study findings:**

This brief, non-glp study provides limited information due to the small number of dogs used. Ulcerations characteristic of other immunosuppressants are seen throughout the gastrointestinal tract, contributing to the diarrhea and dehydration also seen. This study also highlights the limitations of the use of dogs for studies with RAD due to the gastrointestinal distress caused by RAD, similar to that seen with rapamycin.

**Study title:** A comparative 2-week oral (gavage) toxicity study in the rat with a micro-emulsion and a solid dispersion

**Key study findings:** No major toxicologic differences between the two forms of RAD used in this rat study were demonstrated.

**Study no:** (b) project 617951 (203-078)
**Volume #, and page #:** v. 9, p. 1
**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 7 Feb 1996
**GLP compliance:** yes
**QA report:** yes (x ) no ( )
**Drug, lot #, radiolabel, and % purity:** SDZ RAD microemulsion: batch no. Y182 0895, na, 99.6%; SDZ RAD solid dispersion: batch no. 95701, na, 100.1%
**Formulation/vehicle:** SDZ RAD microemulsion: 5% glucose solution; SDZ RAD solid dispersion, water

**Methods** (unique aspects)**:**
**Dosing:**

Species/strain: rat/ HanIbm:WIST (b) (4)
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: none
Age: 9 weeks
Weight: males, 266-313 g; females, 150-209 g
Doses in administered units: SDZ RAD microemulsion:1.5, 15.0 mg/kg; SDZ RAD solid dispersion: 1.5, 15.0 mg/kg
Route, form, volume, and infusion rate: oral gavage, 5 ml/kg

**Observations and times:**

Clinical signs: twice daily
Body weights: pretest, twice weekly on study
Food consumption: weekly
Ophthalmoscopy: day 13
EKG: not performed
Hematology: not performed
Clinical chemistry: not performed
Urinalysis: not performed
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: day 14/15, 0.5, 1, 2, 7 and 24 h after dosing
Other: na

Results:

| Dose (mg/kg) | control micro-emulsion | Microemulsion 1.5 | Microemulsion 15.0 | control solid dispersion | solid dispersion 1.5 | solid dispersion 15.0 |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| HED (mg/kg) | | | | | | |
| Mortality | All animals survived until terminal sacrifice | | | | | |
| Clinical signs | No treatment-related signs were seen | | | | | |
| Body weights | | | dec | | | dec |
| Food consumption | | | dec | | | dec |
| Ophthalmo-scopy | | No treatment-related effects were seen | | | | |
| EKG | Not performed | | | | | |
| Hematology | Not performed | | | | | |
| Clinical chemistry | Not performed | | | | | |
| Urinalysis | Not performed | | | | | |
| Organ weights | | Pituitary, dec<br>Pancreas, dec<br>Thymus, dec | Brain, dec<br>Pituitary, dec<br>Pancreas, dec<br>Thymus, dec<br>Spleen, dec<br>Prostate, dec<br>Testes, dec | | Pituitary, dec, males<br>Pancreas, dec<br>Thymus, dec<br>Uterus, dec | Brain, dec<br>Pituitary, dec<br>Pancreas, dec<br>Thymus, dec<br>Spleen, dec<br>Prostate, dec<br>Testes, dec<br>Uterus, dec |
| Gross pathology | | | Seminal vesicles, small, males | | | Seminal vesicles, small, males |

| | | | | | | |
|---|---|---|---|---|---|---|
| Histopathology | | Brain: distension of lateral ventricles<br>Heart: focal/multifocal myocardial degeneration<br>Lungs: alveolar histiocytes<br>Testes: tubular degeneration<br>Prostate: Atrophy<br>Uterus: atrophy<br>Vagina: atrophy<br>Adrenals: Vacuolation cortex<br>Spleen; dec germinal centers, lymphoid atrophy<br>Bone marrow: hypocellularity/atrophy<br>Thymus:cortical/ medullary atrophy<br>Lymph nodes: absence of germinal centers | Brain: distension of lateral ventricles<br>Heart: focal/multifocal myocardial degeneration<br>Lungs: alveolar histiocytes<br>Testes: tubular degeneration, Multinucleated giant cells<br>Epididymides: Oligospermia<br>Prostate: Atrophy Prostate: Atrophy Uterus: atrophy<br>Vagina: atrophy<br>Adrenals: Vacuolation cortex<br>Spleen; dec germinal centers, lymphoid atrophy<br>Bone marrow: hypocellularity/atrophy<br>Thymus:cortical/ medullary atrophy Lymph nodes: absence of germinal centers<br>Mammary: Lobuloalveolar atrophy, males<br>Eye: swelling of cortical lens fibers | | Brain,: distension of lateral ventricles, females<br>Heart: focal/multifocal myocardial degeneration, females<br>Prostate: Atrophy<br>Uterus: atrophy<br>Adrenals: Vacuolation cortex<br>Spleen; dec germinal centers, lymphoid atrophy<br>Bone marrow: hypocellularity/atrophy<br>Thymus:cortical/medullary atrophy<br>Lymph nodes: absence of germinal centers | Brain: distension of lateral ventricles<br>Heart: focal/multifocal myocardial degeneration, females<br>Testes: tubular degeneration, Multinucleated giant cells<br>Epididymides: Oligospermia<br>Prostate: Atrophy<br>Prostate: Atrophy<br>Uterus: atrophy<br>Vagina: atrophy<br>Adrenals: Vacuolation cortex<br>Spleen; dec germinal centers, lymphoid atrophy<br>Bone marrow: hypocellularity/atrophy<br>Thymus:cortical/medullary atrophy<br>Lymph nodes: absence of germinal centers<br>Mammary: Lobuloalveolar atrophy, males<br>Eye: swelling of cortical lens fibers |
| Toxicokinetics<br>AUC (ng.h/ml)<br>Males<br><br>Females | | <br><br>324<br><br>226 | <br><br>7816<br><br>7288 | | <br><br>262<br><br>226 | <br><br>5735<br><br>8954 |

**Conclusions:** 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 were seen in both including brain, spleen, testes, and prostate at the 15 mg/kg dose and pituitary, pancreas, thymus and uterus at 1.5 and 1.5.0 mg/kg. Seminal vesicles were reduced in size in males at 15 mg/kg. Histopathologic findings at 1.5 and 15.0 mg/kg included atrophy of lymphoid tissues (thymus, spleen, lymph nodes and bone marrow), atrophy of male and female reproductive organs, myocardial degeneration, swelling of brain ventricles, increased incidence of alveolar histiocytes in the lung and vacuolation of the adrenal cortex. At 15.0 mg/kg, swelling of the cortical lens fibers of the eye occurred. 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.

**Study title:** A 2-week oral (gavage) dose-range-finding study in minipigs

**Key study findings:** Due to the small group sizes, it is difficult to draw definitive conclusions about the results of this study.

**Study no:** 212DFP (BS-610)
**Volume #, and page #:** v. 16, p. 17
**Conducting laboratory and location:** Novartis Pharma AG
Basel, Switzerland
**Date of study initiation:** 3 Sept 1996
**GLP compliance:** yes
**QA report:** yes ( ) no ( x )

**Drug, lot #, radiolabel, and % purity:** X035 0396, na, 86.8%
**Formulation/vehicle:** 9.1% solid dispersion
**Methods** (unique aspects)**:**

**Dosing:**

Species/strain: minipig, Gottingen SPF
#/sex/group or time point (main study): 1
Satellite groups used for toxicokinetics or recovery: na
Age: 4-5 mo
Weight: 9.3-11.3 kg
Doses in administered units: 0, 0.5, 1.5, 5 mg/kg
Route, form, volume, and infusion rate: oral gavage, 5 ml/kg, na

**Observations and times:**

Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: not performed
EKG: days 10/11
Hematology: day 14
Clinical chemistry: day 14
Urinalysis: not performed
Gross pathology: at necropsy
Organs weighed: see histopathology table
Histopathology: at necropsy
Toxicokinetics: day 14 at 1, 3, 7 and 14 h after dosing
Other: na

**Results:**

Mortality: 1 female (5 mg/kg) sacrificed day 3-poor health, tremors, sedation, weight loss, increased sedimentation rate; necropsy: multiple granulomas with pneumonia
Clinical signs: no treatment related effects seen
Body weights: no treatment related effects seen
Food consumption: no treatment related effects seen
Ophthalmoscopy: not performed
Electrocardiography: no treatment related effects seen
Hematology: all treated males and females, decreased platelets, lymphocytes
Clinical chemistry: increased creatinine, females, 0.5, 1.5 mg/kg, males, 5 mg/kg;

Albumin, (-globulin decreased , males 1.5 mg/kg, females, 1.5, 5 mg/kg
Urinalysis: not performed
Organ weights: lower weights, pituitary, testes, treated males; spleen, adrenal, pituitary, ovaries, uterus, treated females
Gross pathology: lung nodule, female, 1.5 mg/kg

Histopathology:

| Finding | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
|---|---|---|---|
| thymic cortical lymphocytolysis | all | all | All |
| Lung: foreign body granuloma | | 1F | 1M, 1F |
| Seminiferous tubules: vacuolated germ cells | 1M | 1M | 1M |
| Leydig cell hyperplasia | 1M | 1M | 1M |

Toxicokinetics:

| | 0.5 mg/kg | 1.5 mg/kg | 5 mg/kg |
|---|---|---|---|
| AUC (0-24h) M:<br>ng.h/ml F: | 1454<br>1694 | 5731<br>6271 | 5007<br>* |
| Cmax (ng/ml) M:<br>F: | 88<br>91 | 473<br>418 | 424<br>* |

* value not calculated due to single surviving female

**Conclusion:**
One high dose female died on day 3; weight loss was noted prior to treatment and pneumonitis was seen at necropsy with foreign body granuloma. Immunosuppression may have exaggerated a preexisting condition. Otherwise, all minipigs survived until necropsy.
Drug-induced effects included an increased creatinine, females, 0.5, 1.5 mg/kg, males, 5 mg/kg; albumin and (-globulin decreased, males 1.5 mg/kg, females, 1.5, 5 mg/kg; decreased weights, pituitary, testes, treated males; spleen, adrenal, pituitary, ovaries, uterus, treated females; thymic cortical lymphocytosis seen in all treated groups. Testicular effects were seen in all treated males including vacuolated cells in the seminiferous tubules and Leydig cell hyperplasia.
The small numbers of minipigs in each group permits only generalization concerning the effects seen, especially immunosuppression and testicular effects.

**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 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) by toxicity. A NOEL was not determined in this study.

**Study no:** 971033
**Volume #, and page #:** v. 16, p. 117
**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
**Methods** (unique aspects)**:**

**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 mo
Weight: 6.7-8.2 kg
Doses in administered units: 0, 1.5, 5.0, 15.0 mg/kg
Route, form, volume, and infusion rate: oral gavage, not provided, na

**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 5 mg/kg group; diarrhea, 1 male day 2-4, sedation, tremor, 1 male, week 3;
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 prestudy 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 creatinine, "2- and $1-globulins, decreased phosphorus, alkaline phosphatase, (-globulins 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 |
| Lg intestine:<br>erosion<br>atrophy | | | <br>3/3 m, 1/3 f<br>3/3 m, 2/3 f |
| Spleen:<br>lymphoid depletion | | <br>1/3 f | <br>3/3 m |
| Pancreas:<br>vacuolated cells<br>necrosis | | <br>3/3 m, 3/3 f<br>1/3 f | <br>3/3 m, 3/3 f<br>2/3 m, 1/3 f |
| Thymus: | | | |

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

Toxicokinetics:
The following parameters were determined, day28/29:

| Dose (mg/kg) | sex | n | Cmax (ng/ml) | AUC (0-24h) (ng.h/ml) |
|---|---|---|---|---|
| 1.5 | M<br>f | 3<br>3 | 145.2<br>153.4 | 2937.2<br>2402.3 |
| 5.0 | M<br>F | 3<br>3 | 399.7<br>346.5 | 6163.7<br>5950.9 |
| 15.0 | M<br>F | 1<br>5 | 459.3<br>397.5 | 7982.6<br>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) by toxicity. A NOEL was not determined in this study.

**Study title:** 28-day oral gavage toxicity study in juvenile cynomolgus monkeys with a 2-week reversibility period

**Key study findings:** The small numbers of animals/sex/group and significance of differences for both hematology and clinical chemistry between groups prior to treatment diminishes the value of this study. Increased fibrinogen was seen in the high dose group as well as reduced lung and thymus weights. Doses selected in this study may be too low to see expected histopathology changes due to immunosuppression.

**Study no:** 1463-019 (203-070)

**Volume #, and page #:** v19, p1

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

(b) (4)

**Date of study initiation:** 25 July 1996

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

**Drug, lot #, radiolabel, and % purity:** XO 35 0396, na, not provided
**Formulation/vehicle:** solid dispersion with hydroxymethyl cellulose in water
**Methods** (unique aspects)**:**

**Dosing:**

Species/strain: cynomolgus monkey (b) (4)

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

Satellite groups used for toxicokinetics or recovery: 2 (control, HD)

Age: 10 months
Weight: 0.8- 1.5 kg
Doses in administered units: 0, 0.1, 0.25, 0.50 mg/kg
Route, form, volume, and infusion rate: oral, dispersion, 5 ml/kg

**Observations and times:**

Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: before start of dosing, final week of treatment, and the end of recovery
EKG: before start of dosing, final week of treatment, and the end of recovery
Hematology: before start of dosing, final week of treatment, and the end of recovery
Clinical chemistry: before start of dosing, final week of treatment, and the end of recovery

Urinalysis: before start of dosing, final week of treatment, and the end of recovery; with a 16 h collection period for each

Gross pathology: at necropsy
Organs weighed: at necropsy, see table
Histopathology: at necropsy, see table
Toxicokinetics: not performed
Other: not performed

**Results:**

Mortality: all survived until terminal sacrifice
Clinical signs: no treatment related effects were seen
Body weights: no treatment related effects were seen
Food consumption: no treatment related effects were seen
Ophthalmoscopy: no treatment related effects were seen
Electrocardiography: no treatment related effects were seen

Hematology: fibrinogen was elevated in 0.5 mg/kg m and f; no other treatment related effects were seen. For numerous parameters, treated groups had values significantly different than those of controls prior to dosing, reducing the value of any comparative changes during treatment.

Clinical chemistry: For numerous parameters, treated groups had values significantly different than those of controls prior to dosing, reducing the value of any comparative changes during treatment. Otherwise no treatment-related effects were seen.

Urinalysis: No treatment-related effects were seen.

Organ weights: Lung weights were reduced in 0.5 mg/kg males, with a similar trend in females. Thymus weights were reduced in males and females receiving 0.5 mg/kg.

Gross pathology: No treatment-related effects were seen.
Histopathology: No treatment-related effects were seen.
Toxicokinetics: not performed.

**Summary of individual study findings:**

The small numbers of animals/sex/group and significance of differences for both hematology and clinical chemistry between groups prior to treatment diminishes the value of this study. Increased fibrinogen was seen in the high dose group as well as reduced lung and thymus weights. These effects are probably related to immunosuppression. Doses selected in this study may be too low to see expected histopathology changes due to immunosuppression.

**Study title:** SDZ RAD:Toxicity study by oral gavage administration to cynomologus monkeys for 26 weeks followed by a four week reversibility period
**Key study findings:** 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. A NOEL was not observed in this study.

**Study no:** 96/SMP078/1067 (203-072)
**Volume #, and page #**: v. 21, 22, 23
**Conducting laboratory and location:** (b) (4)

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

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

**Drug, lot #, radiolabel, and % purity:** Y213 1095, na,

**Formulation/vehicle:** solution in 5% dextrose
**Methods** (unique aspects)**:** none

**Dosing:**

Species/strain: cynomolgus monkeys (b) (4)
#/sex/group or time point (main study): 4
Satellite groups used for recovery: controls, 2; 0.1 mg/kg, 4
Age: 31-39 months (1st treatment phase), 11-15 months (recovery group)
Weight: 1.69-2.47 kg (1st treatment phase), 1.28-1.75kg (recovery group)
Doses in administered units: 0.5, 1.5, 5 mg/kg; 0.l mg/kg (recovery)
Route, form, volume, and infusion rate: oral gavage, 5 ml/kg, na

**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: na

**Results:**

| Dose (mg/kg) | 0 | 0.5 | 1.5 | 5.0 (treatment terminated after week 9) | 0.1 (recovery) |
|---|---|---|---|---|---|
| HED (mg/kg) | 0 | 0.2 | 0.6 | 2.0 | 0.04 |
| Mortality | | | 2 killed; 1 week 14, 1 week 25; both approx. 25% body weight loss | 1 killed week 9, abrasions/skin ulcerations | |
| Clinical signs | | Skin ulceration | Skin ulceration; piloerection | Skin ulceration; piloerection | Skin ulceration |
| Body weights | | | | 5 , 2 loss of body weight (~10%) | |
| Food consumption | | | | food consumption decreased, > week 3 | |
| Ophthalmoscopy | | Unaffected by treatment | | | |
| electrocardiography | | Unaffected by treatment | | | |
| Hematology | | | Decreased hemoglobin, packed cell volume<br>Increased fibrinogen<br>Decreased albumin<br>Decreased phosphorus<br>sl increased cholesterol | decreased hemoglobin, packed cell volume<br>increased fibrinogen<br>decreased albumin | |
| Clinical chemistry | | | | decreased phosphorus<br>sl increased cholesterol | |
| Urinalysis | | Unaffected by treatment | | | |
| Organ weights | | | Decreased thymus weight<br>sl increase pancreas weight<br>sl decrease testes weight | decreased thymus weight<br>sl increase pancreas weight | |
| Gross pathology | | skin lesions | Skin lesions | | |
| Histopathology | | pancreas: degranulation of exocrine cells 1m<br>sm intestines: accumulation of macrophage in villi | Thymus: cortical and medullary atrophy<br>Lymph node atrophy<br>Pancreas: degranulation of exocrine cells 3m<br>Spleen : atrophy of germinal centers<br>sm intestines: accumulation of macrophage in villi<br>myocardial degeneration (1) | thymus: cortical and medullary atrophy<br>lymph node atrophy<br>spleen : atrophy of germinal centers<br>sm intestines: accumulation of macrophage in villi<br>myocarditis (1)<br>myocardial necrosis (2)<br>pancreas: degenerated islet | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | cells; degranulation of exocrine cells<br>adrenals: cytoplasmic vacuolation<br>ovaries: reduced follicular development, increased atresia | |
| Toxicokinetics<br>AUC (ng.h/ml)<br>Males week 1<br>Week 13<br>Week 26<br><br>Females week 1<br>Week 13<br>Week 26 | | <br><br>389<br>280<br>358<br><br>564<br>332<br>466 | <br><br>1096<br>777<br>1106<br><br>1133<br>779<br>1218 | <br><br>4049<br>4913 (week 9)<br><br><br>3504<br>3322 (week 9)<br>np | <br><br>83<br>53<br>145<br><br>112<br>np<br>233 |
| | | | | | |

**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. A NOEL was not observed in this study.

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

**Key study findings:** Study findings included diarrhea, decreased body weights and food consumption, increased fibrinogen, increased % band cells, decreased total protein, glucose in urine, decreased thyroid/parathyroid weights, decreased epididymes 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

**Study no:** 1463-045

**Volume #, and page #:** v. 25 p. 1

**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:** X011 0937/X176 1297, na**,**

**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:** na
**Age:** 2-9 yrs
**Weight**: 3.0-5.1 kg (males), 2.7-5.3 kg (females)
**Doses in administered units:** 0.1, 0.3, 0.9 mg/kg
**Route, form, volume, and infusion rate:** oral gavage, 5 ml/kg, na

**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 @ 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) |
|---|---|---|---|---|
| Mortality | | 1% , sacrificed moribund day 225 (edema, hunched posture, poor physical condition) | | 1 %, sacrificed moribund, day 142 (chronic diarrhea, hunched posture, poor physical condition)<br>2 &, sacrificed day 183, day 258 (chronic diarrhea, emaciation, poor physical condition) |
| Clinical signs | | | % diarrhea | %,& diarrhea |
| Body weights | | & sl decrease | %,& sl decrease | %,& sl decrease |
| Food consumption | | | | Decreases in animal sacrificed for morbidity prior to sac |
| Ophthalmosco py | | No treatment-related effect observed | | |
| Electrocardiogr aphy And blood pressure | | No treatment-related effect observed | | |

| | | | | |
|---|---|---|---|---|
| Hematology | | % % band cells increased | % sl increased fibrinogen<br>%,& %band cells increased | %,&increased fibrinogen<br>%,& %band cells increased |
| Clinical chemistry | | | %decreased total protein | % decreased total protein |
| Urinalysis | | | 1& glucose | 1& glucose |
| Organ weights | | % decreased thyroid/parathyroid<br>% decreased epididymes | % decreased thyroid/parathyroid<br>% decreased epididymes | % decreased thyroid/parathyroid<br>% decreased epididymes |
| Gross pathology | | 1 % (moribund) ascites, hydropericardium, renal and hepatic enlargement | 3 % congested lg intestine, mesenteric ln enlargement | 1 %, 2& (moribund) mesenteric ln enlargement, congestion of intestinal vessels<br>1% lung, white-yellow focus<br>1&, cecal ulceration |
| Histopathology | | | 3 %, 2&: acute inflammation lg intestine<br>2%: immaturity of testes<br>(1% immaturity of prostate, seminal vesicles) | 2& inflammation of colon /cecum<br>2%, 2&: stomach inflammation/erosion<br>all %: testicular immaturity |
| Toxicokinetics<br>AUC (ng.h/ml)<br>Males | | | | |
| Week 1 | | 1.740 | 135.857 | 362.596 |
| Week 26 | | 57.739 | 270.747 | 690.809 |
| Week 39 | | 87.809 | 349.909 | 941.299 |
| Week 52 | | 98.028 | 275.571 | np |
| Females | | | | |
| Week 1 | | 26.056 | 161.315 | 732.490 |
| Week 26 | | 78.013 | 144.396 | 395.736 |
| Week 39 | | 80.465 | 246.685 | 471.777 |
| Week 52 | | 59.561 | 176.222 | np |
| Cmax (ng/ml)<br>Males | | | | |
| Week 1 | | 0.696 | 13.814 | 27.900 |
| Week 26 | | 5.389 | 24.776 | 67.599 |
| Week 39 | | 6.476 | 36.393 | 84.921 |
| Week 52 | | 8.532 | 24.092 | np |
| Females | | | | |
| Week 1 | | 8.759 | 31.214 | 86.079 |
| Week 26 | | 15.795 | 15.816 | 37.157 |
| Week 39 | | 11.984 | 35.445 | 47.824 |
| Week 52 | | 10.135 | 20.541 | np |

**Summary of individual study findings:**

Study findings included diarrhea, decreased body weights and food consumption, increased fibrinogen, increased % band cells, decreased total protein, glucose in urine, decreased thyroid/parathyroid weights, decreased epididymes 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. The findings of this study appear related to the immunosuppressive action of SDZ RAD and appear similar to the related drug rapamycin.

**Study title:** Toxicity study by intravenous (bolus) administration to HanIbm Wistar rats for 2 weeks

**Key study findings:** Rats receiving intravenous treatment for two weeks showed some signs of immunosuppression (decreased leukocytes, depletion of lymphocytes from thymic cortex) as well as increased cholesterol and triglycerides

**Study no:** SPM115/970022 (203-071)

**Volume #, and page #:** v. 27 p.1
**Conducting laboratory and location:** (b) (4)

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

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

**Drug, lot #, radiolabel, and % purity:** Y187 0895, na, 96.9%
**Formulation/vehicle:** 2% microemulsion, 1 ml diluted with 3 ml Sandimmune placebo. 0.5 ml of this mixture added to 49.5 ml of 5% glucose solution
**Methods** (unique aspects)**:** none

**Dosing:**

Species/strain: rat/hanIbm Wistar, (b) (4)
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: 6/sex/group=toxicokinetic sampling
Age: 35-42 days
Weight: males, 174-209 g; females141-180 g
Doses in administered units: 0.005, 0.01, 0.05 mg/kg
Route, form, volume, and infusion rate: intravenous, control, 0.05 mg/kg=1ml/kg; 0.005 mg/kg=0.1 ml/kg; 0.01mg/kg=0.2 ml/kg

**Observations and times:**

Clinical signs: daily
Body weights: one week before treatment, 1st day of treatment, twice weekly
Food consumption: week of acclimation, weekly during treatment
Ophthalmoscopy: before treatment, groups 1, 4 during week 2 of treatment
EKG: not performed
Hematology: week 2
Clinical chemistry: week 2
Urinalysis:week 2, for 16 h
Gross pathology: at necropsy
Organs weighed: at necropsy, see table
Histopathology: at necropsy, see table
Toxicokinetics: day 14, 5, 15, 30 min, 1, 4, 14 h after dosing
Other: not performed

**Results:**

| Dose (mg/kg) | 0 | 0.005 | 0.1 | 0.05 |
|---|---|---|---|---|
| HED (mg/kg) | 0 | 0.0003 | 0.0006 | 0.003 |
| Mortality | | All rats survived until terminal sacrifice | | |

| | | | | |
|---|---|---|---|---|
| Clinical signs | | No treatment-related effects were seen | | |
| Body weights | | No treatment-related effects were seen | | |
| Food consumption | | No treatment-related effects were seen | | |
| Ophthalmoscopy | | No treatment-related effects were seen | | |
| Electrocardio-graphy | | Not conducted | | |
| Hematology | | & sl decreased prothrombin times | & sl decreased prothrombin times | & sl decreased leukocyte sl decreased prothrombin times;<br>% sl increased hemoglobin, neutrophils,<br>increased prothrombin time |
| Clinical chemistry | | &sl increased glucose, creatinine | & sl increased glucose, creatinine | & sl increased glucose, creatinine<br>%sl increased cholesterol, Triglycerides |
| Urinalysis | | & sl high pH | | |
| Organ weights | | | % increased lung | % increased lung |
| Gross pathology | | | | 3% dark lung |
| Histopathology | | | | 6% thymus: loss of lymphocytes in outer cortex |
| Toxicokinetics<br>AUC (ng.h/ml)<br>Males<br><br>Females | | | | <br><br>109.4<br><br>96.3 |

**Conclusions:**
Rats receiving intravenous treatment for two weeks showed some signs of immunosuppression (decreased leukocytes, depletion of lymphocytes from thymic cortex) as well as increased cholesterol and triglycerides. A NOAEL was seen at all doses (0.005 – 0.05 mg/kg, HED=0.0003-0.003 mg/kg).

**Study title:** Two-week intravenous infusion toxicity study in minipigs

**Key study findings:** Two-week intravenous treatment with RAD in minipigs did not produce toxic effects. Pharmacologic effects due to the immunosuppressive activity of the drug were seen including increased fibrinogen and cholesterol, and decreased spleen and thymus weights.

**Study no:** Lab no. 19404 (203-075)
**Volume #, and page #:** v. 28, p.1

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

**Date of study initiation:** 3 Sept 1996

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

**Drug, lot #, radiolabel, and % purity:** Y187 0895, na**,**

**Formulation/vehicle:** solution in Sandimmune, 250 mg/5ml diluted in 5% aqueous glucose solution
**Methods** (unique aspects)**:** a Vascular Access Port (VAP) Model GPV with a catheter outlet was surgically implanted over the upper part of the scapula. The jugular vein was catheterized into the vena cava with the reservoir outlet. The access canula was secured by suture and the incision closed. Recovery followed for one week.

**Dosing:**

Species/strain: minipig/Gottingen SPF, (b) (4)

#/sex/group or time point (main study): 3
Satellite groups used for toxicokinetics or recovery: na
Age: 3 months
Weight: 5.6-7.5 kg
Doses in administered units: 0, 0.05, 0.1, 1.0 mg/kg

Route, form, volume, and infusion rate: intravenous, control= 20 ml/kg, 0.05 mg/kg= 1 ml/kg, 0.1 mg/kg= 2ml/kg, 1.0 mg/kg= 20 ml/kg

**Observations and times:**

Clinical signs: daily
Body weights: day of arrival, day of surgery, 1 week before dosing, 1st day of dosing, weekly during treatment
Food consumption: during dosing
Ophthalmoscopy: prior to treatment, final week of treatment
EKG: prior to treatment, 1st day of dosing, last day of dosing
Hematology: prior to treatment, after last dose
Clinical chemistry: prior to treatment, after last dose
Urinalysis: prior to treatment, after last dose
Gross pathology: at necropsy
Organs weighed: at necropsy, see table
Histopathology: at necropsy, see table
Toxicokinetics: 1st dose, 5 min, last dose, 5 min, 24 h
Other: na

| Dose (mg/kg) | 0 | 0.05 | 0.1 | 1.0 |
|---|---|---|---|---|
| HED (mg/kg) | 0 | 0.045 | 0.09 | 0.9 |
| Mortality | | | 1% sacrificed day 13, yellow diarrhea, shivering, vocalization, dehydration | |
| Clinical signs | No treatment-related effects were observed | | | |
| Body weights | | Sl decrease | | |
| Food consumption | No treatment-related effects were observed | | | |
| Ophthalmoscopy | No treatment-related effects were observed | | | |
| Electrocardiography | No treatment-related effects were observed | | | |
| Hematology | | | | Sl increase fibrinogen,%& |
| Clinical chemistry | | 1% increased cholesterol | 1%, 1& increased cholesterol | 2% increased cholesterol |
| Urinalysis | No treatment-related effects were observed | | | |
| Organ weights | | | 1% thymus decreased<br>1% spleen decreased | 3%, 2& thymus decreased<br>2& spleen decreased |
| Gross pathology | No treatment-related effects were observed | | | |

| Histopathology | No treatment-related effects were observed | | | |
|---|---|---|---|---|
| Toxicokinetics<br>Cmax (ng/ml) /dose<br>Cmin (C24) /dose<br>Males | | 5972<br>390 | 4787<br>781 | 481<br>65 |
| Females | | 6968<br>696 | 4163<br>434 | 569<br>73 |

**Summary of individual study findings:**

Two-week intravenous treatment with RAD in minipigs did not produce toxic effects. Pharmacologic effects due to the immunosuppressive activity of the drug were seen including increased fibrinogen and cholesterol, and decreased spleen and thymus weights. A NOAEL was seen at all doses (0.05, 0.1, 1.0 mg/kg).

**Study title:** 4-week toxicity study in cynomolgus monkeys by intravenous infusion followed by a 2-week reversibility period

**Key study findings:** This 4 week intravenous study in cynomolgus monkeys displayed numerous toxicities including expected immunosuppressive effects to the spleen (reduced germinal centers), lymph nodes (reduced germinal centers) and thymus (cortical atrophy, lymphocytolysis) as well as adrenal hypertrophy and inflammation of the heart and digestive tract.

**Study no:** SAZ 523/960379 (203-079)

**Volume #, and page #:** v. 29, p44

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

**Date of study initiation:** 15 Nov 1994

**GLP compliance:** yes

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

Drug, lot #, radiolabel, and % purity: SDZ RAD, batch no. 94901, na, 93.8%

**Formulation/vehicle:** solution in Cremaphor EL and ethanol, in 5% glucose solution

**Methods** (unique aspects)**:**

**Dosing:**

Species/strain: cynomolgus monkeys, (b) (4)

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

Satellite groups used for toxicokinetics or recovery: 2, control, 10 mg/kg

Age: 16-29 months (estimated; this may indicate wild caught monkeys)

Weight: 2.1-3.1 kg

Doses in administered units: 0.3, 1.0, 3.0 mg/kg

Route, form, volume, and infusion rate: intravenous, infusion, 5 ml/kg, 2 h

**Observations and times:**

Clinical signs: daily

Body weights: 1 week prior to dosing, twice weekly during treatment
Food consumption: daily
Ophthalmoscopy: predose, week 4, recovery
EKG: predose, week 4, recovery
Hematology: predose, week 4, recovery
Clinical chemistry: predose, week 4, recovery
Urinalysis: predose, week 4, recovery
Gross pathology: at necropsy
Organs weighed: at necropsy, see table
Histopathology: at necropsy, see table
Toxicokinetics: days 1, 28 @1, 3, 5, 7 and 24h post dosing
Other: na

Results:

| Dose (mg/kg) | 0 | 0.3 | 1.0 | 3.0 |
|---|---|---|---|---|
| HED (mg/kg) | 0 | | | |
| Mortality | All animals survived until terminal sacrifice | | | |
| Clinical signs | | Huddled posture | Huddled posture<br>Tremors during infusion<br>Piloerection<br>Loose stool& | Huddled posture<br>Tremors during infusion<br>Piloerection<br>Loose stool |
| Body weights | | Weight loss | Weight loss, 2/3 % 3/3& | Weight loss-all |
| Food consumption | | | decreased | decreased |
| Ophthalmoscopy | No treatment-related effect | | | |
| Electrocardiography | No treatment-related effect | | | |
| Hematology | | Increased leukocytes-&<br>Increased fibrinogen | Increased leukocytes-all<br>Increased fibrinogen | Increased leukocytes-all<br>Increased fibrinogen<br>Decreased PCV, Hb, RBC |
| Clinical chemistry | | | Increased plasma globulin<br>Decreased albumin Elevated glucose-% | Increased plasma globulin<br>Decreased albumin<br>Elevated glucose<br>Increased CPK<br>Increased triglycerides |
| Urinalysis | | | | & increased protein |
| Organ weights | | Decreased thymus | Decreased thymus | Decreased thymus<br>%decreased testes<br>Increased adrenals |
| Gross pathology | | Epi- and endocardial reddening-2/6<br>Red/pale foci on kidneys-1/6 | Epi- and endocardial reddening-4/6<br>Red/pale foci on kidneys-2/6 | Pale liver-3/6<br>Epi- and endocardial reddening-3/6 Reddening of stomach antrum 2/6<br>Red/pale foci on kidneys-2/6 |
| Histopathology | | Spleen:reduced germinal centers 5/6<br>Lymph nodes: reduced Germinal centers<br>Thymus: cortical atrophy, lymphocytolysis<br>Myocardial vacuolation/degeneration 3/6 | Spleen:reduced germinal centers-5/6<br>Lymph nodes: reduced Germinal centers<br>Thymus: cortical atrophy, lymphocytolysis<br>Myocardial vacuolation/degeneration 4/6<br>Endocarditis 1/6<br>Myocarditis 2/6 | Adrenals: hypertrophy of zona fasiculata and reticularis 6/6<br>Spleen:reduced germinal centers-6/6<br>Lymph nodes: reduced Germinal centers Thymus: cortical atrophy, lymphocytolysis<br>Pancreas: acinar |

| | | | | |
|---|---|---|---|---|
| | | Endocarditis 1/6<br>Myocarditis 1/6<br>Epicarditis 1/6<br>Spleen: absence of germinal centers-6/6<br>Thymus:cortical atrophy 5/6<br>Stomach, duodenum: vacuolated cells/nuclear debris, lamina proprea 4/6<br>Cecum, jejunum, ilium: vacuolated cells/nuclear debris, lamina proprea 6/6<br>Colon: vacuolated cells/nuclear debris, lamina proprea 6/6 | Epicarditis 1/6<br>Spleen: absence of germinal centers-6/6<br>Thymus:cortical atrophy 6/6<br>Stomach, duodenum: vacuolated cells/nuclear debris, lamina proprea 6/6<br>Jejunum, ilium: vacuolated cells/nuclear debris, lamina proprea 6/6<br>Colon: vacuolated cells/nuclear debris, lamina proprea 5/6 | degeneration, vacuolation 3/3&<br>Endocardial hemorrhage1/6<br>Myocardial vacuolation/degeneration 4/6<br>Endocarditis 2/6<br>Myocarditis 3/6<br>Epicarditis 1/6<br>Spleen: absence of germinal centers-6/6<br>Thymus:cortical atrophy 3/6<br>Stomach, duodenum: vacuolated cells/nuclear debris, lamina proprea 6/6<br>Jejunum, ilium: vacuolated cells/nuclear debris, lamina proprea 6/6<br>Colon: vacuolated cells/nuclear debris, lamina proprea 5/6<br>Lymph node, thymus, and digestive tract findings were present in recovery animals |
| Toxicokinetics<br>AUC (ng.h/ml)<br>Males day 1<br>Week 4<br><br>Females day 1<br>Week 4 | | <br><br>644<br>1232<br><br>963<br>1975 | <br><br>4066<br>3388<br><br>4187<br>4155 | <br><br>5326<br>7666<br><br>5072<br>5600 |

**Summary of individual study findings:**

This 4 week intravenous study in cynomolgus monkeys displayed numerous toxicities including expected immunosuppressive effects to the spleen (reduced germinal centers), lymph nodes (reduced germinal centers) and thymus (cortical atrophy, lymphocytolysis) as well as adrenal hypertrophy and inflammation of the heart and digestive tract. The origin of these monkeys is uncertain. Infection, typical of wild-caught monkeys, may complicate any inflammation findings. A NOEL was not determined in this study.

**Study title:** 4-week toxicity study in cynomolgus monkeys by intravenous infusion followed by a 2-week reversibility period

**Key study findings:** Cynomolgus monkeys displayed numerous toxicities including expected immunosuppressive effects to the spleen (reduced germinal centers), lymph nodes (reduced germinal centers) and thymus (cortical atrophy) as well as inflammation of the heart. The uncertain origin of the monkeys (wild caught? first generation colony?) may indicate endogenous viral infection exacerbated by immunosuppression producing myocarditis

**Study no:** SAZ 539/962684 (203-083)

**Volume #, and page #:** v. 30, p1

Conducting laboratory and location: (b) (4)

(b) (4)

**Date of study initiation:** 17 April 1996

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

**Drug, lot #, radiolabel, and % purity:** SDZ RAD, Y187 0895
**Formulation/vehicle:** solution in Cremaphor EL and ethanol, in 5% glucose solution
**Methods** (unique aspects)**:**

**Dosing:**
**Species/strain: cynomolgus** monkeys (b) (4)
**#/sex/group or time point (main study):** 3
**Satellite groups used for toxicokinetics or recovery:** 2 (control, 0.1 mg/kg)
**Age:** 15-21 months (estimated; this may indicate wild caught monkeys)
**Weight:** 2.0-3.1 kg
**Doses** in administered units: 0.05, 0.1 mg/kg
**Route, form, volume, and infusion rate:** intravenous, infusion, 5 ml/kg, 2 h

**Observations and times:**
**Clinical signs:** daily
**Body weights:** weekly during treatment, recovery
**Food consumption:** weekly during treatment, recovery
**Ophthalmoscopy:** prior to dosing, treatment week 4, recovery week 2
**EKG**: prior to dosing, treatment week 4, recovery week 2
**Hematology**: prior to dosing, treatment week 4, recovery week 2
**Clinical chemistry**: prior to dosing, treatment week 4, recovery week 2
**Urinalysis:** prior to dosing, treatment week 4, recovery week 2 (16 h collection period)
**Gross pathology**: at necropsy
**Organs weighed**: at necropsy, see table
**Histopathology:** at necropsy, see table
**Toxicokinetics:** days 1, 28; 0, 1, 3, 6, 22 h following infusion
**Other:** na

Results:

| Dose (mg/kg) | 0 | 0.05 | 0.1 | Recovery (control, 0.1 mg/kg) |
|---|---|---|---|---|
| HED (mg/kg) | 0 | | | |
| Mortality | All animals survived until terminal sacrifice | | | |
| Clinical signs | | Huddled posture | Huddled posture | |
| Body weights | | | Decrease 4/5%, 3/5 & | |
| Food consumption | | Sl decrease | | |
| Ophthalmoscopy | No treatment-related effect was observed | | | |
| Electrocardiography | No treatment-related effect was observed | | | |
| Hematology | | decreased PT, %, week 4<br>increased fibrinogen, week 4 | 5/5 & increased leukocytes, week 4<br>decreased PT, % | |

| | | | | |
|---|---|---|---|---|
| | | | increased fibrinogen, week 4 | |
| Clinical chemistry | | Decreased inorganic phosphorus, week 4<br>increased calcium, week 4 | Decreased inorganic phosphorus, week 4<br>Decreased lipase, week 4<br>increased calcium, week 4 | |
| Urinalysis | | | Increased urinary protein, 1 &, week 4 | |
| Organ weights | | Thymus-decreased<br>Uterus sl decreased | thymus -decreased<br>Uterus sl decreased | |
| Gross pathology | | Heart-Red foci, epi- and endocardial area- 3/3 % | Red foci, epi- and endocardial area , 1%, 3&<br>kidney -red foci, 1%, 1&<br>liver-white area, 1& | Red area ventricle 1% |
| Histopathology<br>Heart-myocarditis, ventricles | Heart-myocarditis, ventricles 2/3%<br>Spleen-vacuolated cells in red pulp, 2/3%, 2/3 & | Heart- myocarditis, ventricles 2/3%<br>Lymph nodes-absence of germinal centers 6/6<br>Spleen-absence of germinal centers 5/6<br>vacuolated cells in red pulp, 3/3%, 2/3 &<br>Thymus-cortical atrophy 1/3 % | Heart-myocarditis, ventricles 3/3%, 2/3&<br>Auricles 1/3%<br>Lymph nodes-absence of germinal centers 6/6<br>Spleen-absence of germinal centers 6/6<br>vacuolated cells in red pulp, 2/3%, 2/3 &<br>Thymus-cortical atrophy 2/3%, 3/3 & | Heart- myocarditis<br>Control, ½<br>0.1 mg/kg, 2/2<br>lymph nodes- absence of germinal centers<br>0.1 mg/kg, ½<br>thymic atrophy-<br>control, ½<br>0.1 mg/kg, 2/2 |
| Toxicokinetics<br>AUC<br>(ng.h/ml)/dose<br>Males week 4<br><br>Females week 4 | | <br><br>6552<br><br>8761 | <br><br>10607<br><br>11795 | |

**Summary of individual study findings:**

This 4 week intravenous study in cynomolgus monkeys displayed numerous toxicities including expected immunosuppressive effects to the spleen (reduced germinal centers), lymph nodes (reduced germinal centers) and thymus (cortical atrophy) as well as inflammation of the heart. The uncertain origin of the monkeys (wild caught? first generation colony?) may indicate endogenous viral infection exacerbated by immunosuppression producing myocarditis. A NOEL was not determined in this study.

**Study title:** RAD 001, rapamycin and Neoral: 2-week exploratory study in rats

**Summary of individual study findings:**

In this exploratory study, comparisons of RAD, rapamycin, Neoral and combinations were examined in rats. Typical immunosuppressive effects were seen in all groups including thymic, splenic and lymph node effects. Combinations produced more marked effects on immunosuppression, myocardiopathy and renal effects. Cholesterol was increased in groups receiving either RAD or rapamycin.

**Study title:** A 4-week oral (gavage) exploratory combination study in rats
Non-glp exploratory study not reviewed

**Study title:** Single dose oral toxicokinetic study in rats
Non glp exploratory study not reviewed

**Study title:** 4-week oral toxicity in rats

**Key study findings:** The predicted immunosuppressive effects on spleen, thymus and lymph nodes were observed in all treated groups. Body weight was reduced in males receiving 10 mg/kg FTY720 (an experimental immunosuppressant under development by the sponsor). The lungs were the site of smooth muscle hypertrophy (FTY effect) while infiltrates consisting of macrophages and granulocytes occurred in RAD treated groups. Arterial/cardiac lesions previously seen in prior FTY studies occurred in this study at lower doses.

**Study no:** 991036 (BS-570)
**Volume #, and page #:** v. 53, p. 1
**Conducting laboratory and location:** Novartis Pharma AG
Basel, Switzerland
**Date of study initiation:** 21 June 1999
**GLP compliance:** yes
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD, X176 1297, na, 92.2%; FTY-720, 98905, na, 100

**Formulation/vehicle: solution/water**
**Methods** (unique aspects)**:**

**Dosing:**
Species/strain: rats/Crl:Wist Han, (b) (4)
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: 6
Age: about 8 weeks
Weight: %181-245 g, &135-190 g
Doses in administered units: FTY720/RAD: vehicle/vehicle, 0.3/0.5, 10/20, 10/1.5, 10/vehicle mg/kg

Route, form, volume, and infusion rate: oral gavage, total 10 ml/kg, na

**Observations and times:**
Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: prior to study, end of treatment
EKG: not performed
Hematology: end of treatment, end of recovery
Clinical chemistry: end of treatment, end of recovery
Urinalysis: end of treatment, end of recovery
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: days 1, 2, last week of treatment
Other: na

Results:

| Dose (mg/kg) FTY/RAD | 0/0 | 0.3/0.5 | 10/0.5 | 10/1.5 | 10/0 |
|---|---|---|---|---|---|
| HED (mg/kg) | 0 | | | | |
| **Mortality** | | | | 1& sacrificed moribund day | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 18 | |
| **Clinical signs** | | | | piloerection | piloerection |
| **Body weights** | | | % decreased | % decreased | % decreased |
| **Food consumption** | | | % decreased-all study & decreased wk 1, 2 | % decreased-all study & decreased wk 1, 2 | % decreased-all study & decreased wk 1, 2 |
| **Ophthalmoscopy** | No treatment-related effects | | | | |
| **EKG** | Not performed | | | | |
| **Hematology**<br>Lymphopenia<br>Neutrophils<br>RBC<br>Hemoglobin<br>Hematocrit | | all<br>inc, all<br>inc, &<br>inc, &<br>inc, & | all<br>inc, all<br>inc, &<br>inc, &<br>inc, & | all<br>inc, all<br><br>inc, %, &<br>inc, %, & | all<br>inc, all<br><br>inc, % |
| **Clinical chemistry**<br>Bilirubin<br>Serum urea<br>Cholesterol<br>Triglycerides<br>$-, (-globulin<br>Glucose<br>Sodium<br>Potassium<br>Phosphate | | Bilirubin-dec, &<br>Cholesterol inc, %<br>$-, (-globulin-dec, all | Serum urea-inc<br>Bilirubin-dec, &<br>Cholesterol inc, %<br>Triglycerides inc, %<br>Sodium-inc, %<br><br>$-, (-globulin-dec, all | Serum urea-inc<br>Bilirubin-dec, &<br>Cholesterol inc, %<br>Triglycerides inc, %<br>Glucose dec, %<br>Phosphate dec, %<br>Sodium-inc, %<br>Potassium-inc, %<br>$-, (-globulin-dec, all | $-, (-globulin-dec, all |
| **Urinalysis** | | | | | |
| | | | % Sm spleen Oliguria, ketones | % Oliguria, ketones | |
| | | | | | |
| **Organ weights** | | Spleen sl inc, all<br>Prostate sl dec<br>Ovary sl dec<br>Uterus sl dec | Lung inc, all<br>Spleen sl inc, all<br>Prostate sl dec<br>Ovary sl dec<br>Uterus sl dec | Lung inc, all Spleen sl inc, both<br>Prostate sl dec<br>Ovary sl dec<br>Uterus sl dec<br>Pituitary sl dec, both | Spleen sl inc, all |
| **Gross pathology** | | | Sm spleen | Sm spleen<br>Sm thymus<br>Sm prostate | Sm spleen |
| **Histopathology** | | Lungs:granulocyte infiltration<br>Thymus: cortical atrophy, both<br>Spleen: atrophy white pulp<br>Lymph nodes: Atrophy | arteries; fibrinoid necrosis, periarteritis, arteriopathy, %<br>Heart: necrotizing arteritis, arteriopathy, 2/10 %<br>Lungs: sm muscle hypertrophy, both<br>Interstitial collagenization, both | arteries; fibrinoid necrosis, periarteritis, arteriopathy, Both<br>Heart: necrotizing arteritis, arteriopathy, 9/10 %, 6/10 &<br>Lungs: sm muscle | Lungs: sm muscle hypertrophy, both<br>Interstitial collagenization, both<br>Alveolar macrophage infiltration |

| | | | | | |
|---|---|---|---|---|---|
| | | Uterus: atrophy & | granulocyte infiltration<br>kidney: necrotizing arteritis/ateriopathy<br>Thymus: cortical atrophy, both<br>Spleen: atrophy white pulp<br>Lymph nodes: Atrophy<br>Prostate/seminal vesicles: decreased secretion<br>Uterus: atrophy & | hypertrophy, both<br>Interstitial collagenization, both granulocyte infiltration<br>kidney: necrotizing arteritis/ateriopathy<br>Thymus: cortical atrophy, both<br>Spleen: atrophy white pulp<br>Lymph nodes: Atrophy<br>Prostate/seminal vesicles: decreased secretion<br>Mammary gland: Decreased development, &<br>Uterus: atrophy &<br>Pituitary: atrophy, pars intermedia | granulocyte infiltration<br>Spleen: atrophy white pulp<br>Lymph nodes: Atrophy<br>Prostate/seminal vesicles: decreased secretion |
| **Toxicokinetics**<br>RAD values were available only for 10/1.5 mg/kg group; this renders comparisons difficult for the effect of RAD on FTY720 concentrations without comparators | | | | | |

**Conclusions:**

The predicted immunosuppressive effects on spleen, thymus and lymph nodes were observed in all treated groups. Body weight was reduced in males receiving 10 mg/kg FTY720. The lungs were the site of smooth muscle hypertrophy (FTY effect) while infiltrates consisting of macrophages and granulocytes occurred in RAD treated groups. Arterial/cardiac lesions previously seen in prior FTY studies occurred in this study at lower doses. A NOEL was not seen in this study.

**Study title:** FTY720 in combination with RAD001: 4-week oral (gavage) toxicity in male monkeys with a 4-week recovery period

**Key study findings:** This combination study demonstrated toxicities in cynomolgus monkeys previous seen including myocardial degeneration. As FTY increased RAD exposure over the course of treatment, this may have been exacerbated. Pancreatic toxicity (acinar degeneration) was also previously seen in earlier studies. Malarial infection of the monkeys complicates study interpretation. The importance of pathogen-free animals for immunosuppression studies cannot be overemphasized. Clinical chemistry effects included decreased total protein which may be related to decreased food consumption and elimination difficulties. Decreased serum calcium and potassium may also be related. Decreased serum phosphorous was also previously seen. The expected lymphoid effects were seen in all treatment groups

Study no: 997062 **(US-75415)**

**Volume #, and page #:** v. 56, p.1
**Conducting laboratory and location:** Novartis Pharmaceutical Corporation,
East Hanover, New Jersey
**Date of study initiation:** 14 June 1999
**GLP compliance: yes**
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity: FTY:** batch no. 98905, RAD batch no. X176 1297; na; FTY, 100%, RAD, 92.2%
**Formulation/vehicle:** FTY: purified water; RAD: 1.35 mg/ml HPMC and 0.15 mg/kg lactose in purified water

**Methods** (unique aspects)**:**
**Dosing:**
Species/strain: cynomolgus monkey/ (b) (4)
#/sex/group or time point (main study): 3 %
Satellite groups used for toxicokinetics or recovery: 2 %
Age: 3-4.5 yr
Weight: 2.4-4.6 kg
Doses in administered units: (FTY/RAD): control, 3.0/0. 0/1.5, 0.5/1.5, 3.0/1.5 mg/kg
Route, form, volume, and infusion rate: oral gavage, 2ml/kg each, FTY and RAD + 3 ml water flush

**Observations and times:**
Clinical signs: daily
Body weights:weekly
Food consumption: daily
Ophthalmoscopy: pretest, week 5
EKG: pretest, week 4
Hematology: pretest, week 4, recovery week 4
Clinical chemistry: pretest, week 4, recovery week 4
Urinalysis: pretest, week 4, recovery week 4
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics:post dosing, days 1, 2, during week 4 @ 0, 1, 2, 4, 8, 12 and 24 h postdose
Other: na

Results:

| Dose FTY/RAD (mg/kg) | 0 | | 3.0/0 | | 0/1.5 | | 0.5/1.5 | | 3.0/1.5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| HED (mg/kg) | 0 | | | | | | | | | |
| Sex ( /sex) | m | f | m | f | m | f | M | f | m | f |
| Mortality | All animals survived until terminal sacrifice | | | | | | | | | |
| Clinical signs | | | Soft feces<br>Reduced feces | | Soft feces<br>diarrhea | | Reduced feces | | Diarrhea<br>Reduced feces | |
| Body weights | | | No treatment-related effect was seen | | | | | | | |
| Food consumption | | | Sl Reduced | | | | | | | |
| Ophthalmoscopy | | | No treatment-related effect was seen | | | | | | | |
| EKG | | | No treatment-related effect was seen | | | | | | | |
| Hematology | | | Total WBC dec<br>Lymphocyte dec<br>Malaria parasites in RBC | | | | | | | |
| Clinical chemistry | | | Total protein dec<br>Calcium dec | | Calcium dec | | Total protein dec<br>Calcium dec | | Total protein dec<br>calcium dec<br>potassium dec | |
| Urinalysis | | | No treatment-related effect was seen | | | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Organ weights | | Thymus dec<br>Spleen dec<br>Testes inc | Thymus dec<br>Testes dec | Thymus dec<br>Spleen dec<br>Testes dec | Thymus dec<br>Testes dec |
| Gross and Histopathology | Malarial pigment , macrophages, Kupfer cells | Lymphoid atrophy (lymph nodes, thymus, spleen)<br><br>Malarial pigment , macrophages, Kupfer cells | Pancreas:<br>dec granules 2/3<br>diffuse acinar atrophy 1/3<br>Lymphoid atrophy (lymph nodes, thymus, spleen)<br>Malarial pigment , macrophages, Kupfer cells | Sm thymus<br>Heart: myofiber degeneration 1/3<br>Lymphoid atrophy (lymph nodes, thymus, spleen)<br>Malarial pigment , macrophages, Kupfer cells | Sm thymus<br>Heart: myofiber degeneration 3/3<br>Pancreas: dec granules 2/3<br>Lymphoid atrophy (lymph nodes, thymus, spleen)<br>Malarial pigment , macrophages, Kupfer cells |

| | | | | |
|---|---|---|---|---|
| Toxicokinetics<br>AUC (ng.h/ml) | | | | |
| FTY day 1-2 | 832 | Na | 185 | 949 |
| Week 4 | 3367 | Na | 610 | 2456 |
| RAD day 1-2 | na | 385 | 840 | 1023 |
| Week 4 | na | 686 | 1201 | 1425 |

Conclusions: This combination study demonstrated toxicities in cynomolgus monkeys previous seen including myocardial degeneration. As FTY increased RAD exposure over the course of treatment, this may have been exacerbated. Pancreatic toxicity (acinar degeneration) was also previously seen in earlier studies. Malarial infection complicates study interpretation. The importance of pathogen-free animals for immunosuppression studies cannot be overemphasized. Clinical chemistry effects included decreased total protein which may be related to decreased food consumption and elimination difficulties. Decreased serum calcium and potassium may also be related. Decreased serum phosphorous was also previously seen. The expected lymphoid effects were seen in all treatment groups. A NOEL was not seen in this study.

**Study title:** RAD001 and methotrexate: 4-week oral combination toxicity study in rats

**Key study findings:** This 4-week combination study of RAD and MTX in rats demonstrated toxicity to the heart, thymus, and lymph nodes, all findings seen previously (RAD, heart, lymph nodes, thymus; MTX, thymus). The combinations at the doses used, did not appear to exacerbate these toxicities, nor were pharmacokinetic effects seen due to the combination.

**Study no:** 0110059
**Volume #, and page #:** v. 56, p. 1
**Conducting laboratory and location:** Novartis Pharma AG,
Basel, Switzerland
**Date of study initiation:** 7 May 2001
**GLP compliance: yes**
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD, lot no. 1010001004, na, 100.3%; methotrexate, lot no. 129H0842, na, 99.3%
**Formulation/vehicle:** RAD; millipore water; methotrexate (MTX), millipure water

**Methods** (unique aspects)**:**

**Dosing:**

Species/strain: rats/ Crl:WI (GLX/BRL/HAN) IGS BR, (b) (4)
#/sex/group or time point (main study): 5
Satellite groups used for toxicokinetics or recovery: 5
Age: 8 weeks
Weight: 150.2-264.2 g
Doses in administered units: RAD/MTX: 0, 0.75/0, 0/0.4, 0.5/0.2, 0.75/0.4 mg/kg
Route, form, volume, and infusion rate: oral gavage, 5ml/kg/drug or vehicle

**Observations and times:**

Clinical signs: daily
Body weights: daily-treatment, weekly, recovery
Food consumption: daily-treatment, weekly, recovery
Ophthalmoscopy: pretest, day 26, end of recovery
EKG: na
Hematology: daily-treatment, weekly, recovery
Clinical chemistry: daily-treatment, weekly, recovery
Urinalysis: daily-treatment, weekly, recovery
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: days 1, 30 @ 1, 2, 4, 7 and 24 h after dosing
Other: na

Results:

| Dose (mg/kg)RAD/MTX | 0 | 0.75/0 | 0/0.4 | 0.5/0.2 | 0.75/0.4 |
|---|---|---|---|---|---|
| HED (mg/kg) | | | | | |
| Sex ( /sex) | | | | | |
| Mortality | 1 & after blood sampling | All animals survived until terminal sacrifice | | | |
| Clinical signs | | | | | |
| Body weights | | Dec, % | | Dec, % | Dec, % |
| Food consumption | No treatment-related effects were seen | | | | |
| Ophthalmoscopy | No treatment-related effects were seen | | | | |
| EKG | Not conducted | | | | |
| Hematology | | RBC-inc<br>Hemoglobin-inc<br>Hematocrit-inc<br>Neutrophil-inc,%<br>Lymphocyte-inc, % | | RBC-inc<br>Hemoglobin-inc<br>Hematocrit-inc<br>Neutrophil-inc,%<br>Lymphocyte-inc,% | RBC-inc<br>Hemoglobin-inc<br>Hematocrit-inc<br>Neutrophil-inc,%<br>Leukocyte-inc, % |
| Clinical chemistry | | Creatine kinase-dec<br>Cholesterol-inc, %<br>Protein-inc, &<br>Albumin-inc, & | | Creatine kinase-dec<br>Cholinesterase-inc, &<br>Protein-inc, & | Creatine kinase-dec<br>Cholesterol-inc, %<br>Cholinesterase-inc, &<br>Protein-inc, &<br>Albumin-inc, & |
| Urinalysis | No treatment-related effects were seen | | | | |
| Organ weights | | Thymus-dec | | Thymus-dec | Thymus-dec |

| | | | | | Adrenals-inc, % |
|---|---|---|---|---|---|
| Gross and Histopathology | | Heart-myocardial degeneration, %<br>Thymus: lymphocytolysis<br>Mesenteric lymph node: germinal center-dec | Thymus: lymphocytolysis | Thymus: lymphocytolysis<br>lymph node: germinal center-dec | Heart-myocardial degeneration, %<br>Thymus: lymphocytolysis<br>lymph node: germinal center-dec |
| Toxicokinetics<br>Day 30<br>AUC (ng.h/ml)<br>Males<br>RAD<br>MTX<br><br>Females<br>RAD<br>MTX | | <br><br><br><br>26.1<br>38.4<br><br><br><br>22.4<br>38.4 | | <br><br><br><br>14.6<br>45.9<br><br><br><br>23.9<br>39.6 | <br><br><br><br>23.0<br>59.8<br><br><br><br>18.2<br>32.4 |

Conclusions: This 4-week combination study of RAD and MTX in rats demonstrated toxicity to the heart, thymus, and lymph nodes, all findings seen previously (RAD, heart, lymph nodes, thymus; MTX, thymus). The combinations at the doses used, did not appear to exacerbate these toxicities, nor were pharmacokinetic effects seen due to the combination. As effects were seen in all treatment groups, a NOEL was not seen.

**Study title:** Comparative toxicity study in HanIbm Wistar rats with batches differing in by-product content

**Key study findings:** The immunosuppressive pharmacologic action of RAD was evident in the thymus, spleen, and lymph nodes. Immunosuppression may have allowed infection to result in myocarditis, otherwise it may be due to a direct toxic effect. Effects on the reproductive organs in both males (testes, prostate, epididymides, seminal vesicles) and females (uterus) appear to be a toxic effect on endocrine hormones. Lung eosinophilic deposits from macrophages may be due to immunosuppression or to a direct phospholipidosis effect. The eye effect of swelling/disruption of fibers in anterior cortex of the lens is similar to previously seen lens toxicity. The stressed RAD, presumed to contain the impurity (b) (4) (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.

**Study no:** 96/SPM091/0532 (203-076)

**Volume #, and page #:** v. 65, p. 1

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

**Date of study initiation: GLP compliance:** yes

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

**Drug, lot #, radiolabel, and % purity:** RAD, Y003 0196, 90.5%; Y254 1295, 82.2%; Y174 0895, 82.2%

Formulation/vehicle: 2% microemulsion diluted with 5% glucose solution

**Methods** (unique aspects)**:**

**Dosing:**

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

#/sex/group or time point (main study): 10

Satellite groups used for toxicokinetics or recovery: 7

Age: 42 d

Weight: 107-168 g
Doses in administered units: RAD: 0.25, 5 mg/kg; stressed RAD 0.25, 5 mg/kg
Route, form, volume, and infusion rate: oral gavage, total 5 ml/kg, na

**Observations and times:**

Clinical signs: daily
Body weights: twice weekly
Food consumption: weekly
Ophthalmoscopy: prior to study, day 13
EKG: not performed
Hematology: day 14
Clinical chemistry: day 14
Urinalysis: day 10
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: day 14, 0.5, 1, 3, 7, 24 h after dosing
Other: na

| Dose (mg/kg) | 0 | Vehicle stressed | 0.25 | 5.0 | 0.25 stressed | 5.0 stressed |
|---|---|---|---|---|---|---|
| HED (mg/kg) | 0 | | | | | |
| **Mortality** | All animals survived until sacrifice | | | | | |
| **Clinical signs** | | No treatment-related signs were seen | | | | |
| **Body weights** | | | | Dec gain | | Dec gain |
| **Food consumption** | | | | Dec | | dec |
| **Ophthalmoscopy** | | No treatment-related signs were seen | | | | |
| **EKG** | Not performed | | | | | |
| **Hematology** | | | PCV: inc<br>HgB: inc<br>RBC: inc□□□ | PCV: inc<br>HgB: inc<br>RBC: inc□lymphocytes: dec□□□□□□ | PCV: inc<br>HgB: inc<br>RBC: inc□□□ | |
| **Clinical chemistry** | | | Triglycerides: inc<br>Cholesterol: inc<br>Sodium: inc, females | Amylase: inc<br>Alk phos: dec<br>Triglycerides: inc<br>Cholesterol: inc<br>Sodium: inc<br>Phosphorus: dec<br>Albumin: dec, males | Triglycerides: inc<br>Cholesterol: inc | Amylase: inc<br>Alk phos: dec<br>Triglycerides: inc<br>Cholesterol: inc<br>Sodium: inc, females<br>Phosphorus: dec<br>Albumin:dec |
| **Urinalysis** | | No treatment-related signs were seen | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Organ weights** | | | | Thymus:dec<br>Spleen: dec<br>Lung: inc<br>Males: dec epididymides, prostate, seminal vesicle, testes<br>Females: dec uterus, cervix, ovary | | Thymus: dec<br>Spleen: dec<br>Lung: inc<br>Males: dec epididymides, prostate, seminal vesicle, testes<br>Females: dec uterus, cervix, ovary, pituitary |
| **Gross pathology** | | No treatment-related signs were seen | | | | |
| **Histopathology** | | | | Thymus: medullary atrophy 9/10 m 10/10 f<br>Mandibular lymph nodes: dec lymphoid activity<br>Heart: myocarditis, 2 m<br>Spleen: extramedullary hematopoesis, 7 f<br>Lungs; eosinophilic deposits, 6f<br>Eyes: swelling/disruption Fibers in anterior cortex of lens, 9m, 10 f<br>Uterus: atrophy, 3f<br>Seminal vesicles: dec secretion, 8m | Uterus: atrophy, 3f | Thymus: medullary atrophy; 10/10 m, 8/10 f<br>Mandibular lymph nodes: dec lymphoid activity<br>Heart: myocarditis, 4 m<br>Spleen: extramedullary hematopoiesis, 9 f<br>Lungs; eosinophilic deposits 6 m, 4f<br>Eyes: swelling/disruption Fibers in anterior cortex of lens, 5m, 10 f<br>Uterus: atrophy, 5f<br>Seminal vesicles: dec secretion, 8m<br>Testes: loss of germ layer, seminiferous tubules, 2m |
| **Toxicokinetics AUC h*ng/ml** | | | | Males=276<br>Females=368 | | |

**Conclusions:** The immunosuppressive pharmacologic action of RAD was evident in the thymus, spleen, and lymph nodes. Immunosuppression may have allowed infection to result in myocarditis, otherwise it may be due to a direct toxic effect. Effects on the reproductive organs in both males (testes, prostate, epididymides, seminal vesicles) and females (uterus) appear to be a toxic effect on endocrine hormones. Lung eosinophilic deposits from macrophages may be due to immunosuppression or to a direct phospholipidosis effect. The eye effect of swelling/disruption of fibers in anterior cortex of the lens is similar to previously seen lens toxicity. The stressed RAD, presumed to contain the impurity (b) (4) (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. The lack of quantified (b) (4) and lack of pharmacokinetic data for the stressed RAD groups leaves the issue of stressed RAD open to further studies.

**Study title:** A comparative 2-week oral (gavage) toxicity study in the rat with two different batches

**Key study findings:** Both batches of RAD exerted expected immunosuppressive effects on thymus, spleen lymph nodes. Shared toxicities included male reproductive (testes, prostate gland, seminal vesicles; mammary gland) and female reproductive (pituitary, uterus, vagina) showing toxicity of 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. The toxicity profiles of each of these batches are nearly identical.

**Study no:** (b) project 634678 (203-077)
**Volume #, and page #:** v. 66, p.1
**Conducting laboratory and location:** Novartis Pharm AG
Preclinical Safety: Toxicology/Pathology
WS-2881
P.O. Box
CH-4002 Basel
Switzerland
**Date of study initiation:** 29 Aug 1996
**GLP compliance:** yes
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD, batch nos. X081 0596, X096 0796, na
**Formulation/vehicle:** solution/water

**Methods** (unique aspects)**:**
**Dosing:**
Species/strain: rat/HanIbm: WIST (SPF), (b) (4)
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: none
Age: 9 weeks
Weight: males, 203-298 g; females, 132-200 g
Doses in administered units: batch no. X081 0596, 1.5, 15.0 mg/kg; batch no. X096 0796, 1.5, 15.0 mg/kg
Route, form, volume, and infusion rate: oral gavage, 5 ml/kg, na

**Observations and times:**
Clinical signs: daily
Body weights: twice pretest, twice weekly during treatment
Food consumption: weekly
Ophthalmoscopy: day 13
EKG: not performed
Hematology: not performed
Clinical chemistry: not performed
Urinalysis: not performed
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: not performed
Other: na

Results:

| Dose (mg/kg) | 0 | batch no. X081 0596, 1.5 mg/kg | batch no. X081 0596, 15.0 mg/kg | batch no. X096 0796, 1.5 mg/kg | batch no. X096 0796, 15.0 mg/kg |
|---|---|---|---|---|---|
| HED (mg/kg) | | 0.25 | 2.5 | 0.25 | 2.5 |
| Mortality | | All animals survived until necropsy | | | |
| Clinical signs | | No treatment-related signs were seen | | | |
| Body weights | | Dec wt gain, m | Dec, m<br>Dec wt gain, f | Dec wt gain, m | Dec, m<br>Dec wt gain, f |
| Food consumption | | Dec, m | dec | Dec, m | dec |

| | | | | | |
|---|---|---|---|---|---|
| Ophthalmoscopy | | No treatment-related signs were seen | | | |
| EKG | | Not performed | | | |
| Hematology | | Not performed | | | |
| Clinical chemistry | | Not performed | | | |
| Urinalysis | | Not performed | | | |
| Organ weights | | Pituitary, dec, f<br>Thymus, dec<br>Spleen, dec, f<br>Ovaries, dec, f<br>Uterus, dec, f | Brain; dec<br>Pituitary, dec, f<br>Thymus, dec<br>Spleen, dec<br>Prostate, dec, m<br>Ovaries, dec, f<br>Uterus, dec, f | Pituitary, dec, f<br>Thymus, dec<br>Spleen, dec, f<br>Ovaries, dec, f<br>Uterus, dec, f | Brain; dec<br>Pituitary, dec, f<br>Thymus, dec<br>Spleen, dec<br>Prostate, dec, m<br>Ovaries, dec, f<br>Uterus, dec, f |
| Gross pathology | | | Seminal vesicles reduced size, m | | Seminal vesicles reduced size, m |
| Histopathology | Heart: focal/multifocal Myocardial degeneration<br>Adrenals: microvesicular vacuolation | Brain: distension of lateral ventricles<br>Heart: focal/multifocal Myocardial degeneration<br>Testes: tubular atrophy, 2m<br>Aspermia, 1m<br>Prostate: diffuse atrophy<br>Seminal vesicles: diffuse atrophy, 2m<br>Uterus: atrophy, f<br>Vagina:atrophy, f<br>Adrenals: microvesicular vacuolation, cortex z vesiculata<br>Spleen: lymphoid depletion<br>Bone marrow: atrophy/hypocellularity<br>Thymus: cortical, medullary atrophy<br>Lymph nodes: absence germinal centers | Brain: distension of lateral ventricles<br>Heart: focal/multifocal Myocardial degeneration<br>Testes: tubular atrophy, 2m<br>Multinucleated giant cell, 1m<br>Oligospermia, 1m<br>Prostate: diffuse atrophy<br>Seminal vesicles: diffuse atrophy, 10m<br>Uterus: atrophy, f<br>Vagina:atrophy, f<br>Adrenals: microvesicular vacuolation cortex z vesiculata<br>Spleen: lymphoid depletion, dec extramedullary hematopoiesis<br>Bone marrow: atrophy/hypocellularity Thymus: cortical, medullary atrophy<br>Lymph nodes: | Brain: distension of lateral ventricles<br>Heart: focal/multifocal Myocardial degeneration<br>Prostate: diffuse atrophy<br>Seminal vesicles: diffuse atrophy, 1m<br>Uterus: atrophy, f<br>Vagina:atrophy, f<br>Adrenals: microvesicular vacuolation cortex z vesiculata<br>Spleen: lymphoid depletion<br>Bone marrow: atrophy/hypocellularity<br>Thymus: cortical, medullary atrophy<br>Lymph nodes: absence germinal centers<br>Mammary gland; lobuloalveolar atrophy, 4m | Brain: distension of lateral ventricles<br>Heart: focal/multifocal Myocardial degeneration<br>Prostate: diffuse atrophy<br>Seminal vesicles: diffuse atrophy, 10 m<br>Uterus: atrophy, f<br>Vagina:atrophy, f<br>Adrenals: microvesicular vacuolation cortex z vesiculata<br>Spleen: lymphoid depletion, dec extramedullary hematopoiesis<br>Bone marrow: atrophy/hypocellularity<br>Thymus: cortical, medullary atrophy<br>Lymph nodes: absence germinal centers<br>Mammary gland; lobuloalveolar atrophy, 5m |

| | | | | | |
|---|---|---|---|---|---|
| | | | absence germinal centers<br>Mammary gland; lobuloalveolar atrophy, 4m<br>Eyes; swelling of cortical lens fibers | | Eyes; swelling of cortical lens fibers |
| Toxicokinetics | | Not performed | | | |

Conclusions: Both batches of RAD exerted expected immunosuppressive effects on thymus, spleen lymph nodes. Shared toxicities included male reproductive (testes, prostate gland, seminal vesicles; mammary gland) and female reproductive (pituitary, uterus, vagina) showing toxicity of 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. The toxicity profiles of each of these batches are nearly identical. No NOEL was seen in this study.

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

**Key study findings:** Both batches of RAD exerted expected immunosuppressive effects on thymus, spleen and lymph nodes. Other toxicities included male reproductive (testes, prostate gland, seminal vesicles, epididymides; mammary gland) and female reproductive (pituitary, ovaries, uterus, vagina) showed toxicity of 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 no:** 991094
**Volume #, and page #:** v. 66, p. 352
**Conducting laboratory and location:** Novartis Pharm AG
Preclinical Safety: Toxicology/Pathology
WS-2881
P.O. Box
CH-4002 Basel
Switzerland
**Date of study initiation:** 10 Nov 1999
**GLP compliance:** yes
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD, batch no. X033 0199 (batch 1), na, 92.9%; batch no. X153 1098 (batch 2), na, 96.9%
**Formulation/vehicle:** dispersion of RAD in demineralized water

**Methods** (unique aspects)**:**
**Dosing:**
Species/strain: rats? Crl:Wist Han, (b) (4)
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: none
Age: 7 weeks
Weight: 112-184 g
Doses in administered units: 0, 1.5(batch 1), 15 (day1)-5 (batch 1), 15 (day1)-5 (batch 2) mg/kg
Route, form, volume, and infusion rate: oral gavage, 10 ml/kg, na

**Observations and times:**

Clinical signs: daily
Body weights: daily
Food consumption: weekly
Ophthalmoscopy: pretest, end of treatment
EKG: not performed
Hematology: day 23
Clinical chemistry: day 23
Urinalysis: day 23
Gross pathology: at necropsy
Organs weighed: at necropsy
Histopathology: at necropsy, see table
Toxicokinetics: not performed
Other: na

Results:

| Dose (mg/kg) | 0 | 1.5 mg/kg (batch 1) | 15 (day1), 5 mg.kg batch 1 | 15 (day1)-5 batch 2 |
|---|---|---|---|---|
| HED (mg/kg) | | 0.25 | | 25.0, 0.85 |
| Mortality | All rats survived until necropsy | | | |
| Clinical signs | | No treatment related signs were seen | | |
| Body weights | | Dec body weight gain | Dec body weight gain | Dec body weight gain |
| Food consumption | | Dec | Dec | Dec |
| Ophthalmoscopy | | No treatment related signs were seen | | |
| EKG | Not performed | | | |
| Hematology | | RBCs: inc<br>Hematocrit: inc<br>Hemoglobin:inc<br>Platelets: dec m<br>Lymphocytes: dec<br>Neutrophils: inc | RBCs: inc<br>Hematocrit: inc<br>Hemoglobin:inc<br>Platelets: dec<br>Lymphocytes: dec<br>Neutrophils: inc | RBCs: inc<br>Hematocrit: inc<br>Hemoglobin:inc<br>Platelets: dec<br>Lymphocytes: dec<br>Neutrophils: inc |
| Clinical chemistry | | Cholesterol: inc, m<br>Triglycerides: inc, m | Cholesterol: inc, m<br>Triglycerides: inc, m | Cholesterol: inc, m<br>Triglycerides: inc, m |
| Urinalysis | | Leukocytes observed in urine of males | | |
| Organ weights | | Thymus:dec<br>Pituitary:dec | Thymus:dec<br>Spleen: dec<br>Pituitary:dec<br>Adrenals:dec, f<br>Testes:dec, m<br>Prostate:dec, m<br>Ovaries:dec,f<br>Uterus:dec, f<br>Brain:dec | Thymus:dec<br>Spleen:dec<br>Pituitary:dec<br>Adrenals: dec, f<br>Testes:dec, m<br>Prostate:dec, m<br>Ovaries:dec, f<br>Uterus:dec, f<br>Brain:dec |
| Gross pathology | | Seminal Thymus:sm | Thymus:sm<br>Testes:sm<br>Thymus:sm<br>Prostate:sm<br>Alopecia/scars: 1m | Thymus:sm<br>testes:sm<br>Thymus:sm<br>Alopecia/scars: 3f |
| | | seminal vesicles: | Heart: myocardial | Heart: myocardial |

| | | | | |
|---|---|---|---|---|
| Histopathology | | dec secretion<br>kidney: hyaline droplet formation, m | degeneration<br>Lungs:eosinophilic material in alveoli<br>Thymus:cortical/ medullary atrophy<br>lymph nodes: absence of germinal centers<br>ovaries: dec follicles, hypertrophy of interstitial cells<br>uterus: atrophy<br>testes: atrophy<br>epididymides:atroph y<br>seminal vesicles: dec secretion<br>mammaries: dec development, m<br>salivary glands; depletion secretory granules<br>eyes: swelling of lenticular fibers<br>kidney: vasculopathy; hyaline droplet formation, m<br>bone: depletion of cortical bone(femur)<br>thyroid: inc sm follicles | degeneration<br>Lungs:eosinophilic material in alveoli<br>Thymus:cortical/med ullary atrophy<br>lymph nodes: absence of germinal centers<br>ovaries: dec follicles, hypertrophy of interstitial cells<br>uterus: atrophy<br>testes: atrophy<br>epididymides:atroph y<br>seminal vesicles: dec secretion<br>mammaries: dec development, m<br>salivary glands; depletion secretory granules<br>eyes: swelling of lenticular fibers<br>kidney: hyaline droplet formation, m<br>bone: depletion of cortical bone (femur)<br>thyroid: inc sm follicles |
| Toxicokinetics | Not performed | | | |

CONCLUSIONS: Both batches of RAD exerted expected immunosuppressive effects on thymus, spleen and lymph nodes. Other toxicities included male reproductive (testes, prostate gland, seminal vesicles, epididymides; mammary gland) and female reproductive (pituitary, ovaries, uterus, vagina) showed toxicity of 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. No NOEL was seen in this study. This study extends the findings of the 2-week study at 1.5 and 15 mg/kg to 1.5 and 5 mg/kg at 4 weeks.

### 3.4.4 GENETIC TOXICOLOGY

**Study title:** SDZ RAD 666: mutagenicity test using Salmonella typhimurium (batch control)

**Key findings:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study no:** 10/96 (203-040)
**Study type** (if not reflected in title)**:**
**Volume #, and page #:** v. 67, p. 1
**Conducting laboratory and location:** Sandoz Pharm Ltd.
CH-4002 Basel
Switzerland
**Date of study initiation:** January 31, 1996
**GLP compliance:** yes
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** SDZ RAD 666, batch 95905, na, 95.4%
**Formulation/vehicle:** solution, DMSO

**Methods:**

Strains/species/cell line: Salmonella typhimurium, strains TA1535, TA97a, TA98, TA100, TA102 (b) (4)

Dose selection criteria:
- Basis of dose selection: results of previous experiments
- Range finding studies: np

Test agent stability: stable in DMSO at room temperature 24 h
Metabolic activation system: S9 rat liver homogenate, induced in vivo by Araclor 1254
Controls:
- Vehicle: DMSO
- Negative controls: none
- Positive controls: 2-aminoanthracene, N-methyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, benz(a)pyrene, 2-nitrofluorene, mitomycin C
- Comments: a standard bacterial reversion test appears to have been used

Exposure conditions:
- Incubation and sampling times: 3 day incubation followed by counting
- Doses used in definitive study: 1562.5, 3125, 6250, 12500, 25000 : g/ml
- Study design: a standard bacterial reversion test appears to have been used

Analysis:
- No. of replicates: 3
- Counting method: image analyzer counts of revertant bacterial colonies

Criteria for positive results: normal range solvent counts, effective positive controls, with response for at least concentration/strain twice that of controls

**Summary of individual study findings:**

Study validity: criteria for validity met
Study outcome: SDZ RAD treatment did not meet criteria for positive reversion

**Genetic toxicology summary:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study title:** SDZ RAD 666: mutagenicity test using Salmonella typhimurium (batch control)

**Key findings:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study no:** 52/96 (203-062)

**Study type** (if not reflected in title)**:**
**Volume #, and page #:** v. 67 p. 43
**Conducting laboratory and location:** Sandoz Pharm Ltd.
CH-4002 Basel
Switzerland
**Date of study initiation:** July 16, 1996
**GLP compliance: yes**
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** SDZ RAD, batch 96908, na, 102.2%
**Formulation/vehicle:** solution in DMSO

**Methods:**

Strains/species/cell line: Salmonella typhimurium, strains TA1535, TA97a, TA98, TA100, TA102 (b) (4)

Dose selection criteria:
- Basis of dose selection: previous experiments
- Range finding studies: none

Test agent stability: 24 h in DMSO
Metabolic activation system: S9 rat liver homogenate, induced in vivo by Araclor 1254
Controls:
- Vehicle: DMSO
- Negative controls: none
- Positive controls: 2-aminoanthracene, N-methyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, benz(a)pyrene, 2-nitrofluorene, mitomycin C
- Comments: a standard bacterial reversion test appears to have been used

Exposure conditions:
- Incubation and sampling times: 3 days followed by counting
- Doses used in definitive study: 1562.5, 3125, 6250, 12500, 25000 : g/ml
- Study design: a standard bacterial reversion test appears to have been used

Analysis:
- No. of replicates: 3
- Counting method: image analyzer

Criteria for positive results: normal range solvent counts, effective positive controls, with response for at least concentration/strain twice that of controls

**Summary of individual study findings:**

Study validity: positive controls were effective
Study outcome: SDZ RAD treatment did not meet criteria for positive reversion response

**Genetic toxicology summary:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study title:** SDZ RAD (solid dispersion): mutagenicity test using Salmonella typhimurium (batch control)

**Key findings:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study no:** 66/96 (203-063)
**Study type** (if not reflected in title)**:**
**Volume #, and page #:** v. 67, p. 80
**Conducting laboratory and location:** Sandoz Pharm Ltd.
CH-4002 Basel
Switzerland
**Date of study initiation:** Aug 28, 1996
**GLP compliance: yes**
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** SDZ RAD (solid dispersion), batch no. X096 0796, na, 104.3%
**Formulation/vehicle: solution in DMSO**

**Methods:**

Strains/species/cell line: Salmonella typhimurium, strains TA1535, TA97a, TA98, TA100, TA102 (b) (4)

Dose selection criteria:
Basis of dose selection: previous experiments
Range finding studies: none
Test agent stability: 24 h at room temperature
Metabolic activation system: S9 rat liver homogenate, induced in vivo by Araclor 1254
Controls:
Vehicle: DMSO
Negative controls: none
Positive controls: 2-aminoanthracene, N-methyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, benz(a)pyrene, 2-nitrofluorene, mitomycin C
Comments: a standard bacterial reversion test appears to have been used
Exposure conditions:
Incubation and sampling times: 3 day incubation followed by counting
Doses used in definitive study: 14.54, 72.72, 363.60, 1818, 9090 : g/ml
Study design: a standard bacterial reversion test appears to have been used
Analysis:
No. of replicates: 3
Counting method: image analyzer
Criteria for positive results: normal range solvent counts, effective positive controls, with response for at least concentration/strain twice that of controls

**Summary of individual study findings:**

Study validity: positive controls were effective
Study outcome: SDZ RAD did not meet criteria of an increased reversion rate (doubling)

**Genetic toxicology summary:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay.

**Study title:** RAD 001: mutagenicity test using Salmonella typhimurium (batch control)

**Key findings:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study no:** 001801 (BS-482)
**Study type** (if not reflected in title)**:**
**Volume #, and page #:** v67, p. 121
**Conducting laboratory and location:** Sandoz Pharm Ltd.
CH-4002 Basel

Switzerland
**Date of study initiation:** Jan 26, 2000
**GLP compliance: yes**
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD 001 (solid dispersion 2%), batch X033 0199, na, 95.0%
**Formulation/vehicle: solution in DMSO**

**Methods:**
Strains/species/cell line: Salmonella typhimurium, strains TA1535, TA97a, TA98, TA100, TA102 (b) (4)
Dose selection criteria:
Basis of dose selection: previous experiments
Range finding studies: none
Test agent stability: not tested; no precipitation on plates
Metabolic activation system: S9 rat liver homogenate, induced in vivo by Araclor 1254
Controls:
Vehicle: DMSO
Negative controls: none
Positive controls: 2-aminoanthracene, N-methyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, benz(a)pyrene, 2-nitrofluorene, mitomycin C
Comments: a standard bacterial reversion test appears to have been used
Exposure conditions:
Incubation and sampling times: 3 day incubation followed by counting
Doses used in definitive study: 12.5, 25, 50, 100, 200 : g/ml
Study design: standard bacterial reversion assay
Analysis:
No. of replicates: 3
Counting method: image analyzer
Criteria for positive results: normal range solvent counts, effective positive controls, with response for at least concentration/strain twice that of controls

**Summary of individual study findings:**
Study validity: positive controls effective
Study outcome: SDZ RAD did not meet criteria of an increased reversion rate (doubling).

**Genetic toxicology summary:** SDZ RAD (solid dispersion) did not cause a positive response in this bacterial reversion assay

**Study title:** SDZ RAD (solid dispersion) Chromosomal aberration test with V79 Chinese hamster cells

**Key findings:** SDZ RAD (dispersion) was not positive in this chromosomal aberration study.

**Study no:** Z63 (203-067)
**Study type** (if not reflected in title)**:**
**Volume #, and page #:** v. 67, p. 148
**Conducting laboratory and location:** Sandoz Pharm Ltd.
CH-4002 Basel
Switzerland
**Date of study initiation:** Feb 9, 1996
**GLP compliance: yes**
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** SDZ RAD solid dispersion, batch X0960796, na, 104.3%
**Formulation/vehicle:** solution in DMSO

**Methods:**

Strains/species/cell line:B79 Chinese hamster cells
Dose selection criteria:
Basis of dose selection: previous data of cell growth and depression of mitotic index
Range finding studies: none
Test agent stability: at least 24 h in DMSO
Metabolic activation system: S9 rat liver homogenate, induced in vivo by Araclor 1254
Controls:
Vehicle: DMSO
Negative controls: none
Positive controls: ethylmethanesulphonate, cyclophosphamide
Comments: controls appear appropriate
Exposure conditions:
Incubation and sampling times:3h, 20 h
Doses used in definitive study: without metabolic activation, 136, 224, 368 : g/ml, 3 h; 20, 100, 141, 200 : g/ml, 20 h; with metabolic activation, 100, 464, 1000 : g/ml, 3 h; 292, 382, 500 : g/ml, 3 h
Study design: following one of the above treatment/incubation schemes, cells were harvested onto slides, stained with giemsa, microscopically examined and scored for aberrations

Analysis:
No. of replicates: 1
Counting method: mitotic index determined by image analyzer, used to select highest dose reducing mitotic index to 50-70% of solvent control. Those slides remaining microscopically examined for aberrations
Criteria for positive results: statistically significant increase in frequency of metaphase with aberrant chromosomes, exceeding historical controls, reproducible between replicates, with evidence of dose-response

**Summary of individual study findings:**
Study validity: positive controls were effective
Study outcome: RAD 001did not increase incidence of aberrant cells
**Genetic toxicology summary:**
SDZ RAD (dispersion) was not positive in this chromosomal aberration study.

**Study title:** RAD001:Chromosomal aberration test with V79 Chinese hamster cells
**Key findings:** RAD001 was not positive in this chromosomal aberration study.

**Study no:** 001831 (BS-725)
**Study type** (if not reflected in title)**:** bacterial reversion mutation assay
**Volume #, and page #:** v. 67, p.199
**Conducting laboratory and location:** Novartis Pharma Ltd.
Basle, Switzerland
**Date of study initiation:** December 23, 1999
**GLP compliance: yes**
**QA reports:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity: RAD, batch X033 0199, na, 95.0%**
**Formulation/vehicle: 1% DMSO solution**

**Methods:**
Strains/species/cell line: V79 Chinese hamster cells
Dose selection criteria:
Basis of dose selection: previous chromosomal aberration studies with RAD
Range finding studies: not performed
Test agent stability: 24h in 2 mg/ml DMSO solution, or 0.02 phosphate buffer solution.
Metabolic activation system: S9 rat liver homogenate, induced by Araclor 1254
Controls:

Vehicle: 1% DMSO
Negative controls: none
Positive controls: ethyl methanesulphonate, cyclophophamide
Comments: none

Exposure conditions:
Incubation and sampling times: continuous, 20 h treatment; pulse, 3 h treatment+17 h incubation (with and without S9)
Doses used in definitive study:
Without activation:
3 h treatment+17 h incubation: 500, 1000, 2000 : g/ml
20 h treatment: 500, 1587.4, 2000 : g/ml
20 h treatment: 639, 793.7, 1000 : g/ml
20 h treatment: 500, 1259.9, 1587.4, 2000 : g/ml
with activation:
3 h treatment+ 17 h incubation: 793.7, 1587.4, 2000 : g/ml
3 h treatment+ 17 h incubation: 630, 793.7, 1000 : g/ml
3 h treatment+ 17 h incubation: 793.7, 1259.9, 2000 : g/ml
Study design: following one of the above treatment/incubation schemes, cells were harvested onto slides, stained with giemsa, microscopically examined and scored for aberrations

Analysis:
No. of replicates: one
Counting method: mitotic index determined by image analyzer, used to select highest dose reducing mitotic index to 50-70% of solvent control. Those slides remaining microscopically examined for aberrations

Criteria for positive results: statistically significant increase in frequency of metaphase with aberrant chromosomes, exceeding historical controls, reproducible between replicates, with evidence of dose-response.

**Summary of individual study findings:**
Study validity: positive controls were effective
Study outcome: RAD 001did not increase incidence of aberrant cells

**Genetic toxicology summary:** RAD001 was not positive in this chromosomal aberration study.

### 3.4.5. Carcinogenicity

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

**Key study findings**: RAD administration for 104 weeks provided immunosuppression –related pathologies and previously seen reproductive effects with only one neoplastic finding in females, osteoma of the femur.

Adequacy of the carcinogenicity study and appropriateness of the test model: The study appears to have 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 104-week oral mouse study is considered adequate by the executive carcinogenicity assessment committee; however, this protocol was not judged adequate by the executive CAC due to an insufficient MTD being set by the sponsor, who had already begun the study. 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 zero-level controls.

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 #**: v.31-37, p 1

**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%
**CAC concurrence**: no

**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, (b) (4)
Number/sex/group (main study): 60
Route, formulation, volume: oral gavage, suspension in HPMC, 10ml/kg
Frequency of dosing:
Satellite groups used for toxicokinetics or special groups: 15
Age: 35-42 days
Animal housing: individual
Restriction paradigm for dietary restriction studies: na
Drug stability/homogeneity: np
Dual controls employed: yes
Interim sacrifices: none scheduled
Deviations from original study protocol:

**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

| | Males (60/group) | | | | | Females (60 /group) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| week | Control 1 | Control 2 | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg | Control 1 | Control 2 | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg |
| 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

| Group and sex/number of animals | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mode of death | 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 |
| Killed in extremis | 16 | 19 | 22 | 23 | 17 | 18 | 16 | 28 | 17 | 17 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Found dead | 8 | 9 | 4 | 1 | 1 | 4 | 6 | 1 | 2 | 0 |
| Killed for humane reasons | 3 | 3 | 1 | 4 | 4 | 8 | 9 | 7 | 11 | 6 |
| total | 27 | 31 | 27 | 28 | 22 | 30 | 31 | 36 | 30 | 23 |

Clinical signs: No treatment-related signs were seen through the study.
Opthamology and hematology had 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 | | | | | number of animals with swellings | total number of swellings | mean weeks 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.

Histopathology:

Non-neoplastic:

| **Males** | c | c | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg |
|---|---|---|---|---|---|
| **Testes** | | | | | |
| 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 |
| **epididymides** | | | | | |
| Hypospermia | 13 | 11 | 15 | 8 | 24 |
| **Sciatic nerve** | | | | | |
| Axonal degeneration | 17 | 13 | 19 | 20 | 25 |
| **Females** | | | | | |
| **thymus** | | | | | |
| involution | 11 | 10 | 7 | 11 | 19 |
| **Sciatic nerve** | | | | | |
| Axonal degeneration | 17 | 16 | 18 | 17 | 26 |

Neoplastic:

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

| | males control 1 | males 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 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Adrenal cortex** | | | | | | | | | | |
| Cortical adenoma | 0/26<br>4/33<br>4/59 | 3/31<br>3/29<br>6/60 | 3/25<br>0/33<br>3/58 | 4/27<br>2/32<br>6/59 | 0/21<br>3/38<br>3/59 | 0/30<br>1/30<br>1/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 1/30<br>0/30<br>1/60 | 0/23<br>0/37<br>0/60 |
| Neuroblastoma | 0/26<br>0/33<br>0/59 | 1/31<br>0/29<br>1/60 | 0/25<br>0/33<br>0/58 | 0/26<br>0/33<br>0/59 | 0/21<br>0/38<br>0/59 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| Cortical adenocarcinoma | 0/26<br>0/33<br>0/59 | 0/31<br>0/29<br>0/60 | 0/25<br>0/33<br>0/58 | 0/26<br>0/33<br>0/59 | 0/21<br>0/38<br>0/59 | 1/30<br>0/30<br>1/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **Adrenal medulla** | | | | | | | | | | |
| Pheochroma cytoma | 0/26<br>1/33<br>1/59 | 1/31<br>0/29<br>1/60 | 0/25<br>0/33<br>0/58 | 0/27<br>0/32<br>0/59 | 0/21<br>0/38<br>0/59 | 0/30<br>1/30<br>1/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **brain** | | | | | | | | | | |
| astrocytoma | 0/27<br>0/23<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 1/22<br>0/38<br>1/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 1/36<br>0/24<br>1/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **cecum** | | | | | | | | | | |
| Adenoma | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/26<br>0/33<br>0/59 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>1/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/35<br>0/24<br>0/59 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **femur** | | | | | | | | | | |
| osteoma | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 1/30<br>0/30<br>1/60 | 1/23<br>1/37<br>2/60 |
| **Hardarian gland** | | | | | | | | | | |
| Adenoma | 0/27<br>3/33<br>3/60 | 3/31<br>2/29<br>5/60 | 5/27<br>3/32<br>8/59 | 0/28<br>3/32<br>3/60 | 2/22<br>3/38<br>5/60 | 2/30<br>1/30<br>3/60 | 4/31<br>4/29<br>8/60 | 1/36<br>2/24<br>3/60 | 2/30<br>2/30<br>4/60 | 2/23<br>1/37<br>3/60 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Carcinoma | 2/27<br>1/33<br>3/60 | 0/31<br>0/29<br>0/60 | 1/27<br>0/32<br>1/59 | 1/28<br>1/32<br>2/60 | 0/22<br>0/38<br>0/60 | 1/30<br>1/30<br>2/60 | 0/31<br>2/29<br>2/60 | 1/36<br>1/24<br>2/60 | 1/30<br>2/30<br>3/60 | 0/23<br>3/37<br>3/60 |
| **jejunum** | | | | | | | | | | |
| adenoma | 0/25<br>0/33<br>0/58 | 0/30<br>0/29<br>0/59 | 0/24<br>0/33<br>0/57 | 0/28<br>0/32<br>0/60 | 0/21<br>0/38<br>0/59 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/25<br>0/24<br>0/59 | 0/30<br>0/30<br>0/60 | 0/23<br>1/37<br>1/60 |
| carcinoma | 0/25<br>1/33<br>1/58 | 0/30<br>0/29<br>0/59 | 0/24<br>0/33<br>0/57 | 0/28<br>0/32<br>0/60 | 0/21<br>0/38<br>0/59 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/25<br>0/24<br>0/59 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **kidneys** | | | | | | | | | | |
| Cortical adenoma | 0/27<br>1/33<br>1/60 | 0/31<br>0/29<br>0/60 | 0/27<br>1/33<br>1/60 | 2/28<br>0/32<br>2/60 | 0/22<br>2/38<br>2/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| Cortical carcinoma | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 1/28<br>1/32<br>2/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **liver** | | | | | | | | | | |
| Hepatocellular Adenoma | 3/27<br>12/33<br>15/60 | 4/31<br>4/29<br>8/60 | 0/27<br>4/33<br>4/60 | 3/28<br>9/32<br>12/60 | 2/22<br>8/38<br>10/60 | 0/30<br>1/30<br>1/60 | 0/31<br>1/29<br>1/60 | 0/36<br>2/24<br>2/60 | 1/30<br>1/30<br>2/60 | 0/23<br>1/37<br>1/60 |
| Hemangioma | 0/27<br>0/33<br>0/60 | 0/31<br>1/29<br>1/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| Hemangiosarcoma | 1/27<br>0/33<br>1/60 | 0/31<br>1/29<br>1/60 | 0/27<br>0/33<br>0/60 | 1/28<br>0/32<br>1/60 | 0/22<br>0/38<br>0/60 | 0/30<br>1/30<br>1/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>1/30<br>1/60 | 0/23<br>0/37<br>0/60 |
| Hepatocellular carcinoma | 3/27<br>3/33<br>6/60 | 3/31<br>2/29<br>5/60 | 5/27<br>4/33<br>9/60 | 3/28<br>2/32<br>5/60 | 2/22<br>3/38<br>5/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| Cholangiocellular carcinoma | 0/27<br>0/33<br>0/60 | 1/31<br>0/29<br>1/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **Lungs** | | | | | | | | | | |
| Pulmonary adenoma | 1/27<br>6/33<br>7/60 | 5/31<br>9/29<br>14/60 | 3/27<br>11/33<br>14/60 | 7/28<br>7/32<br>14/60 | 3/22<br>11/38<br>14/60 | 4/30<br>6/30<br>10/60 | 3/31<br>3/29<br>6/60 | 3/36<br>3/24<br>6/60 | 2/30<br>6/30<br>8/60 | 1/23<br>6/37<br>7/60 |
| Pulmonary carcinoma | 3/27<br>4/33<br>7/60 | 7/31<br>2/29<br>9/60 | 5/27<br>0/33<br>5/60 | 3/28<br>0/32<br>3/60 | 2/22<br>0/38<br>2/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 2/36<br>0/24<br>2/60 | 1/30<br>1/30<br>2/60 | 1/23<br>0/37<br>1/60 |
| **Mammary area Cranial/caudal** | | | | | | | | | | |
| carcinoma | 0/27<br>0/33<br>0/60 | 1/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 1/30<br>2/30<br>3/60 | 3/31<br>0/29<br>3/60 | 1/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **ovaries** | | | | | | | | | | |
| adenoma | | | | | | 0/30<br>2/30<br>2/60 | 0/31<br>1/29<br>1/60 | 0/36<br>1/24<br>1/60 | 0/30<br>1/30<br>1/60 | 0/23<br>0/37<br>0/60 |
| luteoma | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>1/24<br>1/60 | 0/30<br>1/30<br>1/60 | 0/23<br>2/37<br>2/60 |
| Granulosa cell tumor-B | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>1/37<br>1/60 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| hemangioma | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 1/23<br>0/37<br>1/60 |
| Granulosa cell tumor-M | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>1/24<br>1/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **pancreas** | | | | | | | | | | |
| Islet cell adenoma | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/26<br>0/33<br>0/59 | 0/28<br>1/32<br>1/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/30<br>0/29<br>0/59 | 0/26<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **pituitary** | | | | | | | | | | |
| adenoma | 0/27<br>1/33<br>1/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>1/30<br>1/60 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/28<br>0/30<br>0/58 | 0/23<br>2/37<br>2/60 |
| **Seminal vesicles** | | | | | | | | | | |
| adenoma | 0/33<br>0/27<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>2/38<br>2/60 | | | | | |
| **spleen** | | | | | | | | | | |
| hemangiosarcoma | 0/27<br>0/33<br>0/60 | 0/31<br>1/29<br>1/60 | 0/26<br>0/33<br>0/59 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/30<br>0/29<br>0/59 | 0/26<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **stomach** | | | | | | | | | | |
| Squamous cell papilloma | 0/27<br>1/33<br>1/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 1/28<br>0/32<br>1/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **Submandibular salivary glands** | | | | | | | | | | |
| Myoepithelial tumor | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/32<br>0/59 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 1/30<br>0/29<br>1/59 | 0/35<br>0/24<br>0/59 | 0/29<br>0/30<br>0/59 | 0/23<br>0/37<br>0/60 |
| **Testes** | | | | | | | | | | |
| Interstitial cell tumor | 1/27<br>2/33<br>3/60 | 1/31<br>0/29<br>1/60 | 0/27<br>0/33<br>0/60 | 0/28<br>1/32<br>1/60 | 1/22<br>0/38<br>1/60 | | | | | |
| hemangiosarcoma | 0/27<br>1/33<br>1/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | | | | | |
| **thymus** | | | | | | | | | | |
| thymoma | 0/27<br>1/31<br>1/58 | 0/24<br>0/27<br>0/57 | 0/26<br>0/32<br>0/58 | 0/26<br>0/32<br>0/58 | 0/23<br>0/37<br>0/58 | 0/30<br>0/30<br>0/60 | 0/30<br>0/29<br>0/59 | 0/35<br>0/24<br>0/59 | 0/30<br>0/30<br>0/60 | 0/23<br>0/36<br>0/59 |
| **thyroid** | | | | | | | | | | |
| Follicular adenoma | 0/26<br>0/33<br>0/59 | 1/31<br>1/29<br>2/60 | 0/27<br>0/33<br>0/60 | 1/28<br>1/32<br>1/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/29<br>0/30<br>0/59 | 0/23<br>0/37<br>0/60 |
| **Uterine cervix** | | | | | | | | | | |
| Stromal polyp | | | | | | 0/29<br>0/30<br>0/59 | 0/31<br>0/29<br>0/60 | 0/36<br>2/24<br>2/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| fibroma | | | | | | 0/29<br>0/30<br>0/59 | 0/31<br>0/29<br>0/60 | 1/36<br>0/24<br>1/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| leiomyoma | | | | | | 1/29<br>3/30<br>4/59 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>1/37<br>0/60 |

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|---|---|---|---|---|---|---|---|---|---|---|
| leiomyosarcoma | | | | | | 0/29<br>1/30<br>1/59 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 1/23<br>0/37<br>1/60 |
| Stromal sarcoma | | | | | | 0/29<br>0/30<br>0/59 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **uterus** | | | | | | | | | | |
| Stromal polyp | | | | | | 0/30<br>4/30<br>4/60 | 3/31<br>2/29<br>5/60 | 0/36<br>3/24<br>3/60 | 0/30<br>1/30<br>1/60 | 1/23<br>3/37<br>4/60 |
| fibroma | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>1/37<br>1/60 |
| leiomyoma | | | | | | 0/30<br>3/30<br>3/60 | 0/31<br>1/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>1/37<br>1/60 |
| leiomyosarcoma | | | | | | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| adenocarcinoma | | | | | | 0/30<br>1/30<br>1/60 | 1/31<br>1/29<br>2/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| Endometrial sarcoma | | | | | | 2/30<br>0/30<br>2/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 0/23<br>0/37<br>0/60 |
| **abdomen** | | | | | | | | | | |
| Leimysarcoma | 0/1<br>0/0<br>0/1 | 0/3<br>0/0<br>0/3 | 0/2<br>0/0<br>0/2 | 1/1<br>0/0<br>1/1 | 0/2<br>0/0<br>0/2 | 0/1<br>0/0<br>0/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/1<br>0/0<br>0/1 | 0/2<br>0/0<br>0/0 |
| **Buccal cavity** | | | | | | | | | | |
| papilloma | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>1/1<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 |
| **Hemopoietic tumor** | | | | | | | | | | |
| Malignant lymphoma (unclassified) | 0/27<br>1/33<br>1/60 | 1/31<br>0/29<br>1/60 | 1/27<br>0/33<br>1/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 1/31<br>0/29<br>1/60 | 0/36<br>0/24<br>0/60 | 0/30<br>0/30<br>0/60 | 1/23<br>0/37<br>1/60 |
| Histiocytic sarcoma | 1/27<br>0/33<br>1/60 | 1/31<br>0/29<br>1/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 3/22<br>0/38<br>3/60 | 5/30<br>0/30<br>5/60 | 1/31<br>3/29<br>4/60 | 3/36<br>1/24<br>4/60 | 3/30<br>1/30<br>4/60 | 1/23<br>1/37<br>2/60 |
| Follicular center cell lymphoma | 4/27<br>3/33<br>7/60 | 2/31<br>2/29<br>4/60 | 2/27<br>1/33<br>3/60 | 2/28<br>0/32<br>2/60 | 1/22<br>1/38<br>1/60 | 2/30<br>3/30<br>5/60 | 6/31<br>0/29<br>6/60 | 1/36<br>0/24<br>1/60 | 4/30<br>1/30<br>5/60 | 2/23<br>0/37<br>2/60 |
| Granulocytic leukemia | 1/27<br>0/33<br>1/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 1/28<br>0/32<br>1/60 | 1/22<br>1/38<br>1/60 | 1/30<br>0/30<br>1/60 | 1/31<br>0/29<br>1/60 | 1/36<br>0/24<br>1/60 | 2/30<br>0/30<br>2/60 | 3/23<br>0/37<br>3/60 |
| Lymphocyttic/ lymphoblastic lymphoma | 3/27<br>1/33<br>4/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 1/28<br>0/32<br>1/60 | 3/22<br>0/38<br>3/60 | 2/30<br>0/30<br>2/60 | 3/31<br>2/29<br>5/60 | 3/36<br>1/24<br>4/60 | 5/30<br>0/30<br>5/60 | 3/23<br>0/37<br>3/60 |

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|---|---|---|---|---|---|---|---|---|---|---|
| Pleiomorphic lymphoid lymphoma | 0/27<br>0/33<br>0/60 | 0/31<br>0/29<br>0/60 | 0/27<br>0/33<br>0/60 | 0/28<br>0/32<br>0/60 | 0/22<br>0/38<br>0/60 | 0/30<br>0/30<br>0/60 | 0/31<br>0/29<br>0/60 | 0/36<br>0/24<br>0/60 | 1/30<br>0/30<br>1/60 | 0/23<br>0/37<br>0/60 |
| **Musculo-skeletal** | | | | | | | | | | |
| Sarcoma | 0/3<br>0/1<br>0/4 | 0/6<br>0/1<br>0/7 | 1/5<br>0/2<br>1/7 | 0/2<br>0/0<br>0/2 | 0/1<br>0/1<br>0/2 | 1/1<br>0/0<br>1/1 | 0/3<br>0/2<br>0/5 | 0/3<br>0/2<br>0/5 | 0/2<br>0/0<br>0/2 | 0/1<br>½<br>1/3 |
| osteosarcoma | 0/0<br>0/0<br>0/0 | 1/6<br>0/0<br>1/6 | 0/5<br>0/0<br>0/5 | 0/2<br>0/0<br>0/2 | 0/1<br>0/0<br>0/1 | 0/1<br>0/0<br>0/1 | 1/3<br>0/0<br>1/3 | 0/3<br>0/0<br>0/3 | ½<br>0/0<br>1/2 | 0/1<br>0/0<br>0/1 |
| osteoma | 0/0<br>0/1<br>0/1 | 0/0<br>0/1<br>0/1 | 0/0<br>0/2<br>0/2 | 0/0<br>0/0<br>0/0 | 0/0<br>0/1<br>0/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/2<br>0/2 | 0/0<br>0/2<br>0/2 | 0/0<br>0/0<br>0/0 | 0/0<br>½<br>½ |
| **peritoneum** | | | | | | | | | | |
| mesothelioma | 0/0<br>0/0<br>0/0 | 1/3<br>0/0<br>1/3 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/1<br>0/0<br>0/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 |
| **skin** | | | | | | | | | | |
| Hemangiosarcoma<br># Examined at Necropsy not provided | 1/6<br>?<br>1/12 | 0/5<br>?<br>0/13 | 0/9<br>?<br>0/14 | 0/7<br>?<br>0/11 | 0/10<br>?<br>0/21 | 0/16<br>?<br>0/17 | 0/6<br>?<br>0/6 | 0/9<br>?<br>0/9 | 0/11<br>?<br>0/11 | 0/4<br>?<br>0/4 |
| Sarcoma<br># Examined at Necropsy not provided | 0/6<br>?<br>0/12 | 0/5<br>?<br>0/13 | 0/9<br>?<br>0/14 | 0/7<br>?<br>0/11 | 0/10<br>?<br>0/21 | 1/16<br>?<br>1/17 | 0/6<br>?<br>0/6 | 0/9<br>?<br>0/9 | 0/11<br>?<br>0/11 | 0/4<br>?<br>0/4 |
| **thorax** | | | | | | | | | | |
| mesothelioma | 0/2<br>0/0<br>0/2 | 1/3<br>0/0<br>1/3 | 0/1<br>0/0<br>0/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/1<br>0/0<br>0/1 | 0/1<br>0/0<br>0/1 | 0/2<br>0/0<br>0/2 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>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 |
| | 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 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 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. 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% of females after 101 weeks. Survival among treated mice was highest in the high dose groups correlating with lower bodyweight gain. Food consumption was unaffected by treatment. Histopathology findings included treatment-related changes in the thymus, testes, and epididymides. High dose females had thymic involution. Leukocytic infiltration of the renal cortex was reduced in mid- and high dose females and submandibulary salivary gland in treated females appears related to immunosuppression. Reproductive effects were observed in the testes and epididymides of high dose males.
RAD administration for to mice for 104 weeks provided immunosuppression–related pathologies and previously seen reproductive effects with only one neoplastic finding in females, osteoma of the femur.

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

**Key study findings**: RAD administration for 104 weeks provided immunosuppression–related pathologies of the immune tissues and lungs, treatment–related effects on the lens, liver and adrenal gland and previously seen reproductive effects with no neoplastic findings.

Adequacy of the carcinogenicity study and appropriateness of the test model: The study appears to have 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 the zero-level controls.

Evaluation of tumor findings:

**Study no.**: SPM113/973228 (BS-468)
**Volume #, and page #**: v.38., p.1
**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%

**CAC concurrence**:

**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:
Dual controls employed: yes
Interim sacrifices: none scheduled
Deviations from original study protocol:

**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 ( ), no ( )
Toxicokinetics: weeks 4, 13, 26, and 48, 0.5, 1, 2, 4, 7, and 24 h after dosing

**Results**
Mortality:

Animal Survival table

| | Males (60/group) | | | | | Females (60 /group) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| week | Control 1 | Control 2 | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg | Control 1 | Control 2 | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg |
| 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 |

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Deaths during study

| Group and sex/number of animals | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mode of death | 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 |
| Killed in extremis | 9 | 9 | 8 | 5 | 11 | 12 | 15 | 9 | 13 | 3 |
| Found dead | 0 | 3 | 1 | 0 | 0 | 2 | 0 | 3 | 1 | 1 |
| Killed for humane reasons | 15 | 12 | 16 | 7 | 9 | 9 | 8 | 10 | 4 | 5 |
| total | 24 | 24 | 25 | 12 | 20 | 23 | 23 | 22 | 18 | 9 |

**Clinical signs:** No treatment-related signs were seen through the study.

Opthamology a: 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 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 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.

Histopathology:

Non-neoplastic:

| | males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | c | c | 0.1 | 0.3 | 0.9 | C | c | 0.1 | 0.3 | 0.9 |
| **Testes** | 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 spermacytes | 0 | 0 | 0 | 0 | 8 | | | | | |
| Spermatid retention | 0 | 0 | 0 | 0 | 3 | | | | | |
| **epididymides** | 60 | 60 | 60 | 60 | 60 | | | | | |
| Reduced sperm count | 3 | 6 | 5 | 8 | 12 | | | | | |
| **Kidneys** | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Pigmented cortical tubules (lipofuscin) | 0 | 0 | 2 | 16 | 44 | 8 | 11 | 17 | 34 | 51 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **liver** | 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 |
| **lungs** | 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 |
| **Mesenteric lymph nodes** | 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 |
| **thymus** | 60 | 60 | 60 | 60 | 60 | 59 | 59 | 60 | 60 | 60 |
| Atrophy | 6 | 4 | 5 | 11 | 18 | 5 | 3 | 4 | 2 | 8 |
| **ovaries** | | | | | | 60 | 60 | 60 | 60 | 60 |
| Ovarian cyst | | | | | | 5 | 4 | 7 | 6 | 21 |
| **uterus** | | | | | | 60 | 60 | 60 | 60 | 60 |
| Squamous metaplasia | | | | | | 2 | 2 | 3 | 1 | 21 |
| **Hardarian glands** | 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 |
| **pancreas** | 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 |
| **eyes** | 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 |
| **Kidney** | 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 |
| **mammary** | 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:

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

| | males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | c | 0.1 | 0.3 | 0.9 | C | c | 0.1 | 0.3 | 0.9 |
| **Adrenal cortex** | | | | | | | | | | |
| Cortical adenoma | 0/24<br>0/36<br>0/60 | 1/24<br>0/36<br>1/60 | 0/25<br>1/35<br>1/60 | 0/12<br>1/48<br>1/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Adrenal medulla** | | | | | | | | | | |
| Pheochroma cytoma | 2/24<br>2/36<br>4/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>1/37<br>1/60 | 0/22<br>1/38<br>1/60 | 1/18<br>0/42<br>1/60 | 0/9<br>1/50<br>1/59 |
| ganglioneuroma | 0/24<br>1/36<br>1/60 | 0/24<br>0/36<br>0/60 | 1/25<br>0/35<br>1/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/50<br>0/60 |
| **Brain** | | | | | | | | | | |
| Granular cell tumor-b | 1/24<br>1/36<br>2/60 | 0/24<br>1/36<br>1/60 | 0/25<br>0/35<br>0/60 | 0/12<br>1/48<br>1/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>1/37<br>1/60 | 0/22<br>0/38<br>0/60 | 0/18<br>2/42<br>2/60 | 0/9<br>1/51<br>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<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 1/20<br>0/40<br>1/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Astrocytoma | 1/24<br>0/36<br>060 | 0/24<br>0/36<br>0/60 | 3/25<br>0/35<br>3/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>1/38<br>1/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Mixed glioma | 1/24<br>0/36<br>1/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Granular cell tumor-m | 0/24<br>0/36<br>0/60 | 1/24<br>0/36<br>1/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>1/38<br>1/60 | 1/18<br>0/32<br>1/60 | 0/9<br>0/51<br>0/60 |
| Meningial sarcoma | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 1/18<br>0/32<br>1/60 | 0/9<br>0/51<br>0/60 |
| **Colon** | | | | | | | | | | |
| carcinoma | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>1/37<br>1/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **duodenum** | | | | | | | | | | |
| carcinoma | 0/23<br>0/36<br>0/59 | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 1/23<br>0/37<br>1/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **eyes** | | | | | | | | | | |
| Amelanotic melanoma | 0/24<br>0/36<br>0/60 | 0/23<br>0/36<br>0/59 | 0/25<br>0/36<br>0/60 | 1/12<br>0/48<br>1/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Jejunum** | | | | | | | | | | |
| leiomyoma | 0/24<br>1/36<br>1/60 | 0/23<br>0/36<br>0/59 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Kidneys** | | | | | | | | | | |
| Liposarcoma | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 1/18<br>0/42<br>1/60 | 0/9<br>0/51<br>0/60 |
| Tubular sarcoma | 1/24<br>0/36<br>1/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Lymph node mesenteric** | | | | | | | | | | |
| hemangioma | 3/24<br>11/36<br>14/60 | 3/24<br>10/36<br>13/60 | 0/25<br>1/35<br>1/60 | 0/12<br>0/48<br>0/60 | 1/20<br>2/40<br>3/60 | 3/23<br>3/37<br>6/60 | 1/22<br>0/37<br>1/59 | 0/22<br>1/38<br>1/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Liver** | | | | | | | | | | |
| Hepatocellular Adenoma | 0/24<br>1/36<br>1/60 | 0/24<br>0/36<br>0/60 | 0/25<br>1/35<br>1/60 | 0/12<br>2/48<br>2/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 1/23<br>2/37<br>3/60 | 0/22<br>2/38<br>2/60 | 0/18<br>0/42<br>0/60 | 0/9<br>1/51<br>1/60 |
| **Lungs** | | | | | | | | | | |
| Pulmonary adenoma | 0/24<br>0/36<br>0/60 | 0/24<br>2/36<br>2/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>1/40<br>1/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>1/42<br>1/60 | 0/9<br>0/51<br>0/60 |
| **Mammary** | | | | | | | | | | |
| Fibroadenoma | 0/23<br>0/36<br>0/59 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 9/23<br>2/37<br>9/60 | 9/23<br>11/37<br>20/60 | 4/22<br>1/38<br>5/60 | 1/18<br>2/42<br>3/60 | 0/9<br>0/51<br>0/60 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Adenoma | 0/23<br>0/36<br>0/59 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 1/22<br>1/38<br>2/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Carcinoma | 0/23<br>0/36<br>0/59 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 1/23<br>2/37<br>3/60 | 0/23<br>1/37<br>1/60 | 0/22<br>1/38<br>1/60 | 1/18<br>1/42<br>2/60 | 1/9<br>1/51<br>2/60 |
| **Ovaries** | | | | | | | | | | |
| Granulosa cell tumor-b | | | | | | 0/23<br>1/37<br>1/60 | 0/23<br>0/37<br>0/60 | 0/22<br>1/38<br>1/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Undifferentiated gonadal stromal tumor | | | | | | 0/23<br>0/37<br>0/60 | 0/23<br>2/37<br>2/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 1/9<br>0/51<br>1/60 |
| Sertoli cell tumor | | | | | | 0/23<br>1/37<br>1/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| Tubulostromal tumor | | | | | | 0/23<br>1/37<br>1/60 | 0/23<br>1/37<br>1/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>1/51<br>1/60 |
| **Pancreas** | | | | | | | | | | |
| Islet cell adenoma | 0/24<br>3/36<br>3/60 | 2/24<br>0/36<br>2/60 | 1/25<br>0/35<br>1/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 1/22<br>0/37<br>1/60 | 1/23<br>0/37<br>1/60 | 0/22<br>1/38<br>1/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>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<br>2/36<br>2/59 | 1/24<br>0/34<br>1/58 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/19<br>0/35<br>0/54 | 0/20<br>0/34<br>0/54 | 0/22<br>0/36<br>0/58 | 0/20<br>0/38<br>0/58 | 0/18<br>0/42<br>0/60 | 0/9<br>1/48<br>1/57 |
| **Pituitary** | | | | | | | | | | |
| Adenoma | 9/24<br>8/36<br>17/60 | 14/24<br>8/36<br>22/60 | 9/25<br>12/34<br>21/59 | 7/12<br>7/48<br>14/60 | 0/20<br>5/40<br>5/60 | 16/23<br>16/37<br>32/60 | 14/23<br>17/37<br>31/60 | 15/22<br>20/38<br>35/60 | 11/18<br>20/42<br>31/60 | 3/9<br>11/51<br>12/60 |
| ganglioneuroma | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/34<br>0/59 | 0/12<br>0/48<br>0/60 | 0/20<br>1/40<br>1/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| carcinoma | 0/24<br>0/36<br>0/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 1/22<br>0/38<br>1/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Prostate** | | | | | | | | | | |
| adenocarcinoma | 0/24<br>0/36<br>0/60 | 1/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | | | | | |
| **Rectum** | | | | | | | | | | |
| carcinoma | 0/24<br>1/36<br>1/60 | 0/24<br>0/36<br>0/60 | 0/25<br>0/35<br>0/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 0/23<br>0/37<br>0/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Spleen** | | | | | | | | | | |
| Hemangiosarcoma | 0/24<br>0/36<br>0/60 | 0/24<br>1/36<br>1/60 | 1/25<br>0/35<br>1/60 | 0/12<br>0/48<br>0/60 | 0/20<br>0/40<br>0/60 | 1/23<br>0/37<br>1/60 | 0/23<br>0/37<br>0/60 | 0/22<br>0/38<br>0/60 | 0/18<br>0/42<br>0/60 | 0/9<br>0/51<br>0/60 |
| **Submandibular gland** | | | | | | | | | | |

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

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

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Basal cell carcinoma | 0/9<br>0/20<br>0/29 | 0/14<br>0/19<br>0/33 | 0/14<br>0/23<br>0/33 | 1/5<br>0/25<br>1/30 | 0/8<br>0/13<br>0/21 | 0/7<br>0/7<br>0/14 | 0/4<br>0/5<br>0/9 | 0/5<br>1/5<br>1/10 | 0/3<br>0/7<br>0/10 | 0/1<br>0/4<br>0/5 |
| Squamous carcinoma | 0/9<br>0/20<br>0/29 | 1/14<br>0/19<br>1/33 | 0/10<br>0/23<br>0/33 | 0/5<br>0/25<br>0/30 | 0/8<br>0/13<br>0/21 | 1/7<br>0/7<br>1/14 | 0/4<br>0/5<br>0/9 | 1/5<br>0/5<br>1/10 | 0/3<br>0/7<br>0/10 | 0/1<br>0/4<br>0/5 |
| Hemangioma | 0/9<br>0/20<br>0/29 | 0/9<br>0/20<br>0/29 | 0/14<br>0/23<br>0/33 | 0/5<br>0/25<br>0/30 | 0/8<br>0/13<br>0/21 | 0/7<br>1/7<br>1/14 | 0/4<br>0/5<br>0/9 | 0/5<br>0/5<br>0/10 | 0/3<br>0/7<br>0/10 | 0/1<br>0/4<br>0/5 |
| Sarcoma | 0/9<br>0/20<br>0/29 | 0/9<br>0/20<br>0/29 | 0/14<br>0/23<br>0/33 | 0/5<br>1/25<br>1/30 | 0/8<br>0/13<br>0/21 | 0/7<br>0/7<br>0/14 | 0/4<br>0/5<br>0/9 | 0/5<br>0/5<br>0/10 | 0/3<br>0/7<br>0/10 | 0/1<br>0/4<br>0/5 |
| **uretor** | | | | | | | | | | |
| papilloma | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>1/1<br>1/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/1<br>0/1 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>0/0 | 0/0<br>0/0<br>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 trend 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 among treated rats was highest in the mid and high dose groups correlating with lower bodyweight gain. 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. Immunosuppression-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, age-related 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 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.
RAD administration to rats for 104 weeks provided immunosuppression–related pathologies of the immune tissues and lungs, treatment –related effects on the lens, liver and adrenal gland and previously seen reproductive effects with no neoplastic findings.

### 3.4.5 REPRODUCTIVE TOXICOLOGY

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

**Key study findings:** Fertility of male rats was significantly decreased by RAD at 5.0 mg/kg , with histopathologic effects of the testes and epididymides. Sperm head count and testosterone were decreased, without full reversibility after 13-weeks of recovery. Unfortunately, drug levels were not determined at the end of recovery. Testicular atrophy was widely seen at 5.0 mg/kg part 1 rats but was not seen following recovery.

**Study no.:** 7073R (203-094)
**Volume #, and page #:** v. 58, p. 1
**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, (b) (4)
Doses employed: 0, 0.1, 0.5, 5.0 mg/kg
Route of administration: oral gavage
Study design: 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. 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: 1%, 0.1 mg/kg, day 92 during blood sampling; 1%, 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, 1%, 0.1 mg/kg, 1%, 0.5 mg/kg, 11%, 5.0 mg/kg
Body weight: dec week 1, 5.0 mg/kg; dec week 1-13, 0.5 mg/kg; recovery reversed these decreases
Food consumption: dec, %, 5.0 mg/kg part 1 and 2 throughout 13 week treatment, reversed in recovery
Toxicokinetics:

| | 0.1 mg/kg | 0.5 mg/kg | 5.0 mg/kg |
|---|---|---|---|
| AUC (ng.h/ml) | 9.9 | 52.0 | 414.8 |

In-life observations:

| Part 1: | 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: | | | | |
| Males mating | 20 | 20 | 20 | 18 |
| Males with pregnancy | 20 | 19 | 18 | 13 |

| | | | | |
|---|---|---|---|---|
| confirmed in female | | | | |
| Male fertility index | 100 | 95 | 90 | 65 |

| Part 1: | Control | 0.1 mg/kg | 0.5 mg/kg | 5.0 mg/kg |
|---|---|---|---|---|
| Females with viable fetuses | 18/19 | 19/20 | 29/20 | 0/0 |
| Females with fetal death | 1/19 | 1/20 | 0 | 0 |
| Corpora lutea/dam | 13.89 | 12.80 | 13.89 | |
| Implantation /dam | 11.42 | 10.80 | 12.74 | 0 |
| Preimplantation loss/sites | 47/217 | 40/216 | 22/242 | 0 |
| Live fetuses/dam | 10.79 | 9.85 | 11.74 | 0 |
| Dead fetuses/dam | 0.11 | 0 | 0 | 0 |
| Resorptions-early/dam | 0.53 | 0.95 | 1.00 | |
| Resorption –late/dam | 0 | 0 | 0 | 0 |
| malformations | 2 | 0 | 0 | --- |

| Part 2: | 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 |
| malformations | 0 | 0 | 0 | 1 |

Organ weights: males, part1: dec testes, epididymides, prostate, 5.0 mg/kg;
Dec epididymides, rt side, 0.5 mg/kg
part 2: dec testes, epididymides, prostate, 5.0 mg/kg;
Histopathology: part 1: 0.5 mg/kg: testes: inc adluminal positioned irregular and misshapen spermatid nuclei;
kidney : hydronephrosis, tubular hyaline droplets
5.0 mg/kg: testes, testicular atrophy 19/19 males, germ cell depletion
epididymides: oligospermia, aspermia, degeneration of duct epithelium with vacuolation
seminal vesicles: colloid reduced;
kidney: hydronephrosis, tubular hyaline droplets

part 2: testes: 5.0 mg/kg; testicular atrophy, 6/20 males
epididymides: oligospermia, aspermia, vacuolation of duct epithelium
kidney : hydronephrosis, tubular hyaline droplets, all doses;
focal urothelial hyperplasia, 0.1 mg/kg
sperm analysis: part 1: 0.1, 0.5 mg/kg reduced by 10%; 5.0 mg/kg reduced by 90% and testicular head sperm count reduced by 86%
part 2: 5.0 mg/kg sl reduced sperm motility
LH, FSH and testosterone determinations (blood and pituitary): part 1: decreased testosterone, 5.0 mg/kg; all other values unaffected; part 2: decreased testosterone, 5.0 mg/kg, after recovery; all other values unaffected
Terminal and necroscopic evaluations: no histopathologic effects were seen in pituitary

**Conclusions:** Fertility of male rats was significantly decreased by RAD at 5.0 mg/kg , with histopathologic effects of the testes and epididymides. Sperm head count and testosterone were decreased, without full reversibility after 13-weeks of recovery. Unfortunately, drug levels were not determined at the end of recovery. Testicular atrophy was widely seen at 5.0 mg/kg part 1 rats but was not seen following recovery. By functional (mating performance) criteria, a NOAEL was seen at 0.5 mg/kg**.**

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

**Key study findings:** RAD administered orally to pregnant rats had maternal effects consisting of decreased body weight gain at 0.3 and 0.9 mg/kg. This corresponded to increased pre- and post implantation losses in these groups. Intrauterine development was affected at all doses, with reduced numbers of implantation and reduced litter size. Fetal development was affected as evidenced by increased delays in skeletal ossification. Overall malformation incidence in litters and fetuses was not significantly greater than controls in the 0.1 mg/kg group.

**Study no:** 2059K (203-056)
**Volume #, and page #:** v.60, p.1
**Conducting laboratory and location:** Novartis Pharma AG,
Basel, Switzerland

**Date of study initiation:** 14 May, 1996
**GLP compliance:** yes
**QA report:** yes ( x ) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD, batch no. X035 0396, na, 86.8%
**Formulation/vehicle:** solid dispersion 9.1%/water-HPMC

**Methods:**

Species/strain: Wistar (HanIbm:WIST), (b) (4)
Doses employed: 0, 0.1, 03, 0.9 mg/kg
Route of administration: oral gavage
Study design: Females were treated 2 weeks prior to pairing and continued until day 16 p.c. Dams sacrificed on day 21 p.c. and examined with fetuses. Satellite females were sacrificed on day 16 p.c. and examined with fetuses after blood sampling.
Number/sex/group: 20
Parameters and endpoints evaluated: female mating index, female fecundity index, preimplantation loss, post implantation loss

**Results:**

Maternal

| | control | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg |
|---|---|---|---|---|
| HED (mg/kg) | 0 | 0.016 | 0.05 | 0.15 |
| Mortality | All rats survived until terminal necropsy | | | |
| Clinical signs | | Alopecia-1& | Gain dec days 10-16, 16-21 | Gain dec days 10-16, 16-21 |
| Body weight | | | | Dec days 16-21 |
| Food consumption | | 20.0 | 37.5 | 40.5 |
| Toxicokinetics day 18 p.c.<br>AUC (0-24) ng.h/ml | | 18.4 | 61.2 | 225.6 |

fetal

| | control | 0.1 mg/kg | 0.3 mg/kg | 0.9 mg/kg |
|---|---|---|---|---|
| Females with viable fetuses/No. pregnant | 18/20 | 18/20 | 19/19 | 13/20 |

| | | | | |
|---|---|---|---|---|
| Females with fetal death | 1 | 0 | 0 | 7 |
| Implantation /dam | 13.56 | 10.83 | 10.79 | 9.55 |
| Preimplantation loss % | 5.92 | 19.19 | 19.60 | 23.47 |
| Live fetuses/dam | 12.78 | 10.00 | 9.16 | 3.45 |
| Dead fetuses/dam | 0.06 | 0 | 0 | 0 |
| Resorptions-early/dam | 0.61 | 0.72 | 1.63 | 6.10 |
| Resorption –late/dam | 0.11 | 0.11 | 0 | 0 |
| Placental weights mean (g) | 0.67 | 0.69 | 0.70 | 0.69 |
| Fetal weights mean (g) | 4.82 | 4.88 | 4.63 | 4.59 |
| Malformations | | | | |
| fetal incidence | 4 (1.72%) | 5 (2.75%) | 8 (4.60%) | 5 (7.25%) |
| litter incidence | 3 (16.7%) | 3 (16.7%) | 6 (31.6%) | 4 (30.8%) |
| omphacoele | | 1 | | |
| fused placenta | 1 | | | |
| anopthalmia | | | | 1 |
| internal hydrocephalus | | | | 1 |
| external hydrocephalus | 1 | | | |
| dystopia of heart | 1 | | | |
| no truncus | | | | |
| brachiocephalus | | | | 1 |
| head defects | | | 1 | |
| rib cartilage asymmetrical | 1 | | | |
| vertebrae rib defects | 1 | | 2 | |
| thoracic/vertebral defects | | | 1 | 1 |
| thoracic hypoplasia | | | 1 | |
| sternum defects | | 3 | 5 | 1 |
| sternum-cleft | | | | 2 |
| lumbar vertebra body defects | | | 1 | |
| Skeletal ossification Retarded/unossified | | | | |
| Cervical vertebral body | 4 | 9 | 9 | 3 |
| Thoracic ribs | 0 | 1 | 0 | 0 |
| Thoracic vertebral body | 9 | 2 | 16 | 8 |
| Sternum | 1 | 0 | 3 | 1 |
| Forelimb | 3 | 8 | 6 | 7 |
| Ribs | 1 | 0 | 0 | 0 |
| cranium | 2 | 3 | 4 | 0 |

**Summary of individual study findings:** RAD administered orally to pregnant rats had maternal effects consisting of decreased body weight gain at 0.3 and 0.9 mg/kg. This corresponded to increased pre- and post implantation losses in these groups. Intrauterine development was affected at all doses, with reduced numbers of implantation and reduced litter size. Fetal development was affected as evidenced by increased delays in skeletal ossification. Overall malformation incidence in litters and fetuses was not significantly greater than controls in the 0.1 mg/kg group. A NOEL for fetal toxicity was observed at <0.1 mg/kg

**Study title:** An oral embryo-fetal development study in rabbits
**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:** 203-074
**Volume #, and page #:** v.62, p.1
**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 #, radiolabel, and % purity:** RAD, batch no. X035 0396, na, 86.8%
**Formulation/vehicle:** solid dispersion 9.1%/water-HPMC

**Methods:**
Species/strain: female New Zealand hybrid rabbits (Crl: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. Dams were sacrificed on day 29 p.i. and examined with fetuses. Satellite females were sacrificed on day 18 p.i. 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: female mating index, female fecundity index, preimplantation loss, post implantation loss

Maternal findings

| Dose | 0 | 0.05 mg/kg | 0.2 mg/kg | 0.8 mg/kg |
|---|---|---|---|---|
| HED (mg/kg) | 0 | 0.025 | 0.100 | 0.400 |
| 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 | | | Sl red weight gain | Weight loss, day6-18 p.i., overall dec weight gain during pregnancy |
| Food consumption | | | | Reduced during treatment |
| Toxicokinetics day 16 p.c. AUC (0-24) ng.h/ml | | | | |

Fetal findings

| | | | | |
|---|---|---|---|---|
| Females with viable fetuses/No. pregnant | 19/20 | 18/20 | 16/20 | 19/20 |
| Females died/sac during gestation | | 1 | 1 | 1 |
| Females assessable | 19 | 17 | 15 | 18 |
| Females with fetal death | | | | |
| Corpora lutea/dam | 9.68 | 9.76 | 10.67 | 10.78 |
| Implantation /dam | 8.21 | 7.41 | 9.53 | 8.17 |
| Preimplantation loss % | 16.34 | 24.29 | 10.55 | 24.10 |
| Live fetuses/dam | 7.89 | 7.00 | 9.07 | 7.17 |
| Dead fetuses/dam | 0 | 0 | 0 | 1 |
| Resorptions-early/dam | 0.21 | 0.35 | 0.27 | 0.39 |
| Resorption –late/dam | 0.11 | 0.06 | 0.20 | 0.56 |
| Placental weights mean (g) | 6.43 | 7.06 | 6.32 | 6.95 |
| Fetal weights mean (g) | 38.74 | 42.21 | 35.63 | 37.78 |
| Malformations | | | | |
| 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%) |
| -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 |

| | | | | |
|---|---|---|---|---|
| Skeletal ossification Retarded/unossified | | | | |
| 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 |

**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. A NOEL for fetal exposure appears to be 0.2 mg/kg.

**Study title:** An oral pre- and postnatal development study in rats

**Key study findings:** Maternal toxicity (mortality, loss of litter, impaired weight gain, decreased food consumption) was seen in the 0.2 and 0.8 mg/kg groups. This correlated with decreased weight gain in offspring of these groups. Other parameters of intrauterine development were unaffected. The maternal NOEL appears to be 0.03 mg/kg. Fetal weight was decreased in the 0.2 and 0.8 mg/kg groups as noted above. This coincides with maternal toxicity. No other parameters of intrauterine, pre- and post natal development were affected by RAD treatment.

**Study no:** 987105 (US-75392)
**Volume #, and page #:** v.63, p.1
**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 BrlHan:WIST, (b) (4)
Doses employed: 0, 0.03, 0.1, 0.3 mg/kg
Route of administration: oral gavage
Study design: Females were dosed 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; lactation day 21 for culled pups; pups not selected for $F_1$ fertility assessment sacrificed after behavioral tests; gestation day 13 for $F_1$ females selected for fertility assessment; $F_1$ males selected for fertility assessment sacrificed following completion of $F_1$ mating period
Number/sex/group: 25
Parameters and endpoints evaluated: mortality, food consumption, body weight, $F_1$ 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, $F_1$ mating, individual body weights)

Maternal

| dose | 0 | 0.03 mg/kg | 0.10 mg/kg | 0.30 mg/kg |
|---|---|---|---|---|
| HED (mg/kg) | | 0.005 | 0.017 | 0.050 |
| Mortality | All rats survived until terminal sacrifice | | | |
| Clinical signs | | | | |
| Body weight | | | | Sl gain gestation days 6-9 |
| Food consumption | | | Inc days 6-9 | Inc days 18-21 |
| Necropsy findings | | | 1 female (lost litter) had no milk in mammary glands | |
| Delivery data | No treatment-related effects on number of corpora lutea, pre-, post implantation loss, fetal size, litter size or other maternal or developmental parameters | | | |

fetal

| | | | | |
|---|---|---|---|---|
| Body weights | | | Dec days 28, 35, 42, 49, all; days 56, 63 & | Dec days 28, 35, 49 & |
| $F_1$ in-life examinations and measurements (pre-weaning evaluations: viability, mortality, clinical observations, sex ratio, 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, $F_1$ mating) | No treatment-related effect was seen in these studies | | | |

**Conclusions:** Maternal toxicity (mortality, loss of litter, impaired weight gain, decreased food consumption) was seen in the 0.2 and 0.8 mg/kg groups. This correlated with decreased weight gain in offspring of these groups. Other parameters of intrauterine development were unaffected. The maternal NOEL appears to be 0.03 mg/kg. Fetal weight was decreased in the 0.2 and 0.8 mg/kg groups as noted above. This appears to coincide with maternal toxicity. No other parameters of intrauterine, pre- and post natal development were affected by RAD treatment.. Due to the body weight effects at the 0.2 and 0.8 mg/kg doses, a fetal/$F_1$ NOEL was seen at the 0.05 mg/kg dose.

**Study title:** An oral neonatal and juvenile developmental dose range-finding study in rats

**Key study findings:** RAD and rapamycin treatment of juvenile rats on post partum days 7 to 27 demonstrated toxicity as reduced weight and weight gain in all treated groups, and delayed testes decent in males.

**Study no:** 0170129
**Volume #, and page #:** v. 45, p. 245
**Conducting laboratory and location:** Novartis Pharmaceutical Corporation
East Hanover, New Jersey
**Date of study initiation:** 27 Nov 2001
**GLP compliance: yes**
**QA report:** yes ( ) no ( x )
**Drug, lot #, radiolabel, and % purity:** RAD001 microemulsion, batch no. 2990001001, na, 101.2%
**Formulation/vehicle:** solution in 5 % dextrose

**Methods** (unique aspects)**:** On post partum day 0, litters were cross-fostered. Pups were assigned to dose groups and dosed from day 7 to 28 post partum. On day 28, pups were sacrificed and subjected to a gross visceral examination. Maternal rats were sacrificed and necropsied on post partum day 21.

Dosing:
Species/strain: rats, timed pregnant/Wistar Hannover, (b) (4)
#/sex/group or time point (main study): 60 males, 60 female offspring
Satellite groups used for toxicokinetics or recovery: none
Age: time mated females, 11 weeks; offspring: 7 days post partum
Weight: time mated females, 180-225 g; offspring, 12-20 g
Doses in administered units: RAD: 0, 1.5, 3, 5 mg/kg; rapamycin, 3 mg/kg
Route, form, volume, and infusion rate: oral gavage, 2 ml/kg, na

**Observations and times:**
Clinical signs: daily
Body weights: daily on post partum day 0, days 7-28
Food consumption: not performed
Ophthalmoscopy: not performed
EKG: not performed
Hematology: not performed
Clinical chemistry: not performed
Urinalysis: not performed
Gross pathology: at necropsy for offspring
Organs weighed: not performed
Histopathology: not performed
Toxicokinetics: not performed
Other: testes descent evaluation; gene expression of liver, ovary and testes

Results:

| Dose (mg/kg) | 0 | 1.5 | 3 | 5 | 3 (rapamycin) |
|---|---|---|---|---|---|
| HED (mg/kg) | | | | | |
| Mortality | All rats survived until terminal sacrifice | | | | |
| Clinical signs | No treatment-related signs were reported | | | | |
| Body weights | | Dec day 14-29 Males<br>Dec day 12-29 females | Dec day 10-29 males<br>Dec day 10-29 females | Dec day 12-29 males<br>Dec day 10-29 females | Dec day 11-29, males<br>Dec day 10-29 Females |
| Food consumption | Not performed | | | | |
| Ophthalmoscopy | Not performed | | | | |
| EKG | Not performed | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Hematology | Not performed | | | | |
| Clinical chemistry | Not performed | | | | |
| Urinalysis | Not performed | | | | |
| Organ weights | Not performed | | | | |
| Gross pathology | No treatment-related findings were reported | | | | |
| Histopathology | Not performed | | | | |
| Testes decent evaluation, days 20-25 | | Fewer than controls, day 21 | Fewer than controls, 20-21 | Fewer than controls, day 20-22 | Fewer than controls, day 20-22 |
| Gene expression | Samples frozen, no further information reported | | | | |
| Toxicokinetics | Not performed | | | | |

Conclusions: RAD and rapamycin treatment of juvenile rats on post partum days 7 to 27 demonstrated toxicity as reduced weight and weight gain in all treated groups, delayed testes decent in males. A NOEL was not established in this study.

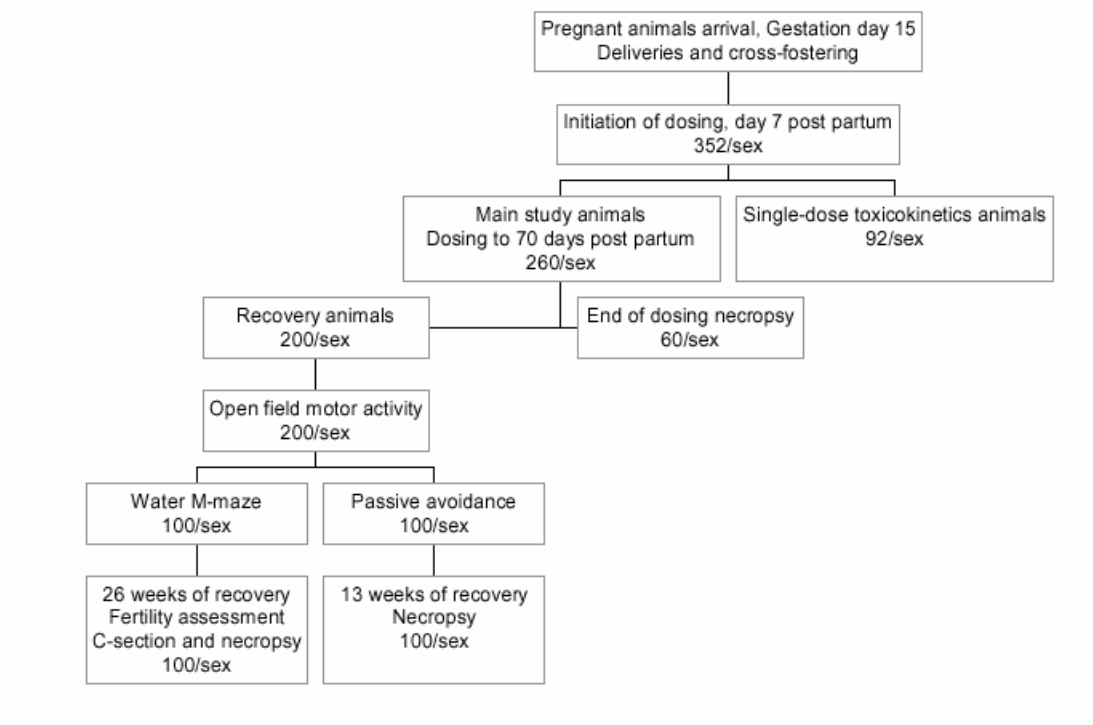
**Study title:** An oral neonatal and juvenile development study in rats with 13- and 26-week recovery periods

**Key study findings:** At doses of 0.15, 0.5 and 1.5 mg/kg RAD and 1.5 mg/kg rapamycin (day 7 to 70 post partum) toxicities were observed at all doses including decreased body weight gain, decreased food consumption and delayed developmental landmarks. Weight gain and food consumption deficits were partially reversed following a recovery period of up top 26 weeks. The 1.5 mg/kg doses of RAD and rapamycin produced toxicities of similar severity including toxicities to the lens of the eye, prostate, blood and immune tissues, testes, epididymides, seminal vesicles, ovaries, uterus, and kidney. Reduced body weight, hyperlipidemia (males), renal mineralization remained after recovery at both doses.

**Study no.:** 0270015
**Volume #, and page #:** electronic submission
**Conducting laboratory and location:** Novartis Pharmaceutical Corp
Hannover, NJ
**Date of study initiation:** 22 Jan 2002
**GLP compliance:** yes
**QA reports:** yes ( x) no ( )
**Drug, lot #, radiolabel, and % purity:** RAD001 2% microemulsion ; 2990001001, na, 101.7%; rapamycin, 2000014007, na, 101.6%
**Formulation/vehicle:** RAD 2% microemulsion in 5% dextrose solution

**Methods:**

Species/strain: rats/ timed-pregnant Wistar-Hannover female, (b) (4)
Doses employed: RAD001: 0.15, 0.5, 1.5 mg/kg; rapamycin: 1.5 mg/kg
Route of administration: oral gavage
Study design:




Terminal sacrifices were scheduled as follows: (refer to study design above)
Day 7/8 for post partum animals
After day 70 post partum for the first 12 main study animals
After at least 13 weeks of recovery for animals assigned to the passive avoidance test
Day 13 of gestation for main study females selected for fertility assessment
Main study females selected for fertility assessment for which evidence of mating not seen sacrificed 11 days after end of mating period
Main study males selected for fertility assessment sacrificed following end of mating period
Maternal animals sacrificed at weaning of litters or litter loss
Males used for mating with main study females sacrificed following end of mating period
Day 13 of gestation for females used for mating with study males selected for fertility assessment
Females used for mating without evidence of mating sacrificed 11 days after end of mating period

Number/sex/group: see above schematic
Parameters and endpoints evaluated:
Mortality: daily
clinical signs: daily
body weight: mainstudy: pp day 0, 4, 7-21, twice weekly until day 70, recovery day 1, 4, 7, 14, 21 until necropsy; mated/fertility assessment: gd 0, 3, 6, 9, 13; single dose animals: pp day 0, 4, 7; maternal animals: gd 0, pp days 0, 7, 14, 21; males and females used for mating w main study animals: prior to mating; mated females not pregnant: gd 0, 3, 6, 9, 13
food consumption: main study; weekly after pp day 28, weekly during recovery; mated females for fertility: gd 0, 3, 6, 9, 13
preweaning evaluations: eye opening, days 14-17 pp; testes descent, days 20-28 pp
post-weaning evaluations: acoustic startle, day 28 pp; pupillary reflex, day 35; vaginal opening, days 32-50; preputial separation, days 42-60; open field motor activity, recovery week 3-4; water maze: recovery week 4-5; passive avoidance, week 4-5; fertility, after 26 weeks of recovery
vaginal cytology: main study females, days 57-70 pp
opthamology: first week after weaning, end of dosing period, after13, 26 weeks recovery
fertility assessment: after 26 weeks recovery

hematology: after 13 weeks recovery
clinical chemistry: after 13 weeks recovery
urinalysis: after 13 weeks recovery
testosterone: after 26 weeks recovery
histopathology: at necropsy, main study animals
sperm counts, motility: at necropsy from main study animals
corpora lutea, uterine implantation examination: day 13 gestation
pharmacokinetics: single dose animals: pp day 7; main study animals, pp day 70, 1, 3, 8, 24 h after last dose

**Results:**

Mortality: due to apparent dosing accidents: control: 1 m, 1 f; RAD 0.5 mg/kg: 1m, 2 f; RAD 1.5 mg/kg: 1 m, 2 f; rapa 1.5 mg/kg: 5 f. sacrificed moribund; rapa 1.5 mg/kg, 1 m, 1 f, gastric ulceration; RAD 0.5 mg/kg 1 m found dead during recovery, day 173 of study; death attributed to renal disease but not explained
Clinical signs: RAD 0.15 mg/kg: unilateral protruding eye, 2 m; RAD 0.5 mg/kg: unilateral protruding eye, 1m, 2 f; unilateral absent testes, 1m; RAD 1.5 mg/kg: unilateral protruding eye, 2 f; unilateral small/absent testes, 1 m; distended abdomen, 1 m, 1 f; dehydration, 1f; rapa 1.5 mg/kg: unilateral protruding eye, 3 m

Body weight: % difference from control

| | RAD 0.15 mg/kg | RAD 0.5 mg/kg | RAD 1.5 mg/kg | Rapa 1.5 mg/kg |
|---|---|---|---|---|
| Dosing day 64 | | | | |
| Males | -5 | -17* | -36* | -33* |
| Females | -3 | -12* | -26* | -23* |
| Recovery week 13 | | | | |
| Males | -3 | -7 | -15* | -11* |
| Females | -2 | -8* | -13* | -12* |
| Recovery week 26 | | | | |
| Males | -2 | -3 | -14* | -11* |
| Females | +3 | -1 | -11* | -5 |

* statistically significant

Food consumption: In males, decreased RAD 0.15 mg/kg days 22-36
RAD 0.5 mg/kg days 22-57
RAD 1.5 mg/kg days 22-64
Rapa 1.5 mg/kg days 22-64
In females, decreased RAD 0.5 mg/kg days 22-50
RAD 1.5 mg/kg days 22-64
Rapa 1.5 mg/kg days 22-64

Toxicokinetics:

| Drug, Dose (mg/kg) | AUC day 7 pp (ng.h/ml) | AUC day 70 pp (ng.h/ml) | Cmax (ng/ml) Day 7 pp | Cmax (ng/ml) Day 70 pp |
|---|---|---|---|---|
| RAD 0.15 | | | | |
| Males | 196 | 16.7 | 10.7 | 1.3 |
| Females | 170 | 5.7 | 9.0 | 1.0 |
| RAD 0.5 | | | | |
| Males | 548 | 71.0 | 35.3 | 8.9 |
| Females | 637 | 61.8 | 31.1 | 7.5 |
| RAD 1.5 | | | | |
| Males | 1600 | 248 | 93.5 | 26.8 |
| Females | 1470 | 265 | 77.8 | 35.0 |
| Rapa 1.5 | | | | |
| Males | 962 | 223 | 57.6 | 17.5 |
| females | 1010 | 245 | 62.5 | 34.7 |

In-life observations:
Offspring:
Pre-weaning observations: dec incidence of eye opening, females, RAD 1.5 mg/kg, rapa 1.5 mg/kg
delayed testes descent, all treated groups
Post-weaning observations: delayed preputial separation: all treated groups
delayed vaginal opening: all treated groups
increased open field activity, RAD 0.15 (f) 0.5 (m), 1.5 (m+f) mg/kg, rapa (m+f)1.5 mg/kg
increased time to criterion, water maze, males, RAD 0.5,1.5 mg/kg, rapa 1.5 mg/kg
Vaginal cytology: inc abnormal estrous cycles: rapa 1.5 mg/kg
Opthalmology: males, females: RAD dose related inc zones of discontinuity, focal cataracts, treatment week 8, recovery week 13, 26; rapa 1.5 mg/kg similar to RAD 1.5 mg/kg
Fertility assessment: no RAD or rapa effect on treated females; males, dec fertility, fecundity at RAD 1.5 mg/kg, rapa 1.5 mg/kg
Hematology: inc WBC: RAD 0.15 mg/kg, 0.5 mg/kg (m)
Dec lymphocytes: RAD 1.5 mg/kg, rapa 1.5 mg/kg; sl inc during recovery, all treated groups
Inc RBC, HGb, HCT; RAD 0.5, 1.5 mg/kg; rapa 1.5 mg/kg
Clinical biochemistry: dec serum protein, albumin: all treated males, females 1.5 mg/kg RAD and rapa; cholesterol and triglycerides: inc, all treated males; testosterone: dec, all treated groups;
Creatinine: inc, 1-2 males in each treatment group
Urinalysis: inc acid urine: males, RAD 0.5, 1.5 mg/kg, rapa 1.5 mg/kg

Terminal and necroscopic evaluations:
Offspring:
Gross pathology: sm epididymides: rapa 1.5 mg/kg
sm testes, prostate, seminal vesicles: all treated males
lung trauma, abdominal distension, gastric ulceration: rats found dead, probably due to gavage error
Histopathology: renal tubular mineralization: all treated groups
Thymic lymphocytes, single cell necrosis: RAD 1.5 mg/kg, rapa 1.5 mg/kg

**Summary of individual study findings:**
At doses of 0.15, 0.5 and 1.5 mg/kg RAD and 1.5 mg/kg rapamycin (day 7 to 70 post partum) toxicities were observed at all doses including decreased body weight gain, decreased food consumption and delayed developmental landmarks. Weight gain and food consumption deficits were partially reversed following a recovery period of up top 26 weeks. The 1.5 mg/kg doses of RAD and rapamycin produced similar toxicities of similar severity including toxicities to the lens of the eye, prostate, blood and immune tissues, testes, epididymides, seminal vesicles, ovaries, uterus, and kidney. Reduced body weight, hyperlipidemia (males), renal mineralization remained after recovery at both doses. Rapamycin produced higher incidence of small epididymides, testes, prostate and seminal vesicles than RAD at the 1.5 mg/kg dose. The small size of the male and female reproductive organs was not accompanied by related histopathology findings.

**3.4.8 SPECIAL TOXICOLOGY STUDIES**

**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 RAD high dose (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 of both strains wee more susceptible 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** #: v. 44, p. 1
**Conducting laboratory and location:** (b) (4)

(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, (b) (4) HanIbm Wistar rats, (b) (4)
**#/sex/group or time point (main study):** 5/strain
**Satellite groups used for toxicokinetics or recovery:** none
**Age:** 6 weeks, 18 weeks (both CD-1 and Han Ibm)
Weight: 6 week CD-1: 229-254g (males); 169-187g (females); 18 week CD-1: 466-526 g (males), 310-339g (females); 6 week HanIbm: 163-183g (males), 114-135g (females); 18 week HanIbm: 410-507g (males), 217-237g (females)
Doses in administered units: 0, 0.5, 5.0 mg/kg Both strains, age groups
Route, form, volume, and infusion rate: oral gavage, 5 ml/kg, na

**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 opthalmoscope 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:

| | CD-1 | | | | HanIbm | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 week | | 18 week | | 6 week | | 18 week | |
| Dose (mg/kg) | 0.5 | 5.0 | 0.5 | 5.0 | 0.5 | 5.0 | 0.5 | 5.0 |
| HED (mg/kg) | 0.084 | 0.84 | 0.084 | 0.84 | 0.084 | 0.84 | 0.084 | 0.84 |
| Mortality | | | | | | 1 male died week 4 no clin signs | | |
| Clinical signs | No treatment-related signs were seen | | | | | | | |
| Body weights | | | | loss | | | | Loss |
| Food consumption | | dec | | dec | | dec | | Dec |
| 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 preformed | | | | | | | |
| Urinalysis | Not performed | | | | | | | |
| Organ weights | | Lungs, inc males Prostate, dec, males Thymus, dec, all Spleen, dec, all | | Lungs, inc, males Prostate, dec, males Uterus, dec, females Spleen, dec, all | | Lungs, inc, males, females Prostate, dec, males Thymus, dec, all Testes, dec, males | | Lungs, inc, males Prostate, dec, males Thymus, dec, all Pituitary , dec, females Testes, dec, males Uterus, dec, females Epididimydes , dec, males |
| 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, the high dose (5.0 mg/kg) exhibited frank toxicity to the lens of both strains as swelling and disruption of the fibers of the anterior cortex. Toxicity to islet cells of the pancrease, 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 wee more susceptible to this toxicity. Myocarditis was exacerbated at both doses and both ages in both strains compared to the incidence in controls. The NOAEL for eye toxicity in this study was 0.5 mg/kg. Otherwise a NOAEL was not observed in this study.

**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 #:** v. 45, p. 1
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

**Methods** (unique aspects)**:**
**Dosing:**

Species/strain: HanIbm rats, males
#/sex/group or time point (main study): 20 (controls, 15 mg/kg), 10 (0.15, 1.5 mg/kg)
Satellite groups used for toxicokinetics or recovery: none
Age: 12 weeks
Weight: 295-369 g
Doses in administered units: 0, 0.15, 1.5, 15 mg/kg
Route, form, volume, and infusion rate: oral gavage, 5 mg/ml, na

**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:

| Dose (mg/kg) | 0 | 0.15 | 1.5 | 15 | 15 | 0 |
|---|---|---|---|---|---|---|
| HED (mg/kg) | | | | | | |
| Mortality | All rats survived until scheduled sacrifice | | | | | |
| Clinical signs | No treatment-related signs were seen | | | | | |
| Body weights | | | | dec | dec | |
| Food consumption | | | dec | dec | dec | |
| Ophthalmoscopy | Not performed | | | | | |
| EKG | | | | Heart rate dec 10% | Heart rate dec 10% | |
| Hematology | | | Inc hematocrit, RBC, HgB Platelets, dec Leukocytes, dec | Inc hematocrit, RBC, HgB Platelets, dec Leukocytes, dec Neutrophils inc | Inc hematocrit, RBC, HgB Platelets, dec Leukocytes, dec Neutrophils inc | |
| Clinical chemistry | | | Dec ASAT Dec CK Dec LDH Cholesterol inc | Dec ASAT Dec CK Dec LDH Cholesterol inc Testosterone dec | Dec ASAT Dec CK Dec LDH Cholesterol inc Testosterone dec | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Urinalysis | | No treatment-related findings reported | | | | |
| Organ weights | | | | | | |
| Gross pathology | | No treatment-related findings reported | | | | |
| Histopathology | | | | | | |
| Thymus | | | | | | |
| Medullary | | | 10/10 | 10/10 | 10/10 | |
| Atrophy | | | | | | |
| 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 | | | | | | |
| Mammary atrophy | 1/10 | | 9/10 | 9/10 | 10/10 | 2/10 |
| Testes | | | | 6/10 | 6/10 | |
| Germ cell degeneration | | | | | | |
| Vacuoles/seminal epithelium | | | 1/10 | 10/10 | 10/10 | |
| Giant multinucleated cells | | | 1/10 | 7/10 | 9/10 | |
| Tubular atrophy | | | | | | |
| Prostate | | | | 1/10 | 3/10 | |
| Atrophy | | | | 2/10 | 3/10 | |
| Seminal vesicles | | | | | | |
| Atrophy | | | | 9/10 | 7/10 | |
| Sternum –bone marrow depletion | | | 5/10 | 8/10 | 9/10 | 1/10 |
| | 1/10 | 1/10 | 2/10 | 5/10 | 8/10 | 1/10 |
| 2-d gel electrophoresis heart tissue | | | | Tubulin-$, "-1-antiproteinase, apolipoprotein E precursor, transeferrin dec Apolipoprotein E inc | | |
| serology | Antibody against Coxsackie virus B3 | | | | | |
| Toxicokinetics | Not performed | | | | | |

Conclusions: 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. Without a basis for the 2-D gel electrophoresis findings, they are of unknown value.

**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:** (b) (4) project 246792 (BS-812)

**Volume #, and page #:** V. 57, p. 1
**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 #, radiolabel, and % purity:** RAD, batch 98902, na, 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 treated with 10% SDS. Two weeks after epidermal application all animals challenged with 50% RAD and vehicle.

**Dosing:**

Species/strain: guinea pig/albino, females, Dunkin Hartley strain, (b) (4)
#/sex/group or time point (main study): 10 treated, 5 control
Satellite groups used for toxicokinetics or recovery:
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.

**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:** (b) (4) Project 246781 (BS-811)
**Volume #, and page #:** v. 57, p. 17
**Conducting laboratory and location:** (b) (4)

**Date of study initiation: 27-30 Oct, 1998**
**GLP compliance: no**
**QA report:** yes ( ) no ( x )
**Drug, lot #, radiolabel, and % purity:** RAD, batch 98902, na, 93.5%

**Formulation/vehicle:** solution in water

**Methods** (unique aspects)**:**
**Dosing:**

Species/strain: rabbit/albino New Zealand White, (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.

**Histopathology Inventory for NDA 21560**

| Study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Species | Dog | 2 wk Mini-pig | 4 wk mini-pig | 28-day cyno | 26 wk cyno | 52 wk cyno | 2 week rat iv | 2 week minipig iv |
| Adrenals | | X* | X* | X* | X* | X* | X* | X* |
| Aorta | | x | X | x | x | x | x | x |
| Bone Marrow smear | | | | x | | x | | |
| Bone (femur) | | x | X | | x | x | x | x |
| Brain | | X* | X* | X* | X* | X* | X* | X* |
| Cecum | | | X | x | x | x | x | x |
| Cervix | | | | x | X* | x | x | |
| Colon | | | X | | x | x | x | x |
| Duodenum | X | x | X | x | x | x | x | x |
| Epididymis | | | X | X* | X* | X* | X* | x |
| Esophagus | X | x | X | x | x | x | | |
| Eye | | x | X | x | x | x | x | x |
| Fallopian tube | | | | | x | | | |
| Gall bladder | | x | X | x | x | x | | x |
| Gross lesions | | | X | | x | | | x |
| Harderian gland | | | | | | | | |
| Heart | | X* | X* | X* | X* | X* | X* | X* |
| Ileum | | x | X | x | x | x | x | x |
| Injection site | | | | | | | | x |
| Jejunum | | x | X | x | x | x | x | x |
| Kidneys | | X* | X* | X* | X* | X* | X* | X* |
| Lachrymal gland | | | | | | | | |
| Larynx | | | | | | | | |
| Liver | | X* | X* | X* | X* | X* | X* | X* |
| Lungs | X | x | X | X* | X* | x | X* | X |
| Lymph nodes, cervical | | | | | | | | |
| Lymph nodes mandibular | | x | X | x | x | x | x | X |
| Lymph nodes, mesenteric | | x | X | x | x | x | x | X |
| Mammary Gland | | x | X | | x | x | x | X |
| Nasal cavity | | | | | | | | |
| Optic nerves | | x | X | x | | x | x | X |
| Ovaries | | X* | X* | X* | X* | X* | X* | X* |
| Pancreas | | X* | X* | X* | X* | x | x | X |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parathyroid | | x | X | X* | X* | X* | X* | X |
| Peripheral nerve | | | | | | | | |
| Pharynx | | | | | | | | |
| Pituitary | | X* | X* | X* | X* | X* | X* | X* |
| Prostate | | X* | X* | X* | X* | X* | X* | X* |
| Rectum | | | | x | x | x | x | X |
| Salivary gland | | x | X | x | X* | x | X* | X |
| Sciatic nerve | | x | X | x | x | | x | X |
| Seminal vesicles | | | X | X* | X* | X* | X* | X |
| Skeletal muscle | | x | X | x | x | x | x | X |
| Skin | | x | X | x | x | x | x | X |
| Spinal cord | | x | X | x | x | x | x | |
| Spleen | | X* | X* | X* | X* | x | X* | X* |
| Sternum | | x | X | x | x | x | x | X |
| Stomach | X | x | X | x | x | x | x | X |
| Testes | | X* | X* | X* | X* | X* | X* | X* |
| Thymus | | X* | X* | X* | X* | X* | X* | X* |
| Thyroid | | x | X* | X* | X* | X* | X* | X* |
| Tongue | | x | X | x | x | x | x | X |
| Trachea | | x | X | x | x | x | x | X |
| Urinary bladder | | x | X | x | x | x | x | X |
| Uterus | | x | X* | X* | X* | X* | X* | X* |
| Vagina | | x | X | | x | x | x | X |
| Zymbal gland | | | | | | | | |
| Standard List | | | | | | | | |
| | | | | | | | | |

**X, histopathology performed**

*, organ weight obtained

| Study | 4 week iv | 4 week iv | 4 week FTY/RAD | 4 wk FTY/RAD | 4 week RAD/MTX | 2yr carcinogen | 2yr carcinogen | |
|---|---|---|---|---|---|---|---|---|
| Species | Cyno | Cyno | Rat | cyno | rat | mouse | rat | |
| Adrenals | X* | X* | X* | X | X* | x | x | |
| Aorta | X | | X | X | x | x | x | |
| Bone Marrow | x | X | X | | | | | |
| Bone (femur) | X | X | | | | | x | |
| Brain | X* | X* | X* | X* | X* | x | x | |
| Cecum | X | X | X | X | x | x | | |
| Cervix | | | | | | x | x | |
| Colon | X | X | X | X | x | x | x | |
| Duodenum | X | X | X | X | x | x | x | |
| Epididymis | x | X | X | X | x | x | x | |
| Esophagus | | X | X | X | x | x | x | |
| Eye | X | X | X | X | x | x | x | |
| Fallopian tube | | | | | | | | |
| Gall bladder | X | X | | X | | x | | |
| Gross lesions | X | X | | | | | | |
| Harderian gland | | | | | | x | x | |
| Heart | X* | X* | X* | X* | X* | x | x | |
| Ileum | X | X | X | X | x | x | x | |
| Injection site | X | X | | | | | | |
| Jejunum | X | X | X | X | x | x | x | |
| Kidneys | X* | X* | X* | X* | X* | x | x | |
| Lachrymal gland | | | X | | x | x | | |
| Larynx | | | | | | | | |
| Liver | X* | X* | X* | X* | X* | x | x | |
| Lungs | X* | X* | X* | X* | X | x | x | |
| Lymph nodes, cervical | X | X | | | | | | |
| Lymph nodes mandibular | | | X | | x | x | x | |
| Lymph nodes, mesenteric | X | X | X | Bronchial | x | x | x | |
| Mammary Gland | | X | X | X | x | x | x | |
| Nasal cavity | | | | | x | | | |
| Optic nerves | X | X | X | | x | x | x | |
| Ovaries | X* | X* | X* | | X* | x | x | |
| Pancreas | X* | X* | X | X | x | x | x | |
| Parathyroid | X* | X* | X* | X* | x | x | x | |
| Peripheral nerve | | | X | | x | | | |
| Pharynx | | | | | | | | |
| Pituitary | X* | X* | X* | X* | X* | | x | |
| Prostate | X* | X* | X* | X* | X* | x | x | |
| Rectum | X | X | X | X | x | x | x | |
| Salivary gland | X | X | X | X | x | x | x | |
| Sciatic nerve | X | X | | X | | x | x | |
| Seminal vesicles | X | X | X | X | x | x | x | |
| Skeletal muscle | X | X | X | X | x | x | x | |
| Skin | X | X | X | X | x | x | x | |
| Spinal cord | X | X | X | X | x | x | x | |
| Spleen | X* | X* | X* | X* | X* | x | x | |
| Sternum | X | X | X | X | x | x | x | |
| Stomach | X | X | X | X | x | x | x | |
| Testes | X* | X* | X* | X* | X* | x | x | |
| Thymus | X* | X* | X* | X* | X* | x | x | |
| Thyroid | X* | X* | X* | X* | X* | x | x | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tongue | X | X | X | X | x | x | x | |
| Trachea | X | X | X | X | x | x | x | |
| Urinary bladder | X | X | X | X | x | x | x | |
| Uterus | X* | X | X* | | X* | x | x | |
| Vagina | X | X | X | | x | x | x | |
| Zymbal gland | | | | | | | | |
| Standard List | | | | | | | | |
| | | | | | | | | |

**X, histopathology performed**

*, organ weight obtained

### 3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

The New Drug Application for Certican is approvable based on the preclinical pharmacology and toxicology submission. Toxicities demonstrated in preclinical studies may be monitored or be superceded by the benefit/risk ratio of clinical concerns supporting use of this drug.
It is recommended that possible impurities arising from the manufacturer or degradation of everolimus be qualified according to ICH guidelines. It is also recommended that the sponsor elucidate the mechanism of toxicity to the female reproductive organs, possibly to determine any hormonal changes caused by everolimus

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/s/

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