CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-088

PHARMACOLOGY REVIEW

MEMORANDUM
May 30, 2007

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 22,088

I concur with Drs. Saber, Verbois, and Leighton that the marketing application for Torisel (temsirolimus) may be approved based on review of submitted nonclinical data. Given that temsirolimus is metabolized to sirolimus, a previously approved drug, it is acceptable to rely on safety data obtained and submitted for the later. The final label does not contain dose or exposure data at which rodent tumors were observed, but these studies were conducted with sirolimus and conversion of comparative data would is not necessary, especially given the indication. I concur with the determination that temsirolimus should be labeled Pregnancy Category D.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

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/s/

Kenneth Hastings
5/30/2007 04:29:26 PM
PHARMACOLOGIST

ADDENDUM REVIEW
NDA 22,088
Temsirolimus

Pharmacology/Toxicology Reviewer: Haleh Saber, Ph.D.
Supervisory Pharmacologist: John Leighton, Ph.D.
Sponsor: Wyeth Pharmaceuticals, Inc.
Date of addendum review submission to DFS: 5/23/07

The following information has been proposed by the sponsor of NDA 22,088 for section 13.1 of the PLR labeling:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with temsirolimus. However, sirolimus, the major metabolite of temsirolimus in humans, was carcinogenic in mice and rats. The following effects were reported in mice and/or rats in the carcinogenicity studies conducted with sirolimus: lymphoma, hepatocellular adenoma and carcinoma, and testicular adenoma.

Temsirolimus was not genotoxic in a battery of *in vitro* (bacterial reverse mutation in *Salmonella typhimurium* and *Escherichia coli*, forward mutation in mouse lymphoma cells, and chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse micronucleus) assays.

In male rats, the following fertility effects were observed: decreased number of pregnancies, decreased sperm concentration and motility, decreased reproductive organ weights, and testicular tubular degeneration. These effects were observed at oral temsirolimus doses ≥ 3 mg/m^2/day (approximately 0.2-fold the human recommended intravenous dose). Fertility was absent at 30 mg/m^2/day.

In female rats, an increased incidence of pre- and post-implantation losses occurred at oral doses ≥ 4.2 mg/m^2/day (approximately 0.3-fold the human recommended intravenous dose), resulting in decreased numbers of live fetuses.

Reviewer's comments:

"In male rats…These effects were observed at oral temsirolimus **doses ≥ 3 mg/m^2/day**".

The dose of ≥ 3 mg/m^2/day temsirolimus has been accepted since fertility effects were evident and statistically significant at 3 mg/m^2/day, although some effects started to manifest at a lower dose (0.6 mg/m^2/day).

"These effects were observed at oral temsirolimus doses ≥ 3 mg/m^2/day (approximately **0.2-fold** the human recommended intravenous dose)".

A comparison of an oral daily dose (animals) and an i.v. weekly dose (humans) is not appropriate. However, the sponsor's language has been accepted. A comparison of the weekly doses taking into account the oral bioavailability (~5%

in rat), results in an estimation that is close to that proposed by the sponsor. Both values show fertility effect in rats at human sub-therapeutic doses.
3/mg/m^2/day x 7 x 0.05= 1.05 mg/m^2/week
1.05 mg/m^2/week (rat) : 14.7 mg/m^2/week (human) = **0.07**

"In female rats, an increased incidence of pre- and post-implantation losses occurred at oral doses ≥ 4.2 mg/m^2/day (approximately **0.3-fold** the human recommended intravenous dose), resulting in decreased numbers of live fetuses."

As previously stated, a comparison of an oral daily dose and an i.v. weekly dose is not appropriate. However, the sponsor's language with regard to the rat/human ratio has been accepted (see previous page).
4.2/mg/m^2/day x 7 x 0.05= 1.47 mg/m^2/week
1.47 mg/m^2/week (rat) : 14.7 mg/m^2/week (human) = **0.1**

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/s/

Haleh Saber
5/23/2007 10:39:54 AM
PHARMACOLOGIST

John Leighton
5/25/2007 12:28:02 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22,088**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**10/05/2006**
PRODUCT:	**Torisel™ (temsirolimus)**
INTENDED CLINICAL POPULATION:	**Advanced Renal Cell Carcinoma**
SPONSOR:	**Wyeth Pharmaceuticals, Inc.**
DOCUMENTS REVIEWED:	**Pharmacology/Toxicology**
REVIEW DIVISION:	**Division of Drug Oncology Products**
PHARM/TOX REVIEWER:	**Haleh Saber, Ph.D.**
PHARM/TOX SUPERVISOR:	**S. Leigh Verbois, Ph.D., Acting Team Leader**
DIVISION DIRECTOR:	**Robert Justice, M.D.**
PROJECT MANAGER:	**Carl Huntley, R.Ph.**

Date of review submission to Division File System (DFS): 3/21/07

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There are no Pharmacology/Toxicology issues which preclude approval of the product for the requested indication.

B. Recommendation for nonclinical studies: None.

C. Recommendations on labeling: Have been provided within the team meetings.

II. Summary of nonclinical findings

Note: Since the proposed route of administration in renal cell carcinoma (RCC) patients is intravenous (i.v.), the studies reviewed were mainly those in which drug was given via the intravenous route. Because the metabolic profile of the drug after oral administration to rats and monkeys resembles that after intravenous administration to humans, few oral nonclinical studies were reviewed. Reproductive toxicology studies were conducted as oral administration only. For a comprehensive understanding of safety issues in humans, toxicities associated with sirolimus (the major metabolite of temsirolimus) should be considered.

A. Brief overview of nonclinical findings

After intravenous dosing, excretion was via hepatobiliary route in rats and monkeys. Based on the i.v. studies conducted with radiolabeled temsirolimus (^{14}C-CCI-779) in rats, distribution of radioactivity was rapid, with a T_{max} of ~5 min for most tissues. The radioactive products remained in tissues for an extended period of time as indicated by the $T_{1/2}$, which ranged from 35 hrs (heart) to 78 hrs (stomach). CYP 3A4 was the major cytochrome P450 involved in metabolism of temsirolimus. Temsirolimus was the major circulating product after intravenous drug administration to rats and monkeys. Temsirolimus and sirolimus were the major products after intravenous drug administration to humans. The metabolic profile of temsirolimus after intravenous drug administration resembled that seen in rats and monkeys after oral administration.

Safety pharmacology studies showed drug-related respiratory effects: single intravenous doses of temsirolimus in male rats, at 0.2-5 mg/kg (1.2-30 mg/m^2) resulted in reduced respiratory rates of 9-11% at 2 hrs post-dose. Respiratory toxicities were also seen in the toxicology studies: alveolar macrophage infiltration and inflammation was observed in the lungs of rats in repeat-dose toxicology studies as short as 14 days. In the single i.v. safety pharmacology study conducted in Cynomolgus monkeys, there was a tendency for reduced heart rate (approximately 10-25%), in the first 1.5 hrs post-dose. Cardiac events in the repeat-dose toxicology studies were seen in rats and consisted of: myocardial degeneration in the 14-day study and increased incidence of cardiomyopathy in the 6-month study.

Toxicities after repeat-dose intravenous toxicology studies in rats and/or monkeys included lymphoid atrophy, increased glucose, pancreatic islet cell vacuolation, increased

cholesterol, increased fibrinogen, myocardial degeneration, GI toxicity, hypokalemia, hypophosphatemia, inflammation and alveolar macrophages in the lung, renal effects (increased BUN and creatinine, tubular degeneration), coagulation effects (increased fibrinogen and aPTT, and reduced platelets), and effects in the male reproductive system (e.g. testicular atrophy and tubular degeneration, hypospermia, and immature epididymides). Of note, exposures to temsirolimus were generally higher in males in both species tested, which resulted in more pronounced toxic effects in male animals. Based on the 6-month oral toxicity study in rats, although toxicities were generally comparable in the intravenous and oral dosing, effects were more evident following i.v. dosing. Hepatotoxicity was more pronounced following oral administration of the drug. Reduced toxic effects after oral dosing may be due to reduced systemic exposures. Toxicokinetic data were not available for the oral study reviewed; however, based on the summary of absorption studies, the oral bioavailability was low and estimated to be only 5% in rats, in 4-cycle toxicity studies.

Temsirolimus was not genotoxic in the Ames Test, the TK+/- mouse lymphoma forward mutation assay, the chromosomal aberration study in Chinese hamster ovary (CHO) cells, and the in vivo mouse micronucleus assay. Although these studies were negative, temsirolimus should be considered potentially carcinogenic in humans due to the data available for sirolimus. According to the labeling for sirolimus (NDA 21,083), the following effects were reported in mice and/or rats in the carcinogenicity studies: lymphoma, hepatocellular adenoma and carcinoma, and testicular adenoma.

Administration of oral temsirolimus to male rats for a period of ~70 days prior to co-habitation and 14 days during co-habitation resulted in reduced fertility in male animals. Treatment of female rats with temsirolimus at doses up to 1 mg/kg or 6 mg/m^2 (2 weeks prior to co-habitation through GD6) did not affect the estrous cycle, mating index, fertility index, or the conception rate. Embryo-fetal reproductive toxicity studies showed uterine and embryo-fetal toxicities in rats and rabbits at human sub-therapeutic exposures. Effects included the following:

- Uterine (rats and rabbits): ↑resorption, ↑post-implantation loss, ↓litter size.
- Embryo-fetal (rat): ↓fetal weight, and ↓ossification of sternabrae and vertebral centra.
- Embryo-fetal (rabbit): ↓fetal weight, omphalocele, fused or bifurcated sternabrae, ↑incidence of notched ribs/ incomplete ossification of pubic bone/ incomplete ossification of frontal bone

B. Pharmacologic activity

Temsirolimus is an analog (ester) of sirolimus (rapamycin [Rapamune®]). In humans, after i.v. or oral dosing, temsirolimus is converted to sirolimus, with a ratio of sirolimus:temsirolimus > 1 (~2.7 after i.v. dosing and ~12 after oral dosing). Therefore, temsirolimus may be considered a pro-drug for sirolimus. After i.v. dosing of temsirolimus to rats and monkeys, sirolimus was either not detected or was detected at low levels.

Primary Pharmacology:

Pharmacologic class: Kinase Inhibitor
Target of the drug: mTOR

For pharmacologic highlights in the product label, the term "Kinase Inhibitor" was chosen as consistent with other products in this class.

Temsirolimus is an inhibitor of the mammalian target of rapamycin (mTOR, also known as FRAP). mTOR is a serine/threonine kinase involved in cell proliferation. This kinase is downstream of multiple pathways, including growth factors, insulin, and nutrients (Hay N & Sonenberg N 2004, Genes & Dev. 18: 1926-1945; Kwon G et al. Diabetes 2004, 53: S225-232).

Temsirolimus inhibited growth of several tumor cells with IC50's ranging from nM's to μM's. In in vitro studies, under the conditions tested, temsirolimus inhibited the mTOR pathway and the production of HIF-2 α transcription factor in the RCC cell line A498.

Secondary Pharmacology: Activity of mTOR has been implicated in the proliferation of lymphocytes. Therefore, effects of temsirolimus on T-cell response were tested in an in vivo study. Under the conditions tested in mice, temsirolimus treatment resulted in transiently delayed T cell response to a contact sensitizing agent, during dosing with the drug.

C. Nonclinical safety issues relevant to clinical use

Nutrient availability influences mTOR. mTOR is downstream of multiple pathways, including growth factors, insulin, and nutrients (e.g. glucose). Therefore, while temsirolimus will inhibit mTOR, the increased blood glucose levels (a side effect of temsirolimus treatment) may activate mTOR. Although clinically unknown at this time, this may result in resistance/insensitivity to the initial dose of temsirolimus if hyperglycemia is not controlled. In addition, when controlling the hyperglycemic conditions in patients, insulin may not be an appropriate treatment, since it may activate mTOR.

In humans, sirolimus is a major metabolite of temsirolimus. The ratio of temsirolimus:sirolimus (AUC) was approximately 2.7 after i.v. dosing. Therefore, for a comprehensive safety evaluation of temsirolimus, toxicities associated with sirolimus should be considered. Although carcinogenicity studies were not conducted with temsirolimus and genotoxicity studies were negative, temsirolimus should be considered potentially carcinogenic in humans due to the data available for sirolimus. According to the labeling for sirolimus, the following effects were reported in mice and/or rats in the carcinogenicity studies: lymphoma, hepatocellular adenoma and carcinoma, and testicular adenoma.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22,088
Review number: 1
Sequence number/date/type of submission: 000/Oct 5, 2006/ Original NDA
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Wyeth Pharmaceuticals, Inc.
Manufacturer for drug substance: [redacted]
Wyeth Pharmaceuticals
64 Maple Street
Rouses Point, NY 12979

[redacted]

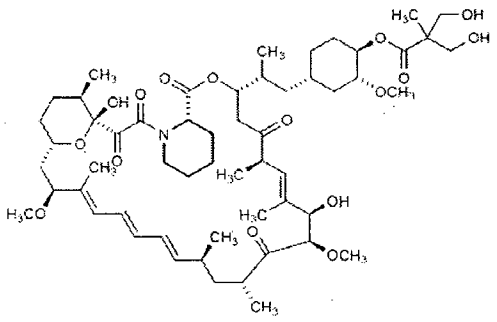
Reviewer name: Haleh Saber, Ph.D.
Division name: Division of Drug Oncology Products
Review completion date: 3/20/2007

Drug:

Trade name: Torisel™
Generic name: temsirolimus
Code name: CCI-779, WAY 130779
Chemical name: Rapamycin 42-ester with 2,2-bis(hydroxymethyl)-propionic acid

Rapamycin 42-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]

CAS registry number: 162635-04-3
Molecular formula/molecular weight: $C_{56}H_{87}NO_{16}$/ 1030.3
Structure:



Relevant INDs/NDAs/DMFs: IND 55,830

Pharmacologic class: kinase inhibitor
Target of the drug: mTOR serine/threonine kinase

Intended clinical population: "Treatment of advanced renal cell carcinoma" (RCC)

Clinical formulation:
"Temsirolimus [redacted] Injection," 25 mg/mL is a non-aqueous, ethanolic, sterile solution. When combined appropriately with separately manufactured, sterile "Diluent for Temsirolimus [redacted] Injection", the concentrate/diluent combination, at a concentration of 10 mg temsirolimus/mL, will be suitable for dilution in 0.9% Sodium Chloride Injection for intravenous administration.

Quantitative composition of "Temsirolimus [redacted] Injection, 25 mg/mL"; nominal fill volume is 1.2 mL.

Ingredient	Function	Unit Dose	
		mg/mL	% w/v
Temsirolimus	Active ingredient	25.00	2.50
dl-alpha tocopherol (vitamin E)	[redacted]	0.75	0.075
Dehydrated alcohol (anhydrous ethanol)		[redacted]	
Anhydrous citric acid		0.025	0.0025
Propylene glycol		503.325	50.33
[redacted]		Ad lib	Ad lib
Total		**923.7 mg (1.0 mL)**	**100% (v)†**

* Used for blanketing the bulk solution, and as inert cover in the filled vials.
† Solution density = 0.9237

Composition of "Diluent for Temsirolimus [redacted] ; nominal fill volume is [redacted]

Ingredient	Unit Formula	Function
Polysorbate 80	[redacted]	
Dehydrated Alcohol (Ethanol, Anhydrous)[a]		
Polyethylene Glycol 400 (Macrogol 400)[b]		
[redacted]		

Route of administration: i.v., 30-60 min infusion
Drug to be administered once weekly at 25 mg

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise. The single dose toxicology study in mice (Report GTR-31708) and the repeat-dose toxicity studies (GTR-30878, GTR-30341, GTR-31183, GTR-30340, and GTR-31575) were reviewed as part of the original IND submission. Dr. David Morse was the secondary reviewer of this NDA.

Studies reviewed within this submission: see Table below
Note: since the proposed route of administration in RCC patients is intravenous, the studies reviewed were mainly those in which drug was given i.v. Because the metabolic profile of the drug after oral administration to rats and monkeys resembles that after i.v. administration to humans, few oral nonclinical studies were also reviewed.

Studies not reviewed within this submission: see Table below

Title	Reviewed	
	Yes	No
PHARMACOLOGY		
PRIMARY		
GTR-32380: Effect of WAY-130779 on the Growth of Human Tumor Cells in vitro	X	
RPT-49843: CCI-779 Potentiates the Inhibitory Effect of the Anti-Angiogenic Drug Interferon-Alpha on the Growth of a Human Renal Cell Carcinoma in Nude Mice	X	
GTR-32469: The Effect of CCI-779 on the Growth of Human Glioblastoma Cells in nu/nu Female Mice		X
GTR-32471: The Effect of CCI-779 on the Growth of Human Prostate Carcinoma Cells in nu/nu Female Mice		X
GTR-32472: The Effect of CCI-779 on the Growth of Human Pancreatic Tumor Cells in nu/nu Female Mice		X
GTR-32473: The Effect of CCI-779 on the Growth of Histologically Diverse Human Tumors in nu/nu Female Mice		X
GTR-32474: Optimization of the Dosing Regimen for CCI-779 in Human Tumor Nude Mouse Xenografts		X
GTR-34985: The Effect of Combining CCI-779 with Standard Chemotherapy on Tumor cell growth in Vitro and in Vivo		X
SECONDARY		
GTR-32595: Effect of WAY-130779 on in Vivo T cell Function as Measured by Contact Sensitivity to Dinitrofluorobenzene (DNFB)	X	

Title	Reviewed	
	Yes	No
GTR-39691: The Effect of Oral Dosing with CCI-779 on the Growth of Histologically Diverse Human Tumors in Nude Mouse Xenografts		X
SAFETY PHARMACOLOGY		
RPT-43607: CCI-779: A Single Dose Intravenous Central Nervous System Safety Pharmacology Study in Male Rats	X	
RPT-43608: A Single Dose Intravenous Respiratory Safety Pharmacology Study in Male Rats	X	
GTR-29466: An Exploratory Single-Dose Intravenous Cardiovascular Study in Male Rats		X
GTR-27099: A Cardiovascular Safety Assessment Study of Single Oral Doses in Conscious Sprague-Dawley Rats	X	
GTR-32073: CCI-779: An Escalation Dose Intravenous Cardiovascular Study in Conscious Male and Female Cynomolgus Monkeys	X	
PHARMACOKINETICS		
Analytical methods and validation*		
In a separate Table; see below	-	-
Absorption		
RPT-40992: CCI-779 (WAY-130779): Pharmacokinetics in nu/nu Female Mice after a Single Intravenous Dose (1 and 20 mg/kg)		X
RPT-44897: CCI-779: Single Oral (Gavage) Dose Pharmacokinetic Study in Male and Female DBA/1J Mice		X
GTR-38567: CCI-779: Pharmacokinetics of Radioactivity Following a Single Oral Dose of ^{14}C-WAY-130779 in Rats		X
RPT-42613: CCI-779: Bioavailability of CCI-779 in Cynomolgus Monkeys: Evaluation of Two Intravenous Formulations (Protocol PS 946008): CCI-779 and Sirolimus Bioanalytical and Pharmacokinetic Data		X
GTR-38462: CCI-779: Pharmacokinetics and Excretion of Total Radioactivity Following a Single Intragastric (7.5 mg/kg) Dose of 14C-WAY-130779 in Male Cynomolgus Monkeys		X
Distribution		
RPT-44598: CCI-779: Tissue Distribution of [14C]-CCI-779-Derived Radioactivity Following a Single 2.5 mg/kg Intravenous Dose of [14C]-CCI-779 in Male Sprague-Dawley and Long-Evans Rats	X	
RPT-44599: CCI-779: Tissue Distribution of [14C]-CCI-779-Derived Radioactivity Following a Single 7.5 mg/kg Intragastric Dose of WAY-130779 in Male Sprague-Dawley and Long-Evans Rats		X
RPT-62965: Temsirolimus: in vitro Protein Binding of [14C]Temsirolimus by Erythrocyte Partitioning		X
RPT-39803: CCI-779: Blood to Plasma Distribution and Redistribution in Mice, Rats, Cynomolgus Monkeys and Humans		X
RPT-49956: Temsirolimus: Transport and Inhibition of P-Glycoprotein Activity in Modified Caco-2 Cell Monolayers		X
RPT-61821: Temsirolimus: Plancental Transfer of [14C]Temsirolimus-Derived Radioactivity Following a Single Oral (1.5 mg/kg) Administered of [14C]Temsirolimus to Gravid Rats		X
Metabolism		
GTR-34738: CCI-770 (WAY-130779): Biotransformation in Male Rats after a Single Intravenous Dose of the 14C-Radiolabeled Drug (2.5 mg/kg)	X	
GTR-38620: CCI-770 (WAY-130779): Biotransformation in Rats after		X

Title	Reviewed	
	Yes	No
a Single Intragastric Dose of the 14C-Radiolabeled Drug (7.5 mg/kg)		
RPT-44613: CCI-779 (WAY-130779): Biliary Excretion and Metabolite Profiling of [14C]WAY-130779 in Male Bile Duct Cannulated Sprague-Dawley Rats Following a Single Intravenous (2.5 mg/kg) or Oral (7.5 mg/kg) Administration	X	
GTR-36527: CCI-779 (WAY-130779): Biotransformation in Cynomolgus Monkeys after a Single Intravenous Dose of the 14C-Radiolabeled Drug (2.5 mg/kg)		X
GTR-38606: CCI-779 (WAY-130779): Biotransformation in Cynomolgus Monkeys after a Single Oral Dose of the 14C-Radiolabeled Drug (7.5 mg/kg)		X
RPT-42535: CCI-779 (WAY-130779): Metabolite Profiles in Human Blood Samples Collected From a Phase 1 Study of Weekly Intravenous Administration of CCI-779 in Patients with Advanced Solid Tumors		X
RPT-62848: Temsirolimus: Metabolic Characterization of Human Whole Blood, Plasma, Urine, and Fecal Samples from Clinical Protocol 3066K1-133-US [An Open-Label, Single-Dose, Non-Randomized Study of the Mass Balance and Metabolic Disposition of Orally and Intravenously Administered 14C-Labeled Temsirolimus in Healthy Male Subjects]		X
RPT-54304: CCI-779 (WAY-130779): In Vitro Metabolism in Liver Microsomes of Male CD-1 Mice	X	
GTR-32279: CCI779 (WAY-130779): Biotransformation in Rat and Human Liver Microsomes, and Preliminary Characterization of Cytochrome P450 Isozyme(s) Involved in Its Metabolism	X	
RPT-36765: CCI-779 (WAY-130779): Identification of Cytochrome P450 Enzymes Involved in Its Metabolism in Human Liver Microsomes Using Chemical Inhibitors		X
RPT-47388: CCI-779 (WAY-130779): Effects on Drug Metabolizing Enzymes in Rat Livers From the 28-Day Oral Toxicity Study		X
RPT-58912: Evaluating the Potential for Induction of CYP3A4 by Temsirolimus Using the CYP3A4 Reporter Gene Assay		X
GTR-39416:CCI-779: The Preliminary Evaluation as an Inhibitor of Human P450 Enzymes		X
RPT-45792: CCI-779 (WAY-130779): Evaluation as an Inhibitor of Cytochrome P450 Enzymes CYP3A4/5, CYP2D6, CYP2C8 and CYP2C9 in Human Liver Microsomes		X
Excretion		
GTR-32603: CCI-779: Single Intravenous Dose Mass Balance Study of 14C-CCI-779 in Male Sprague-Dawley Rats	X	
GTR-38355: CCI-779: Single 14C Oral dose (1.5 mg/kg) Mass Balance Study in Male Rats		X
GTR-38462: CCI-779: Pharmacokinetics and Excretion of Total Radioactivity Following a Single Intragastric (7.5 mg/kg) Dose of 14C-WAY-130779 in Male Cynomolgus Monkeys		X
GTR-33528: CCI-779: Single 14C Intravenous Dose (2.5 mg/kg) Mass Balance Study in Male Monkeys	X	
RPT-62880: Temsirolimus: Mass Balance of Orally (100 μCi) and Intravenously (50 μCi) Administered [14C]-CCI-779 in Healthy Subjects		X
RPT-62848: Temsirolimus: Metabolic Characterization of Human Whole Blood, Plasma, Urine, and Fecal Samples from Clinical		X

Title	Reviewed	
	Yes	No
Protocol 3066K1-133-US		
RPT-44613: CCI-779 (WAY-130779): Biliary Excretion and Metabolite Profiling of [14C]WAY-130779 in Male Bile Duct Cannulated Sprague Dawley Rats Following a Single Intravenous (2.5 mg/kg) or Oral (7.5 mg/kg) Administration		X
Other Pharmacokinetic Studies: Drug Interaction		
GTR-34706: CCI-779 (WAY-130779): Metabolic Interactions with Taxol and Doxorubicin and Inhibition of Cytochrome P450 CYP3A4/5 Catalytic Activity in Human Liver Microsomes		X
RPT-60233: Temsirolimus (CCI-779): Evaluation of Potential Drug Interactions with Letrozole using Human Liver Microsomes		X
TOXICOLOGY		
Single-dose		
GTR-31708: CCI-779: Acute Intravenous Toxicity Study in Mice	X	
GTR-38183: CCI-779: Acute Oral Toxicity Study in Mice		X
GTR-31709: CCI-779: Acute Intravenous Toxicity Study in Rats	X	
GTR-31897: CCI-779: Acute Intravenous Toxicity Study in Rats (Addendum 1)	X	
GTR-38184: CCI-779: Acute Oral Toxicity Study in Rats		X
Repeat-dose		
RPT-44451: CCI-779: Multiple (14 Days) Dose Oral (Gavage) Ranging Study in Mice		X
RPT-53607: CCI-779: Thirteen Week Oral (Gavage) Range Finding Study in Mice		X
GTR-30878:: WAY-130779: A Five-Day Intravenous Pilot Toxicology Study in Rats	X	
GTR-30341: CCI-779: Fourteen-Day Intravenous Dose Ranging Study in Rats	X	
GTR-31183: CCI-779: Four Cycle Intravenous Study in Rats	X	
RPT-43567: CCI-779: 26-Week (One Dose per Week) Intravenous Toxicity Study in Rats with a 13-Week Recovery	X	
GTR-27125: WAY-129327 and WAY-130779: Ten-Day Oral (Gavage) Pilot Toxicology Study in Rats		X
RPT-37674: CCI-779: Four Cycle Oral (Gavage) Toxicity Study in Rats		X
RPT-44048: CCI-779: 28-Day Oral (Gavage) Toxicity Study in Rats		X
RPT-46920: CCI-779: 13-Week Oral (Gavage) Toxicity Study In Rats With A 13-Week Recovery Period		X
RPT-47743: CCI-779: 26-Week Oral (Gavage) Toxicity Study in Rats	X	
GTR-30340: CCI-779: Fourteen-Day Intravenous Dose-ranging Study in Monkeys	X	
GTR-31575: CCI-779: Four Cycle Intravenous Toxicity Study in Monkeys	X	
RPT-43566: CCI-779: 39-Week (One Dose per Week) Intravenous Toxicity Study in Monkeys with a 13-Week Recovery	X	
GTR-37787: CCI-779: Four Cycle Oral (Gavage) Toxicity Study in monkeys		X
RPT-44050: CCI-779: 28-Day Oral (Gavage) Toxicity Study in Cynomolgus Monkeys		X
RPT-46684: CCI-779: 13-Week Oral (Gavage) Toxicity Study in Cynomolgus Monkeys with a 13-Week Recovery Period		X

Title	Reviewed	
	Yes	No
RPT-47744: CCI-779: 39-Week Oral Gavage Toxicity Study In Cynomolgus Monkeys		X
GENOTOXICITY		
GTR-28250: Mutagenicity Assay with WAY 130779-4 in the Salmonella/Mammalian- Microsome Reverse Mutation Screening Assay (Ames Test)		X
GTR-30767: Mutagenicity Test with CCI-779 in the Salmonella – Escherichia coli/Mammalian-Microsome Reverse Mutation Assay With a Confirmatory Assay	X	
GTR-30972: Mutagenicity Test on CCI-779 in the L5178Y TK+/- Mouse Lymphoma forward Mutation Assay with a Confirmatory Assay	X	
GTR-30694: Mutagenicity Test on CCI-779 Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with a Confirmatory Assay with Multiple Harvests	X	
GTR-30826: Mutagenicity Test on CCI-779 in the In Vivo Mouse Micronucleus Assay	X	
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (Embryo-Fetal Development)		
RPT-44151: CCI-779: Oral Gavage Fertility Ranging Study in Male Rats		X
RPT-44150: CCI-779: Oral Gavage Fertility Dose Ranging Study in Female Rats		X
RPT-46442: CCI-779: Oral (Gavage) fertility Study in Male Rats	X	
RPT-45217: CCI-779: Oral Gavage fertility Study in Female Rats	X	
RPT-45432: CCI-779: Intravenous Developmental Toxicity Dose Ranging Study in Rats		X
RPT-45434: CCI-779: Oral (Gavage) Developmental Toxicity Dose Ranging Study in Mated Female Rats		X
RPT-45268: CCI-779: Oral (Gavage) Developmental Toxicity Study in mated Female Rats	X	
RPT-45435: CCI-779: Oral (Gavage) Developmental Toxicity Dose Ranging Study in Mated Female Rabbits		X
RPT-45267: CCI-779: Oral (Gavage) Developmental Toxicity Study in mated Female Rabbits	X	
ADDITIONAL TXICITY STUDIES		
RPT-47806: Two Dose (Dosing on Postnatal Days 8 and 15) Oral (Gavage) Toxicity Study in Neonatal Mice		X
Impurities Qualification of Drug Substance using Lot 76336-126 (modified synthesis)		
GTR-36704: CCI-779: Fourteen Day Intravenous Impurity Qualification Study in Rats		X
GTR-37662: CCI-779: Bacterial Reverse Mutation Test with Salmonella Typhimurium and Escherichia Coli	X	
RPT-38890: CCI-779: In Vitro Mammalian Chromosome Aberration Test	X	
Impurities Qualification of Drug Product using heated stressed Intravenous formulation (Batch 2001B0205)		
RPT-47359: CCI-779: Fourteen Day Intravenous Qualification Study in Rats		X
RPT-48191: CCI-779. Bacterial Reverse Mutation Test with Salmonella typhimurium and Escherichia coli		X
RPT-49608: CCI-779: In Vitro Chromosomal Aberration Study with Human Lymphocytes for Qualification of Intravenous Impurities		X

Title	Reviewed	
	Yes	No
Impurity Qualification of Drug Product Using Oral granulation Formulation Degraded to Contain Seco-Temsirolimus (Lot 24300-016) †		
RPT-49338: CCI-779: Twenty-Eight Day Oral (Gavage) Reformulated Impurity Qualification (Seco-CCI) Study in Rats		X
RPT-52345: CCI-779: Bacterial Reserve Mutation Reformulated Impurity Qualification (Seco-CCI) Study In Salmonella typhimurium and Escherichia coli		X
RPT-53269: In Vitro Chromososme Aberration Reformulated Impurity Qualification Study in Human Peripheral Blood Lymphocytes		X

* Validations of analytical methods were not reviewed in detail. They were used as a cross reference when deemed necessary.
† Lot 24300-016 was a preparation of granulation formulation that mimicked the clinical tablet formulation.

Report Issue Date	Report No.	Cross-Reference	Purpose[a]	Type of Analytical Method	Matrix	Analyte	Range (ng/mL)
Validation Reports							
26 Feb 1998	GTR-32667	4.2.2.1	Validation	LC/MS/MS	Rat whole blood	Temsirolimus	1 to 100
27 Feb 1998	GTR-32723	4.2.2.1	Validation	LC/MS/MS	Monkey whole blood	Temsirolimus	1 to 100
23 Oct 1998	GTR-34589	4.2.2.1	Validation	LC/MS/MS	Rat plasma	Temsirolimus	1 to 100
07 Dec 1998	GTR-34898	4.2.2.1	Validation	LC/MS/MS	Monkey plasma	Temsirolimus	1 to 100
12 Jan 1999	GTR-34999	4.2.2.1	Validation	LC/MS/MS	Monkey whole blood	Temsirolimus	0.25 to 100
05 Oct 1999	GTR-38151[b]	4.2.2.1	Validation	LC/MS/MS	Monkey whole blood	Temsirolimus	0.25 to 100
10 Nov 1999	GTR-38152[b]	4.2.2.1	Validation	LC/MS/MS	Rat whole blood	Temsirolimus	0.500 to 100
14 Nov 2000	RPT-41184	4.2.2.1	Validation	LC/MS/MS	Rat whole blood	Temsirolimus and sirolimus	0.25 to 100
14 Nov 2000	RPT-41154	4.2.2.1	Validation	LC/MS/MS	Monkey whole blood	Temsirolimus and sirolimus	0.25 to 100
03 Oct 2002	RPT-45088	4.2.2.1	Validation	LC/MS/MS	Mouse whole blood	Temsirolimus and sirolimus	0.25 to 100
Report Issue Date	**Report No.**	**Cross-Reference**	**Purpose[a]**	**Type of Analytical Method**	**Matrix**	**Analyte**	**Range (ng/mL)**
Validation Reports (cont'd)							
09 Dec 2002	RPT-47939	4.2.2.1	Validation	LC/MS/MS	Rabbit whole blood	Temsirolimus and sirolimus	0.5 to 500
19 Apr 2006	RPT-63298	4.2.2.1	Cross-validation of RPT-41184	LC/MS/MS	Rat whole blood	Temsirolimus and sirolimus	0.25 to 100
Stability Reports							
18 Feb 1998	GTR-32280	4.2.2.1	Stability evaluation	NA	Mouse, rat, monkey, and human whole blood and plasma	Temsirolimus and sirolimus	NA
04 Mar 2003	RPT-49428	4.2.2.1	Long-term stability evaluation	NA	Monkey whole blood	Temsirolimus and sirolimus	NA
26 Apr 2004	RPT-53153	4.2.2.1	Long-term stability evaluation	NA	Rabbit whole blood	Temsirolimus and sirolimus	NA
07 Apr 2006	RPT-62892	4.2.2.1	Long-term stability evaluation	NA	Rat whole blood	Temsirolimus and sirolimus	NA

a. All method validations and stability evaluations were conducted at Wyeth Research unless otherwise indicated.
b. Validation conducted at [redacted]

GTR = General Technical Report; LC/MS/MS = Liquid chromatography-tandem mass spectroscopy; NA = Not applicable; RPT = Report.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Temsirolimus is an analog (ester) of sirolimus (rapamycin [Rapamune®]). In humans, after i.v. or oral dosing temsirolimus is converted to sirolimus, with a ratio of sirolimus:temsirolimus > 1 (~2.7 after i.v. dosing and ~12 after oral dosing). Therefore, temsirolimus may be considered a pro-drug for sirolimus. After i.v. dosing of temsirolimus to rats and monkeys, sirolimus was either not detected or was detected at low levels. Sirolimus was the major metabolite in rats and monkeys after oral administration of the drug.

Primary Pharmacology: Temsirolimus is an inhibitor of the mammalian target of rapamycin (mTOR, also known as FRAP). Pharmacology studies suggest that temsirolimus and sirolimus work through the same pathway, which includes binding to FKBP-12 protein, hence inhibiting mTOR. mTOR is a serine/threonine kinase that regulates translation and cell division. Increased nutrients (e.g. glucose and amino acids) will result in mTOR activation and cell division. mTOR is downstream of multiple pathways, including growth factors, insulin, and nutrients.

Temsirolimus inhibited growth of several tumor cells with IC50's ranging from nM's to μM's. However, since information on the activity of mTOR, before and after treatment, was not provided for these cells, the mechanism of growth inhibition in these studies is unclear. In the vitro studies, under the conditions tested, temsirolimus inhibited the mTOR pathway and the production of HIF-2 α in the RCC cell line, A498.

Secondary Pharmacology: Activity of mTOR has been implicated in the proliferation of lymphocytes. Therefore, effect of temsirolimus on T-cell response was tested in an in vivo study. Under the conditions tested in mice, temsirolimus treatment resulted in transiently delayed T cell response to a contact sensitizing agent, during dosing with the drug.

2.6.2.2 Primary pharmacodynamics

- Temsirolimus is an mTOR inhibitor. Temsirolimus and rapamycin bind to the same intracellular protein FKB P-12 and therefore, may work through the same mechanism of action. Temsirolimus can block entry into the S phase, resulting in accumulation of cells at the G0/G1 phase.
- The growth inhibition dose-response curve was biphasic. At 0.001-1.0 μM of temsirolimus or sirolimus (over 3 log concentrations), the dose response was flat. At concentrations higher than 1.0 μM, a more complete inhibition of cell growth was observed.
- In the human A498 RCC athymic mouse xenograft model, treatment with temsirolimus as a single agent did not result in tumor regression. A reduction in the rate of tumor growth was observed which was similar at doses of 10-75 mg/kg tested. Under the conditions tested, the combination of temsirolimus and IFN-α induced tumor regressions in the A498 RCC xenograft model.

Drug activity related to proposed indication:
The present NDA is submitted for the use of temsirolimus in treatment of RCC.

Temsirolimus has been shown to have anti-tumor properties; it inhibited growth of several tumor cells with IC50's ranging from nM's to μM's. Since information on the activity of mTOR, before and after treatment, was not provided for these cells, the definitive mechanism of growth inhibition in these studies is unclear. In vitro studies suggest that the mechanism of action of temsirolimus results in part from binding to FKBP-12 and inhibition of the mTOR kinase. mTOR regulates a signaling cascade that controls cell proliferation. The net effect of this compound on cells is to block cell proliferation at the G1-S phase transition of the cell cycle. In the in vitro studies, under the conditions tested, temsirolimus inhibited the mTOR pathway and the production of HIF-2 α transcription factor in the RCC cell line A498. In the A498 RCC athymic mouse xenograft model, treatment with temsirolimus as a single agent resulted in reduced rate of tumor growth but did not cause tumor regression.

Study Title: Effect of WAY-130779 on the growth of human tumor cells *in vitro*

Report# GTR-32380

Effect of temsirolimus on the growth of human tumor cells *in vitro*:
Temsirolimus was tested in vitro for its ability to inhibit the growth of a panel of 22 human tumor cell lines.

TUMOR TYPE	CELL LINE	IC_{50} (μM)
Breast Cancer	BT-474	0.0006
	SKBR-3	0.0007
	MCF7	0.001
Prostate Cancer	PC-3	0.0005
	LNCaP	0.0007
	DU 145	0.001
Melanoma	LOX	0.001
Ovarian Cancer	A2780 S	0.004
	A2780 DDP	0.04
	HTB 161	0.07
Lung Cancer	A549	0.1
	LX-1	2.5
Colon Cancer	CaCo 2	0.004
	HCT-15	0.07
	SW 948	0.05
	MIP 101	0.08
	CX-1	4.4
	SW 620	4.4
	COLO 205	4.8
	LS 174T	4.9
	SW 480	5.9
Leukemia	CCRF-CEM	0.1
	HL-60	5.8

Table excerpted from the package.

- The mean IC50 for all cells tested was 1.4 μM.

- The prostate and breast tumor lines were more sensitive to temsirolimus than the other cell lines tested.
- The MDR-1 over-expressing MIP 101 cell line was more sensitive to temsirolimus than its MDR-1 negative counterpart SW 620, suggesting that temsirolimus is not a substrate for pgp-1 mediated multi-drug resistance. However, studies conducted with modified Caco-2 cells over-expressing pgp (Report rpt-49956) suggested that temsirolimus is subject to pgp-mediated efflux.

Similar experiments were performed by the National Cancer Institute (NCI) on 51 cell lines, derived from human tumors. The mean IC50 in the NCI study was determined to be ≤ 3.3 μM. Table below shows the results of the NCI screen for panels of human central nervous system (CNS) and of the combined NCI and Wyeth panel of breast tumors.

TISSUE TYPE	CELL LINE	IC_{50} (μM)
All	51 cell lines	0.15
CNS	SF-268	2.6
	SF-295	<0.01
	SF-539	<0.01
	SNB-19	<0.01
	SNB-75	<0.01
	U251	<0.01
Breast	MCF7	<0.01
	BT-549	<0.01
	T-47D	<0.01
	BT4M	<0.01
	SKBR-3	<0.01
	MDA-MB-231	5.9
	MDA-MB-435	1.6
	MDA-N	5.9

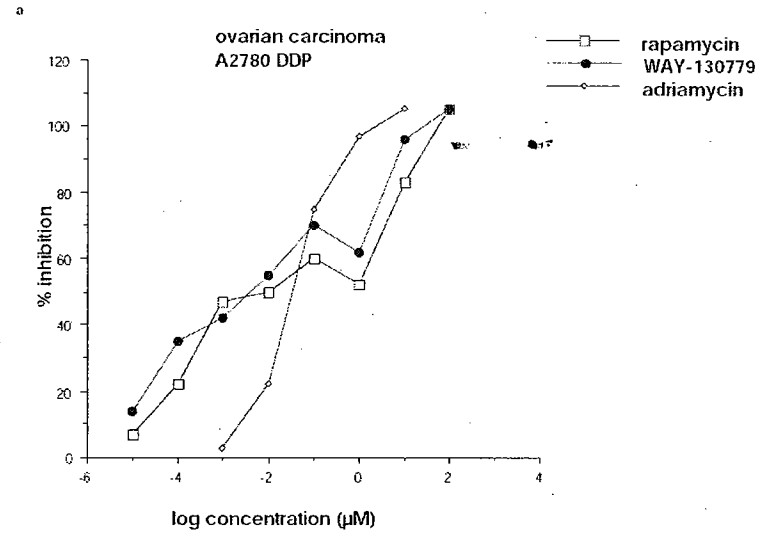
Table excerpted from the package.

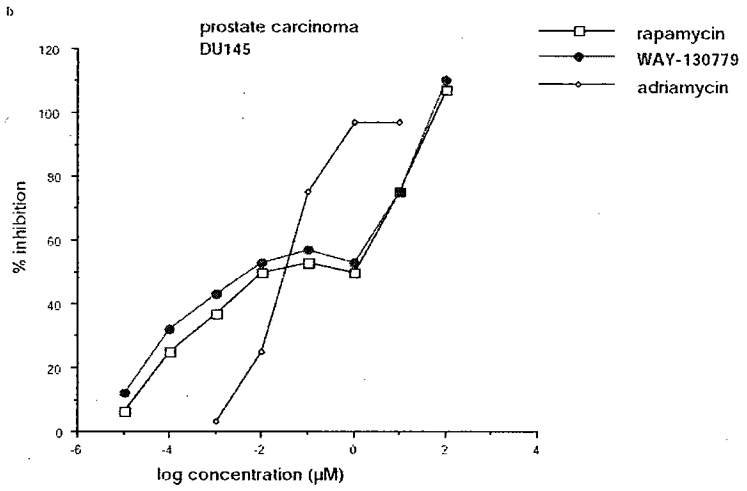
- The mean IC_{50} in the NCI study was determined to be ≤ 3.3 μM.
- For the CNS tumors 5/6 had an IC50 of <0.01 μM and for breast tumor cell lines 5/8 had an IC50 of < 0.01.

Dose-response curves and mechanism of action (in vitro studies)

The human ovarian and prostate carcinoma cell lines, OVCAR-3 and DU-145, were cultured for 48 hours at drug concentrations indicated. Cells were then fixed and stained with sulphorhodamine B dye. The absorbance read at 540 nm is proportional to the cell number. The % inhibition was determined by the ratio of the absorbance at 540 nm for untreated versus drug treated wells.

The dose-response curve for temsirolimus (WAY-130779), adriamycin, and rapamycin in ovarian and prostate carcinoma cell lines is shown in graphs below:



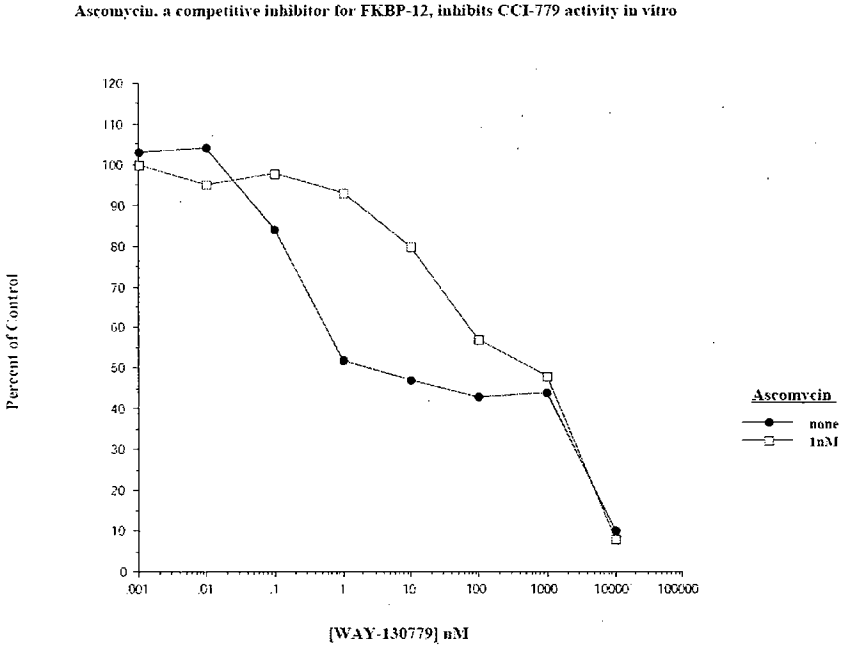


Graphs excerpted from the package.

Ascomycin is a competitive inhibitor of FKBP-12, the rapamycin-binding protein. A study was conducted to evaluate the effect of ascomycin on temsirolimus inhibition of tumor cell (U87MG) growth in vitro. The IC50 for temsirolimus was approximately 1nM. An equivalent concentration (1nM) of ascomycin almost completely blocked the 50% inhibition seen with temsirolimus alone. As the concentration of temsirolimus was increased, temsirolimus was able to overcome the inhibitory effect of ascomycin.

- These data suggest that temsirolimus and rapamycin bind to the same intracellular protein FKBP-12 and therefore, may work through a similar mechanism of action.
- The growth inhibition dose-response curve was biphasic. At 0.001-1.0 µM of temsirolimus or sirolimus (over 3 log concentrations), the dose response was flat.

At concentrations higher than 1.0 μM, a more complete inhibition of cell growth was observed.



U87MG cells ($1x10^5$) were plated on 10% serum containing medium plus or minus 1 nM Ascomycin. The concentration of WAY-130779 was varied as indicated and the effect on ^{3}H-labeled thymidine incorporation determined over the last 6 hours of a 24 hour incubation period. Data are reported as % of ^{3}H-thymidine uptake from control cells.

Graph excerpted from the package.

Temsirolimus induced a G1 arrest in PC3 cells:

To determine if there were a specific point in the cell cycle that temsirolimus exerted its growth inhibitory activity, PC3 (human prostate) cells were stained with the DNA binding dye propidium iodide and analyzed by flow cytometry for DNA content.

Effect of temsirolimus on cell cycle progression with prostate cell lines.

	16 hrs.			**24 hrs.**		
WAY-130779 (μg/ml)	Go/G1	S	G2/M	Go/G1	S	G2/M
none	48	22	29	54	18	28
10	77	3	18	88	2	8

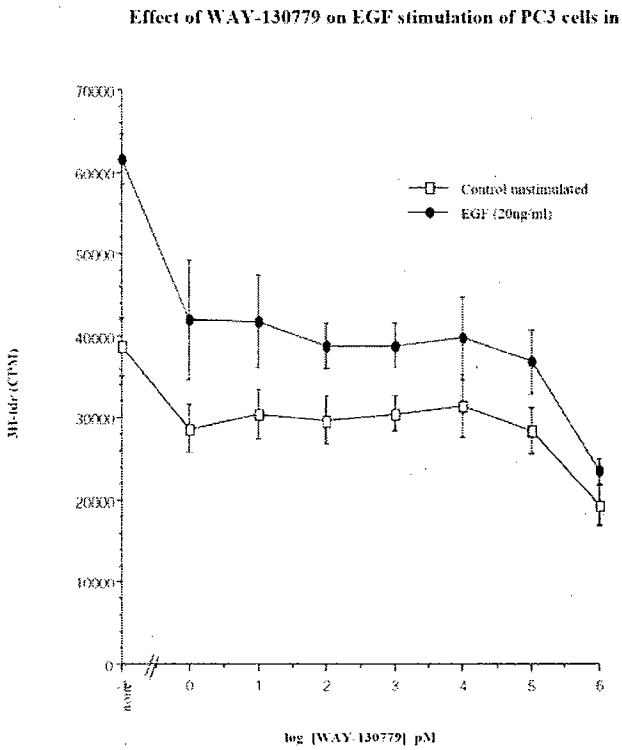


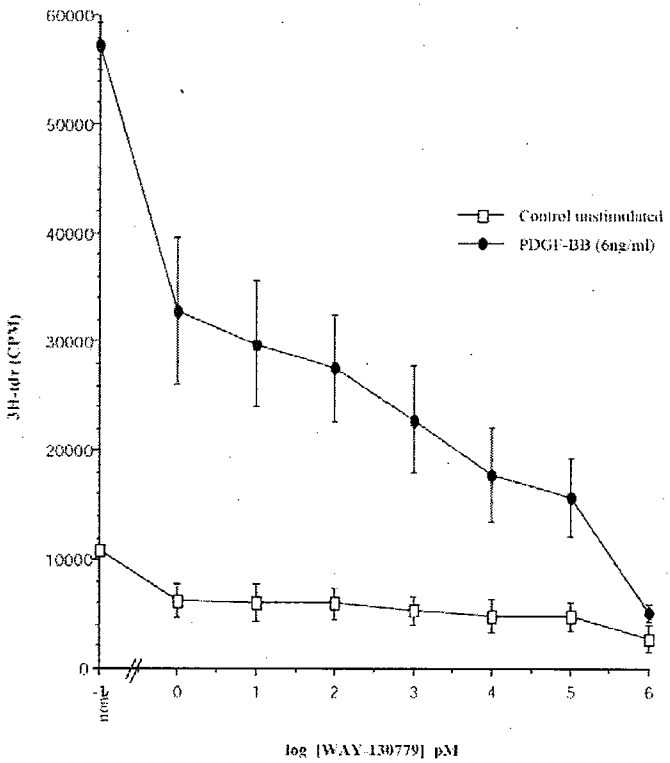
Table and graph excerpted from the package

- An accumulation of cells was observed in the G0/G1 phase of the cycle at 16 hrs after treatment with temsirolimus. By 24 hrs, 88% of the cells had accumulated in G0/G1 suggesting that temsirolimus blocked entry into the S phase. The concentration of temsirolimus was 10 µg/mL (~10 µM).
- When tested for inhibitory effects at low temsirolimus concentrations, inhibition of growth was small below 1 µM. When PC3 cell proliferation was enhanced via EGF treatment, the IC50 of inhibition was about 1 pM.

<u>The Effect of temsirolimus on constitutive and growth factor-induced proliferation of human glioblastoma cells in vitro:</u>

Proliferation of T98G cell line, derived from a human glioblastoma can be enhanced by stimulation with platelet derived growth factor (PDGF). In 3 independent experiments, T98G cells were stimulated with PDGF in serum free medium and the effect of temsirolimus on the PDGF induced proliferation was determined.

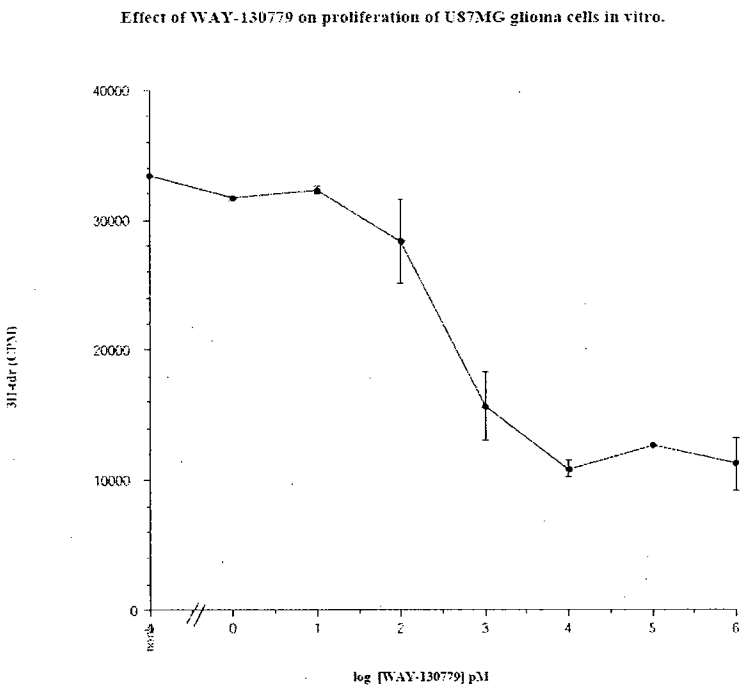
Effect of WAY-130779 on PDGF stimulation of T98G glioma cells in vitro.



Graph excerpted from the package.

Human T98G cells were plated in serum free medium. Cells were either not stimulated or treated with 6 ng/ml of PDGF-BB. Eighteen hours after PDGF treatment cells were pulsed with 1 μCi of tritium labeled thymidine (^{3}H-tdr) for 6 hrs. Thymidine uptake was then quantitated by liquid scintillation spectrometry. Data are the x ± SEM from 3 independent experiments with triplicate determination in each experiment.

- Temsirolimus inhibited the PDGF-induced proliferation of T98G human glioblasma-derived cell line. The IC50 was determined to be 0.5-2.0 pM.



Human U87MG glioma cells were plated in 10% serum containing medium. Cells were pulsed with ^{3}H-tdr. Data are the x ± SEM for thymidine incorporation from 2 independent experiments with triplicate determinations in each experiment.

The Effect of temsirolimus (WAY-130779) on DNA Synthesis of U87MG glioblastoma cells as measured by flow cytometric analysis of DNA content or ^{3}H-thymidine uptake.

Conc of temsirolimus (nM)	Propidium iodide staining		3H-thymidine uptake
	% S phase cells	% changes vs control	% inhibition
0 (control)	13	—	—
1.0	5	↓62%	68%
100	5	↓62%	71%
1000	5	↓62%	74%

Cells were cultured for 30 hours with 10% FBS alone or with WAY-130779 at 1.0 nM - 1.0 mM. The U87MG cells were then stained with propidium iodide to label DNA and the percentage of cells with a greater than 2N but less than 4N content of DNA was determined by flow cytometry.

- Reduced proliferation of U87MG glioma cells was observed at increasing concentrations of temsirolimus. The IC50 was defined to be 1.0 nM. Approximately 65%-70% growth inhibition was observed over the concentration range of 10 nM-1 μM temsirolimus, as defined by ^{3}H-thymidine incorporation. Therefore, over this concentration range the growth inhibitory effect of temsirolimus remained constant.
- The propidium iodide staining of U87MG glioma cells treated with temsirolimus (or untreated) showed that the numbers of cells at the S-phase were reduced by ~62% at temsirolimus concentrations of 1 nM-1 μM. Therefore, over this

temsirolimus range, the growth inhibitory effect of temsirolimus remained constant, correlating with the thymidine incorporation study.

Conclusions:

- Temsirolimus inhibited growth of several tumor cells with IC50's ranging from nM's to μM's.
- The similarity of the dose-response curves for rapamycin and temsirolimus may suggest a similar mechanism of action for the two drugs. Additionally, Ascomycin (a competitive inhibitor of rapamycin-binding protein) inhibited temsirolimus activity. Data obtained in that study suggest that temsirolimus and rapamycin bind to the same intracellular protein FKBP-12 and therefore, may work through the same mechanism of action.
- The growth inhibition dose-response curve was biphasic. At 0.001-1.0 μM of temsirolimus or sirolimus (over 3 log concentrations), the dose response was flat. At concentrations higher than 1.0 μM, a more complete inhibition of cell growth was observed.
- Under the conditions tested, temsirolimus at about 10 μM blocked entry into the S phase. At 24 hrs post-treatment, 88% of PC3 human prostate cells accumulated at the G0/G1 phase. Moreover, the propidium iodide staining of U87MG glioma cells showed that the numbers of cells at the S-phase were reduced by 62% at temsirolimus concentrations of 1 nM-1μM.
- Temsirolimus inhibited the PDGF-induced proliferation of T98G human glioblastoma cell line. The IC50 was determined to be 0.5-2.0 pM.
- Reduced proliferation of U87MG glioma cells was observed at increasing concentrations of temsirolimus. The IC50 was defined to be 1.0 nM.

Study Title: CCI-779 potentiates the inhibitory effect of the anti-angiogenesis drug interferon-alpha on the growth of a human renal cell carcinoma in nude mice

Report# RPT-49843

Test Facility: Wyeth Research
Pearl River, NY 10965

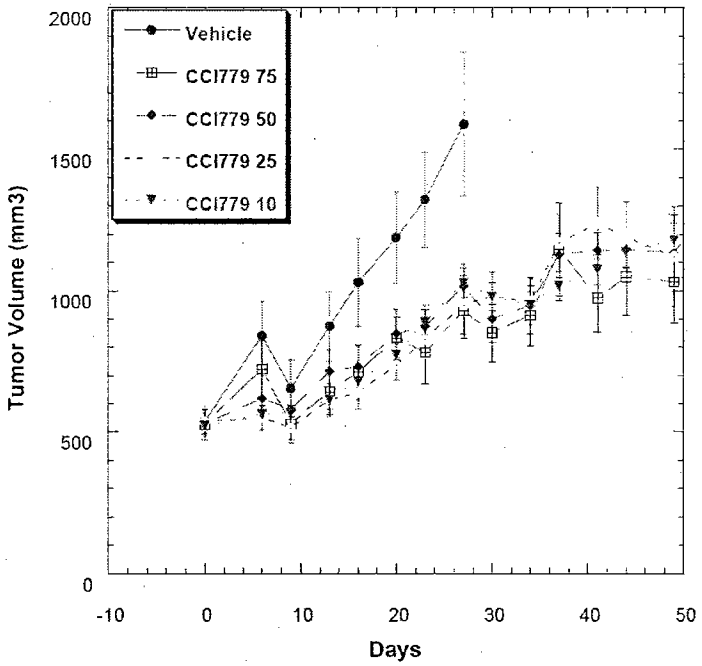
Study design:

The effect of combination of temsirolimus (CCI-779) and interferon-α was studied in human renal cell carcinoma A498 (HTB44) xenograft model. The A498 is a human RCC cell line with loss of von Hippel-Lindau (VHL) tumor suppressor gene. In addition, the effect of temsirolimus on inhibition of the mTOR pathway and production of the angiogenesis factor HIF-2 was assessed in vitro, in A498 cells.

Husbandry	Specifications
Species:	Mouse
Strain:	Nude.
Source:	[redacted]
No. of Animals Per Group:	10
Total No. of Animals:	90
Age and Sex:	6 weeks/female
Weight:	20g
Date Received:	04 April 2001
Acclimation Period:	1 week

Results:

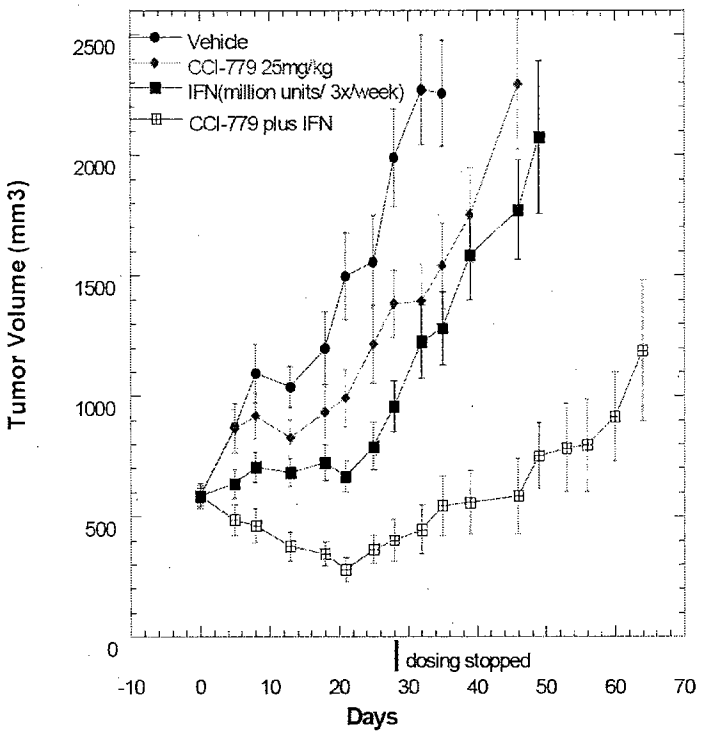
Effect of CCI-779 on A498 Human Tumor Xenograft in Nude Mice



CCI-779 was tested as a single agent. Dosing was from 10 mg/kg to 75 mg/kg i.v. once a week beginning on day 0 when tumors had reached a size of about 500 mm3.

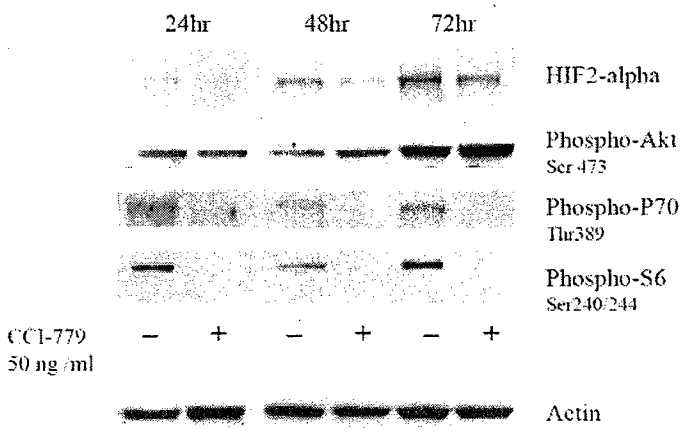
- All doses tested had similar effect. The dose of 25 mg/kg was chosen to combine with interferon-a (IFN-α).

The Effect of 4 Weekly Cycles of CCI-779 and IFN-alpha on the Growth of A498 Human Renal Tumors in Nude Mice



Groups of 10 mice were treated with CCI-779 alone, IFN-α alone, or the combination. IFN-α was given i.p. at 1 million units, 3x per week and CCI-779 i.v. at 25 mg/kg once per week. IFN-α was given on Days 1, 3, and 5 and CCI-779 on Day 6 of each week for 4 weeks. Dosing began after tumors had reached a size of about 500 mm^3.

mTOR Activity and HIF2-alpha Levels are Inhibited by CCI-779 in A498 Renal Carcinoma Cells



Western blot analysis of cells treated with 50 ng/ml CCI-779 (+) or not treated (-)

- For all 3 time-points, a decrease in HIF-2 α protein was observed when A498 cells were treated with temsirolimus, as compared to untreated cells.
- At the same time points, the mTOR pathway was inhibited as shown by decreased phosphorylation of the target protein of mTOR, p70S6k. Phosphorylation of the S6 ribosomal protein was also inhibited confirming the inhibition of the mTOR/p70S6k pathway, since S6 ribosomal protein is a substrate of p70S6 kinase.

Conclusions:

- In the A498 RCC athymic mouse xenograft model, treatment with temsirolimus as a single agent did not result in tumor regression. A reduction in the rate of tumor growth was observed which was similar at doses of 10-75 mg/kg tested.
- Under the conditions tested, the combination of temsirolimus and IFN-α induced tumor regression in the A498 RCC xenograft model.
- In the vitro studies, under the conditions tested, temsirolimus inhibited the mTOR pathway and the production of HIF-2 α in the RCC cell line (A498) over a similar time frame.

2.6.2.3 Secondary pharmacodynamics

Study Title: Effect of WAY-130779 on in vivo T cell function as measured by contact sensitivity to dinitrofluorobenzene (DNFB)

Report# GTR-32595

Methods:

Delayed Hypersensitivity Assay

Contact sensitivity to the hapten dinitrofluorobenzene (DNFB) was used as an assay for delayed hypersensitivity (DTH). Male C3H/Fe/J mice were sensitized with a 0.5% solution of DNFB in acetone:olive oil (4:1). The solution (0.025 ml) was applied to the shaved abdomen of the mice and 0.005 ml was applied to the four foot pads. Four days later, the mice were challenged with 0.001 ml of a 0.2% solution of DNFB (applied on the right foot pad). Twenty-four hours later, the mice were sacrificed and the right and left rear feet were removed and weighed. The amount of swelling was determined by subtracting the weight of the unchallenged foot (left) from that of the challenged foot (right).

Rationale for the dose of temsirolimus: the dose selected was 5 mg/kg i.v. According to the sponsor, this dose was 50 times the minimum effective dose for treatment of a sensitive human tumor in nude mice (U87MG glioblastoma) and equivalent to the minimum effective dose for treatment of a less sensitive human tumor (PC3 prostate) in nude mice.

Results:

Study #1: Effect of temsirolimus (CCI-779, WAY-130779) on the in vivo T cell response to the contact sensitizing agent Dinitro-fluorobenzine (DNFB).

Experiment 1

Drug	*Days dosed	T cell sensitization	n	Foot pad swelling (mg)
Vehicle	5		20	38 ± 2
WAY-130779	5	during drug dosing	5	3 ± 2
WAY-130779	5	1 day after dosing	5	32 ± 5
WAY-130779	5	3 days after dosing	5	32 ± 5
WAY-130779	5	7 days after dosing	5	30 ± 6

Experiment 2

Drug	Days dosed	T cell sensitization	n	Foot pad swelling (mg)
Vehicle	5		20	38 ± 13
WAY-130779	5	during drug dosing	5	5 ± 2
WAY-130779	5	1 day after dosing	5	29 ± 15
WAY-130779	5	3 days after dosing	5	40 ± 2
WAY-130779	5	7 days after dosing	5	31 ± 2

Mice were treated with 5 mg/kg iv temsirolimus (CCI-779, WAY130779) for 5 consecutive days, during the days between sensitization and challenge with DNFB

- In each experiment there was marked ↓swelling indicating suppression of the DTH response, during dosing. Therefore, 5 mg/kg of temsirolimus had immunosuppressive effects.
- A normal T cell response to DNFB could be induced as early as 1 day after temsirolimus treatment. These data show that suppression of in vivo T cell function by temsirolimus was transient.

Study # 2: This study was performed to determine if the cumulative effect of multiple 5 day treatment regimens with temsirolimus would result in more sustained immunosuppression. Mice were treated for 5 consecutive days every other week with temsirolimus (5 mg/kg iv). The effect of treatment with temsirolimus on the DTH reaction was evaluated as before during the sensitization and challenge with DNFB or 1-3 days prior to sensitization for each round of treatment.

Drug	Days dosed	T cell sensitization	n	Foot pad swelling (mg)
Vehicle	5		10	28 ± 4
WAY-130779	5	during drug dosing	5	9 ± 4
WAY-130779	5	3 days after drug dosing	5	22 ± 4
Vehicle	10		10	26 ± 4
WAY-130779	10	during 2nd round of drug dosing	5	5 ± 2
WAY-130779	10	3 days after last drug dose	5	26 ± 5
Vehicle	15		10	36 ± 4
WAY-130779	15	during 3rd round of drug dosing	4	6 ± 2
WAY-130779	15	1 day after last drug dose	5	38 ± 4
WAY-130779	15	3 days after last drug dose	5	33 ± 5

- T cell function in the treated mice returned to normal within 3 days of each of the 3 rounds of temsirolimus treatment. After the third round of treatment, mice could still be fully sensitized to DNFB 1 day after the last drug dose. These data suggest that in mice there was no cumulative damage to the immune system after as many as 3 cycles of bi-weekly temsirolimus administration, in which each administration was for 5 consecutive days.

Conclusions:
Under the conditions tested in mice, temsirolimus treatment resulted in delayed T cell response during dosing with the drug. Delayed T-cell response was transient; response was resumed one day after dosing was stopped. The same effect was observed when temsirolimus was administered in 3 cycles (bi-weekly) to mice, suggesting that cumulative damage to T-cells did not take place under conditions of the experiment. This transient effect may not be directly extrapolated for patients, since protein binding and drug half-life might be different in patients. Plasma protein binding was not evaluated in animal species, hence a comparison cannot be made. A comparison of temsirolimus T1/2 shows that the half-life of the drug after a single dose administration in animal species (Week 1 of the 6-month rat and 9-month monkey toxicology studies) was slightly higher than that observed in patients after single dose administration of 25 mg i.v. (18-33 hrs in rats, 21-27 hrs in monkeys, and ~17 hrs in patients).

2.6.2.4 Safety pharmacology

Summary:
Neurological effects:

- Under the conditions tested, single i.v. doses of temsirolimus did not cause any neurological effect.

Cardiovascular effects:

- Single i.v. doses of temsirolimus at 0.1, 0.5 and 1.5 mg/kg to conscious ♂ and ♀ monkeys did not affect the mean arterial blood pressure, systolic pressure, diastolic pressure, electrocardiograms, spontaneous gross motor activity, or body temperature during a 24-hr post-dose assessment. A dose-dependent ↓ in heart rate (~↓10-25%) was observed in the first 1.5 hrs post-dose. There was high variability throughout the study; therefore, a definite conclusion cannot be made.
- Single oral doses of temsirolimus at up to 3 mg/kg and a single oral dose of sirolimus at 3 mg/kg did not result in toxicologically meaningful changes in the mean arterial blood pressure or heart rate in SD rats. PK parameters were not provided; therefore it is not clear whether adequate systemic exposure was achieved in these tests.

Pulmonary effects:

- A single i.v. dose of temsirolimus in ♂ rats, at 0.2, 1, and 5 mg/kg resulted in ↓respiratory rates of 9-11% at 2 hrs post-dose at all dose levels.

Neurological effects:

Study Title: CCI-779: a single dose intravenous system safety pharmacology study in male rats

Key findings: under the conditions tested, temsirolimus did not cause any neurological effect.

Report: RPT-43607
Compliance: GLP
Conducting facility: [redacted]
Study initiation: June 2001
Test article: CCI-779, Batch/Lot# 0M8601, [redacted] pure

Study design:

Doses: 0.2, 1, 5 mg/kg; single dose i.v. (Day 2 sacrifice)
1.2, 6, 30 mg/m2

Rationale for dose selection:
A dose of 0.1 mg/kg resulted in significant inhibition of tumor growth in a nude mouse glioma model when administered i.v. for 5 consecutive days. The low dose is two fold the efficacious dose in this mouse model. The high dose of 5.0 mg/kg corresponds closely to the weekly dosage used in clinical trials in Europe and is 50 fold the efficacious dose seen in the nude mouse glioma model.

Vehicle control: propylene glycol, ethanol, polysorbate 80, polyethylene glycol, vitamin E, saline

CCI-779 Stock: 25 mg/mL CCI-779, 395 mg/mL ethanol; 0.75 mg/mL vitamin E, and propylene glycol

CCI-779 diluent:	5% (w/v) polysorbate 80; 5% (w/v) polyethylene glycol 400, and sterile water for injection
Dosing formulation:	An intermediate stock solution (5 mg/mL) was prepared by mixing appropriate amounts of the stock solution with the diluent. This intermediate stock was further diluted with saline, to prepare the dosing formulation.
Route, formulation:	i.v.; solution
Dose volume:	4 mL/kg
Species:	SD rats
No. of animals:	4 ♂s/group
Age/weight:	~7 weeks old and 221-267 g

Endpoints evaluated:

- Mortality and clinical signs: twice daily.
- Body weights: at randomization, on the day of dosing and 24 hours post dose.
- A Functional Observational Battery (FOB); blinded: once pre-dose and at approximately 15 minutes, and 2 and 6 hours post-dose.

Qualitative assessments:

Body position	Olfactory response
Convulsions, twitches	Lacrimation
and tremors	Pupil size
Bizarre behavior	Salivation
Ease of removal	Body tone
Vocalization	Extensor thrust
Rearing	Corneal reflex
Gait	Pinna reflex
Palpebral closure	Toe pinch
Piloerection	Tail pinch
Respiratory rate/pattern	Visual placing
Locomotor activity level	Positional passivity
Arousal	Auricular startle
Grooming	Air righting reflex
Defecation/urination	
Diarrhea	
Urinary staining	

Quantitative assessments:

Grip strength - fore and hind limbs
Hind limb splay
Body temperature (rectal)

Results:

- No mortality or toxicologically significant changes in BW were observed.
- FOB did not show test article-related effect.

Cardiovascular effects:

Study Title: CCI-779: An escalating dose intravenous cardiovascular study in conscious male and female cynomolgus monkeys

Key findings:

- Single i.v. doses of 0.1, 0.5 and 1.5 mg/kg of CCI-779 administered to conscious ♂ and ♀ monkeys did not affect the mean arterial blood pressure, systolic pressure, diastolic pressure, electrocardiograms, spontaneous gross motor activity, or body temperature during a 24-hr post-dose assessment. There was no evidence for gender-related effects on these parameters.
- A dose-dependent ↓ in heart rate (~↓10-25%) was observed in the first 1.5 hrs post-dose. However, high variability in measurements throughout the study makes the drug-dependent effect uncertain.

Report: gtr-32073
Compliance: non-GLP
Conducting facility: Wyeth Research; Chazy, NY
Study initiation: November 1997
Test article: CCI-779, Batch/Lot# 0M7612, [redacted] pure

Study Design:
Dose:

Dosage[a] (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)
0 (vehicle control)	0	1
0.1	0.1	1
0.5	0.5	1
1.5	1.5	1

a: based on an active moiety of 100% and corrected for purity

Rationale presented for dose selection:
The doses selected represented 1X, 5X and 15X multiples of the low dose used in a toxicology study'.

Vehicle control: 3% ethyl alcohol and 97% CCI-779 diluent.
CCI-779 diluent: 5% polysorbate 80; 5% polyethylene glycol 400 in sterile water for injection.
CCI-779 stock: 50 mg/mL, made in ethanol
Species: Cynomolgus monkeys
No. of animals: 3/sex/group
Route, formulation: i.v., solution
Dose volume: 1 mL/kg

Endpoints evaluated:
At least 17 weeks before dose initiation, a telemetry transmitter was placed into the peritoneal cavity. The blood pressure catheter was tunneled subcutaneously to the left groin region, placed into the left femoral artery. The electrocardiogram leads (axial lead) were subcutaneously tunneled to the base of right side of neck and near the sternum-5th intercostal space on the left side.

Parameter	Measurement Type	Measurement Method
Systolic blood pressure	Direct	telemetry implant, femoral artery
Diastolic blood pressure	Direct	telemetry implant, femoral artery
Mean arterial blood pressure	Derived	telemetry implant, femoral artery
Spontaneous gross motor activity	Derived	telemetry implant[a]
Electrocardiogram	Direct	telemetry implant[b]
Heart Rate	Derived	telemetry implant, blood pressure waveform
Core temperature	Direct	telemetry implant, peritoneal cavity
Clinical Signs/Mortality	Direct	visual observations
Food Consumption	Direct	visual estimate

a: spontaneous gross motor activity was derived from electrical signal strength, which correlates inversely with gross movement.

b: axial lead was used for the electrocardiogram, with leads placed at base of right side of neck and 5th intercostal space near the sternum on the left side.

- The telemetry data were collected over 20 second periods every 30 minutes for 24 hours before and after each dose.
- Arterial blood pressure (systolic, diastolic and mean), heart rate, electrocardiograms, core body temperature and spontaneous gross motor activity were monitored for 24 hours pre-dose and 24 hours post-dose.
- Clinical signs, mortality and food consumption: at least once daily on the day of dosing.
- Two electrocardiograms from each monkey at each dosage level were evaluated.

Results:

- No mortality and no effect on clinical signs, or food consumption.
- No test article-related effect on blood pressure, gross motor activity, or temperature.
- No effect on electrocardiogram.
- Small dose-dependent ↓ in heart rate was observed in the first 1.5 hrs post-dose. After this time-point, changes were non-dose dependent and/or minimal. Only the first 3 hrs post-dose will be presented here.

Time post-dose	Groups	Heart rate (BPM)	Changes vs vehicle
0.50	Vehicle	236	-
	LD	209	**↓11%***
	MD	203	**↓14%***
	HD	193	**↓18%***
1.00	Vehicle	215	-
	LD	193	**↓10%**
	MD	181	**↓16%***
	HD	163	**↓25%***

1.50	Vehicle	187	-
	LD	163	**↓13%***
	MD	148	**↓21%***
	HD	139	**↓26%***
2.00	Vehicle	163	-
	LD	156	↓4%
	MD	158	↓3%
	HD	222	↑36%*
2.50	Vehicle	144	-
	LD	157	↑8%
	MD	157	↑9%
	HD	167	↑15%
3.00	Vehicle	156	-
	LD	143	↓8%
	MD	158	↑1%
	HD	165	↑6%

* Statistically significant.

Study Title: WAY-130779: A Cardiovascular Safety Assessment Study of Single Oral Doses in Conscious Sprague-Dawley Rats

Key Findings: single oral doses of temsirolimus at up to 3 mg/kg and a single oral dose of sirolimus at 3 mg/kg did not result in toxicologically meaningful changes in the mean arterial blood pressure or heart rate in SD rats. PK parameters were not provided; therefore it is not clear whether adequate systemic exposure was achieved in these tests.

Report # GTR-27099
Compliance: non-GLP
Species: SD rats (4/sex)
Age and weight: ~65-70 days and 305-325 g
Route of administration: Oral gavage (10 mL/kg)

Doses:

Temsirolimus (single dose):	0.3, 1, and 3 mg/kg (1.8, 6, and 18 mg/m2)
Sirolimus (single dose):	3 mg/kg (18 mg/m2)
Vehicle control:	Phosal 50 PG
Rationale for the doses:	temsirolimus doses were selected to be 1x, 3x, and 10x of the pharmacologically efficacious dose.

Assessments: mean arterial blood pressure, heart rate- conducted in conscious rats
Mean arterial blood pressure and heart rate were monitored at one minute intervals from 60 minutes prior to dosing to 300 minutes following dosing.

Results: The effect of temsirolimus on mean arterial blood pressure and heart rate was studied in conscious normotensive rats. Single oral doses of temsirolimus (0.3, 1, and 3 mg/kg, p.o.) or a single oral dose of sirolimus at 3 mg/kg resulted in changes that were

small, non-dose dependent and/or non-time-dependent. PK parameters were not provided (and no toxicity endpoint was incorporated), therefore it is not clear whether adequate systemic exposure was achieved with these drugs.

Pulmonary effects:
Study Title: CCI-779: A single dose intravenous respiratory safety pharmacology study in male rats

Key findings: ↓respiratory rates of 9-11% were observed at 2 hrs post-dose in LD, (0.2 mg/kg) MD (1 mg/kg) and HD (5 mg/kg) rats, when compared to pre-treatment values.

Report# rpt-43608
Compliance: GLP
Conducting facility: [redacted]
Study initiation: June 2001
Test article: CCI-779, Batch/Lot# 0M8601; [redacted] pure

Study Design:

Dose:	0.2, 1, and 5 mg/kg (single dose) 1.2, 6, 30 mg/m2 Rationale for the HD (per sponsor): The high dose corresponds closely to the weekly dosage used in clinical trials in Europe and is 50 fold the efficacious dose seen in the nude mouse glioma model.
Vehicle:	ethanol, polyethylene glycol, propylene glycol, polysorbate 80, vitamin E, saline
CCI-779 Stock:	25 mg/mL CCI-779, 395 mg/mL ethyl alcohol; 0.750 mg/mL vitamin E, and propylene glycol.
CCI-779 Diluent:	5% (w/v) polysorbate 80; 5% (w/v) polyethylene glycol 400, and sterile water for injection.
Dosing formulation:	An intermediate stock solution (5 mg/mL) was prepared by mixing appropriate amounts of the stock solution with the diluent. This intermediate stock was further diluted with saline, to prepare the dosing formulation.
Species:	SD rats; ~7 weeks old; ~210-230 g
No. of animals:	6 ♂s/group
Route, formulation:	i.v., solution
Dose volume:	4 mL/kg
Endpoints evaluated:	Mortality, clinical signs, respiratory function Respiratory Function Measurements: animals were placed in 'head out' plethysmographs and ventilatory parameters (tidal volume, respiratory rate and derived minute volume) were measured for 15 minute periods at the following timepoints: pre-dose, immediately post-dose, 2 and 6 hours post-dose. On one occasion the 6 hour post dose time-point was not recorded due to technical difficulties, therefore these animals were measured at 7.5 hours post-treatment.

The respiratory waveforms were recorded using the Buxco Electronic LS-20 system.

Results

- No mortality or clinical signs were reported.
- No toxicologically significant changes in the mean tidal volume or minute volume were observed.
- The MD and HD groups had statistically significantly ↓respiratory rates compared to the vehicle control group (15% and 17%, respectively) at the 2-hr post-dose assessment. These differences represented a 9% and 11% decrease from the pre-treatment values in each treatment group, respectively. Reduced respiratory rate was seen at LD, at the same time-point, but the change was not statistically significant.

Respiration rate (breaths/min)

	Pre-dose	Immediately post-dose	2 hrs post-dose	6 hrs post-dose
Control	218.8	208.8 (↓5%)	213.3 (↓2%)	212.3 (↓3%)
LD	225	214 (↓5%)	200.3 (↓11%)	202 (↓10%)
MD	197.5	189.8 (↓4%)	*180.5 (↓9%)	172.5 (↓13%)
HD	198.5	192 (↓4%)	*177.5 (↓11%)	214.3 (↑8%)

* Statistically significantly different from control.
(): Changes compared to pre-dose values.

Renal effects: A separate study was not conducted

Gastrointestinal effects: A separate study was not conducted

Abuse liability: not known

2.6.2.5 Pharmacodynamic drug interactions

None.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

See Tables provided under each study reviewed.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Toxicokinetic data were reviewed as part of the toxicology studies. Detailed information can be found under each study reviewed. A brief summary is presented below.

Toxicokinetic data:

6-month, once weekly i.v. toxicology of temsirolimus in monkeys:

- Exposures to temsirolimus were generally higher in ♂s than ♀s.
- The half-life of temsirolimus was rather long, being 18-33 hrs during Week 1 and 37-55 hrs during Week 26.

- Repeated dosing resulted in accumulation of temsirolimus as indicated by increased temsirolimus exposures on Week 26 when compared to Week 1 and the increased half-life on Week 26.

9-month, once weekly i.v. toxicology of temsirolimus in monkeys:

- Exposures (AUC) to temsirolimus were higher in ♂s than ♀s; with the exception of Week 1 HD data
- Exposures to temsirolimus increased with increasing doses; increases were less than dose-proportional
- Repeated weekly dosing did not result in increased AUCs; AUCs on Weeks 1 and 38 were comparable. However, the half-life of temsirolimus increased after repeated dosing: 21-27 hrs on Week 1 and 36-48 hrs on Week 38.

Temsirolimus		6-month rat toxicology		9-month monkey toxicology	
		Week 1	Week 26	Week 1	Week 38
$T_{1/2}$ (hr)		18-33	37-55	21-27	36-48
AUC	♂ (LD-HD)	260-2050	590--3400	1290-5480	1240-6620
	♀ (LD-HD)	185-1860	330-2100	950-6280	1020-4900

AUC: ng.hr/mL

Absorption:

The following is based on the summary data excerpted from the package:

- The oral bioavailability was estimated to be ~5% in rats and ~22% in monkeys, in 4-cycle toxicity studies.
- After a single oral (gavage) dose of [^{14}C]temsirolimus to rats (1.5 mg/kg) or monkeys (7.5 mg/kg), [^{14}C]temsirolimus-derived radioactivity was rapidly absorbed, with time to peak concentration (t_{max}) values in whole blood of 1.5 hours in rats and between 1 and 2 hours in monkeys. The apparent terminal half life ($t_{1/2}$) of total radioactivity was 56 hours in rats and 38 hours in monkeys. Comparisons of the pharmacokinetics from whole blood and plasma from rats and monkeys suggest that the AUC and $t_{1/2}$ were greater in whole blood than plasma and the total clearance (CL_T) was greater in plasma than whole blood.

Distribution:

Based on the study conducted with ^{14}C-temsirolimus in SD and Long-Evans rats (i.v. dosing):

- Distribution of radioactivity in rats was rapid, with a Tmax of 0.083 hrs (~5 min) for most tissues.
- The radioactive products remained in tissues for an extended period of time as indicated by the T1/2, which ranged from 35 hrs (heart) to 78 hrs (stomach). T1/2 was 42 hrs for blood.
- At 168 hrs (7 days) post-dose, although the radioactivity was reduced compared to the maximum concentrations, they were still measurable.

- The following is the order for the exposure (AUC_{0-168}) of radioactivity from the highest to the lowest in SD rats: Thymus (180,626 ng.equiv.hr/g), adrenal/stomach, pituitary, liver, thyroid, large intestine (103,859 ng.equiv.hr/g), pancreas, small intestine, spleen, lymph node, kidney, heart (77,131 ng.equiv.hr/g), salivary gland, lung (72, 125 ng.equiv.hr/g), bone marrow (34,205 ng.equiv.hr/g), skin, brain/muscle, fat, bone, plasma, blood, eye (3,992 ng.equiv.hr/g). The terminal elimination was not observed in testes.
- The exposure level (AUC_{0-168}) of radioactive products in the pigmented skin of Long-Evans rats was comparable to that in the skin of SD rats.
- The tissue:blood ratios were greater than 1 for most tissues, indicating extensive distribution of radioactive products.
- In general, the elimination of radioactive materials from most tissues was slightly slower than the rate from blood.
- The presence of radioactivity in the GI tract following intravenous administration indicates biliary excretion and entero-hepatic recycling.

Plasma protein binding was not evaluated for animal species. ^{14}C-temsirolimus was 85-87% protein bound to male human plasma proteins at concentrations of 10 and 100 ng/mL, using an erythrocyte partitioning technique.

Metabolism:

- Based on the summary of data presented (see section 2.6.5, PK tabulated summary) and the study reviewed (Report # GTR-32279), CYP 3A4 was the major cytochrome P450 involved in metabolism of temsirolimus in human and in rat. CYP2E1 was also involved in the metabolism of the drug.
- For in vitro metabolism using human liver microsomes, for the 3 subjects studied, the amount of cytochrome P450-dependent CCI-779 metabolites varied by more than 20-fold, whereas the amount of non-cytochrome P450-dependent drug-derived products (rapamycin, seco-CCI-779 and seco-rapamycin) varied less (<2 fold).
- Temsirolimus was the major product after i.v. drug administration to rats and monkeys. In rats, the relative amounts of extractable radioactivity were ≥86% in blood and ≥68% in plasma after i.v. dosing. Temsirolimus and sirolimus were the major products after i.v. drug administration to humans. The sirolimus:temsirolimus exposure ratio (AUC) was ~2.7 after i.v. administration in humans. In addition, sirolimus was determined to be present in large amounts (~≥10% of the temsirolimus peak) after oral administrations to rats and monkeys.
- Based on the summary Table excerpted from the package, the metabolite profile of temsirolimus after i.v. drug administration to humans was different than those observed in rats and monkeys after i.v. drug administration and resembled that seen in the animals after p.o. administration.
- Sirolimus or sirolimus-derived products were not detectable after i.v. dosing to rats. Based on summary of data for rats, sirolimus represented between 11% and 23% of the temsirolimus peak after oral dosing.

- The high amount of sirolimus after p.o. administrations to rats, monkeys, and human (sirolimus:temsirolimus ~ 2.71 in humans) was speculated to be due the higher levels of esterase activity in the liver and the GI tract, which can increase the conversion of temsirolimus to sirolimus.
- Following incubations of CCI-779 in mouse liver microsomes in the presence or absence of NADPH, 5 metabolites (M10, M18, M23, M4, and sirolimus) were detected. In addition, an impurity/ degradation product [redacted] was also present.
- After single i.v. administration in rats, between 5 min and 24 hrs post-dose, unchanged drug was the predominant product in both blood and plasma extracts (86%-94% of extractable radioactivity in blood and 70%-88% in plasma). Seco-temsirolimus or M4 (up to 14% in blood and up to 24% in plasma) and hydroxyl-temsirolimus or M10 (up to 4% in blood and up to 15% in plasma) were significant drug-derived products detected.
- After single i.v. dosing in rats, the recoveries of radioactivity from whole blood and plasma were ~96% at 5 minutes, and decreased gradually with time to approximately 50% and 30%, respectively, at 24 hours. In addition to metabolic reactions, degradation of CCI-779 appeared to contribute to the reduction in radioactivity.
- In rats, after single i.v. dosing, while feces contained about 80% f the administered dose (fecal being the major route of excretion), CCI-779 was present at relatively low levels in feces. Drug-derived products in feces were characterized as seco-CCI-779, unidentified polar degradation products, and various oxidative metabolites of CCI-779.
- Following oral dosing in bile duct cannulated SD rats, ~93% of the radioactivity was recovered over 72 hours: ~3% in urine, ~8% in bile, and ~82% in feces.
- Following i.v. dosing in bile duct cannulated SD rats, ~86% of the radioactivity was recovered over 72 hours: 4% in urine, 76% in bile, and ~6% in feces.
- In bile, 11 compounds were detected. Two of these peaks (M21 and M22) were determined to be degradation products of [^{14}C]CCI-779. In some samples, these peaks contained more than 50% of the radioactivity in bile. Seco-CCI-779 (M4) and [redacted] also degradation products of CCI-779, were detected at up to 27.5% of the radioactivity in bile. The major metabolites excreted in the bile, following either i.v. or oral administration of temsirolimus, were characterized as oxidation or reduction products: 3 of the major metabolites were hydroxylated metabolites (M8, M10 and M17), 3 other (M18, M19 and M20) were reduced CCI-779 metabolites.

Excretion:

- Biliary excretion was the major route of excretion in i.v. administration to rats and monkeys, i.e. for absorbed radioactivity (drug and metabolites).
- When drug was given orally, due to low absorption, most of the radioactivity was in the feces.

2.6.4.2 Methods of Analysis
See under individual study reviews.

2.6.4.3 Absorption
The following is based on the summary data excerpted from the package:

- The oral bioavailability was estimated to be ~5% in rats and ~22% in monkeys, in 4-cycle toxicity studies (animals were dosed i.v. or p.o.).
- After a single oral (gavage) dose of [^{14}C]temsirolimus to rats (1.5 mg/kg) or monkeys (7.5 mg/kg), [^{14}C]temsirolimus-derived radioactivity was rapidly absorbed, with time to peak concentration (t_{max}) values in whole blood of 1.5 hours in rats and between 1 and 2 hours in monkeys. The apparent terminal half life ($t_{1/2}$) of total radioactivity was 56 hours in rats and 38 hours in monkeys. Comparisons of the pharmacokinetics from whole blood and plasma from rats and monkeys suggest that the AUC and $t_{1/2}$ were greater in whole blood than plasma and the total clearance (CL_T) was greater in plasma than whole blood.

2.6.4.4 Distribution

Study Title: CCI-779: tissue distribution of [^{14}C]-CCI-779-derived radioactivity following a single 2.5 mg/kg intravenous dose of [^{14}C]-CCI-779 in male Sprague-Dawley and Long-Evans rats

Report# RPT-44598
Testing facility: Wyeth Research, Pearl River, NY
Test article: [^{14}C]-CCI-779, Batch # L20475-54
Radiochemical purity: [redacted]
Chemical purity: [redacted]
Specific activity: 38.7 μCi/mg
Study design:
Species: SD (♂) or Long-Evans (♂) rats
SD: 0.276-0.314 g
Long-Evans: 0.286-0.321 g
Dose: single bolus i.v. dose of 2.5 mg/kg (15 mg/m2; ~100 μCi/kg) ^{14}C-CCI-779
1 mL/kg
Formulation: solution in polysorbate 80 (5% w/w), ethanol (5% v/v) and polyethylene glycol 400 (5% w/w)
Sample collection and techniques:
The distribution of radioactivity to selected tissues was evaluated up to 168 hrs post-dose by:

- Quantitative whole body autoradiography (QWBAR) via phosphor-imaging (n=1/time point) and
- Tissue dissection (n=1/time point) via liquid scintillation counting (LSC).

Tissue samples (n=26) were analyzed at 0.083, 1, 8, 24, 72 and 168 hours post-dose.

The distribution of radioactivity in selected melanin-containing tissues of male Long-Evans (pigmented) rats (n=1/time point) was also characterized by QWBAR. Long-Evans rats (n=1/time point) were sacrificed at 0.083, 1, 8, 24, 72 and 168 hours post-dose.

Results:

- The maximum tissue concentrations (C5min) for [^{14}C]-CCI-779-derived radioactivity in selected tissues of Sprague-Dawley rats ranged from the highest in the adrenal (39305 ng equiv./g) to a lowest in the eyes (68.7 ng equiv./g).
- The following is the order for the C5min levels of radioactivity in tissues (including blood and plasma) from the highest to the lowest in SD rats: adrenal, heart, lung, liver, kidney, thyroid, pancreas, pituitary/salivary gland, spleen, lymph node, small intestine, stomach, bone marrow, muscle/plasma, thymus, large intestine, blood, fat, skin, bone, brain/testes, eyes.
- The following in the order for the exposure (AUC0-168) of radioactivity from the highest to the lowest in SD rats: Thymus (180,626 ng.equiv.hr/g), adrenal/stomach, pituitary, liver, thyroid, large intestine (103,859 ng.equiv.hr/g), pancreas, small intestine, spleen, lymph node, kidney, heart (77,131 ng.equiv.hr/g), salivary gland, lung (72, 125 ng.equiv.hr/g), bone marrow (34,205 ng.equiv.hr/g), skin, brain/muscle, fat, bone, plasma, blood, eye (3,992 ng.equiv.hr/g). The terminal elimination was not observed in testes.
- The half-life of [^{14}C]-CCI-779-derived radioactivity in blood was 42 hours.
- Tmax was 0.083 hrs for most tissues as well as for plasma and blood, indicating rapid distribution.
- In tissues where t1/2 was measurable, the half-life of radioactive material ranged from 35 hrs (heart) to 78 hrs (stomach), indicating that drug or drug-derived material were retained in these tissues for an extended period of time.
- At 168 hours (7 days) post-dose, the concentrations of radioactivity declined greatly as compared to C5min, however, all tissues still had concentrations above the quantitation limit. Tissue concentrations of [^{14}C]-CCI-779- derived radioactivity at 168 hours ranged from a high of 283 ng equiv./g in thymus to a low of 7.83 ng equiv./g in eye and represented 30 and 11% of C5min value, respectively. All tissues had concentrations higher than blood (3.77 ng equiv./g) at 168 hours, indicating slow clearance from tissues and high volume of distribution.
- The tissue:blood ratios were greater than one for majority of tissues, indicating extensive distribution of [^{14}C]-CCI-779-derived radioactivity in tissues of Sprague-Dawley rats. The blood:plasma concentration ratios were greater than one up to 8 hours and less than one from 24 to 168 hours.
- Distribution in melanin-containing tissues: in Long-Evans (pigmented) rats the maximum radioactivity levels in the skin and uveal tract were 2809 and 5625 ng equiv./g, respectively. The maximum radioactivity levels were observed at 0.083 hr. Corresponding exposure levels (AUC_{0-8}) of drug-derived radioactivity in skin and uveal tract were 38,219 and 16,986 ng equiv•hr/g, respectively. The tissue:blood AUC0-t ratios were 3.61 and 1.60 for skin and uveal-tract, respectively. The tissue:blood concentration ratios were greater than one for skin and uveal tract at most of the time points. By 168 hours post-dose, tissue concentrations of [14C]-CCI-779-derived radioactivity in the skin and uveal-tract

had declined to 0.9 and 0.2% of the C5min value, respectively. These data suggest that [14C]-CCI-779-derived radioactivity associates with low affinity to melanin-containing tissues.

Species: Rats (S-D and Long-Evans)
No. and Sex (M/F) of Animals: 1 M time point assay
Feeding Condition: Fed
Vehicle/Formulation: 5% polysorbate 80, 5% ethanol, and 5% PEG 400

Dosage (mg/kg): 2.5
Radionuclide: ^{14}C
Specific Activity: 58.7 µCi/Mg
Analyte/Assay (unit): Total radioactivity, ng eq/g

Method of Administration: IV
Method of Analysis: LSC[a] (S-D and Long-Evans); QWBAR[b] (S-D and Long-Evans)

Sampling times: 5 minutes, and 1, 8, 24, 72, and 168 hours

Strain	Tissues/Organs	C_{5min} (ng eq/g)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-168} (ng eq•h/g)	$AUC_{0-\infty}$ (ng eq•h/g)	Tissue:Blood $AUC_{0-\infty}$ Ratio	Tissue:Blood C_{5min} Ratio
Sprague-Dawley	Blood	1113	0.083	42	6519	6851	1.00	1.00
	Plasma	2025	0.083	38	6981	7101	1.04	1.82
	Adrenal	39,305	0.083	49	125,046	131,565	19.2	35.3
	Bone	738[c]	1	47	14,905	15,946	2.33	0.663
	Bone Marrow	2720	0.083	41	32,782	34,205	4.99	2.44
	Brain	154	0.083	ND[d]	7135	31,807	1.02[e]	0.138
	Eyes	68.7	0.083	69	3214	3992	0.583	0.0617
	Fat	1035[c]	1	51	22,383	24,245	3.54	0.930
	Heart	35,035	0.083	35	75,771	77,151	11.3	31.5
	Kidney	10,272	0.083	43	82,180	86,002	12.6	9.23
	Kidney, Cortex	9616	0.083	53	66,692	71,583	10.4	8.64
	Kidney, Medulla	5619	0.083	42	52,993	55,410	8.09	5.05
	Large Intestine	1699[c]	1	61	88,659	103,859	15.2	1.53
	Liver	15,842	0.083	47	108,343	113,787	16.6	14.2
	Lung	23,596	0.083	36	70,559	72,425	10.6	21.2
	Lymph Node	4696	0.083	37	86,069	89,017	13.0	4.22
	Muscle	2055	0.083	39	30,711	31,886	4.65	1.85
Sprague-Dawley (cont'd)	Pancreas	7268[c]	0.083	36	95,222	97,169	14.2	6.53
	Pituitary	5454[c]	1	36	112,147	115,906	16.9	4.90
	Salivary gland	5460[c]	1	43	72,056	75,115	11.0	4.91
	Skin	1017[c]	1	41	31,625	33,356	4.87	0.914
	Small Intestine	3751[c]	1	47	89,716	96,080	14.0	3.37
	Spleen	4765	0.083	53	87,248	95,563	13.9	4.28
	Stomach	3061	0.083	78	102,753	131,596	19.2	2.75
	Testes	159	0.083	ND[d]	12,953	ND[d]	1.89[e]	0.143
	Thymus	1866[c]	1	59	156,504	180,626	26.4	1.68
	Thyroid	9863	0.083	65	98,394	111,720	16.3	8.86
Long-Evans	Blood	1467	0.083	62	9433	10,583	1.00	1.00
	Plasma	2675	0.083	48	9348	9850	0.931	0.548
	Skin, pigmented	2809	0.083	41	36,744	38,219	3.61	1.05
	Uveal tract	5625	0.083	41	16,263	16,986	1.60	2.10

a. N=1. Analysis by LSC (LLOQ = 0.51 ng eq/g) for blood and plasma (S-D and Long-Evans); bone, brain, fat, kidney, large intestine, small intestine, stomach, and thyroid (S-D).
b. N=1. Analysis by QWBAR (LOQ = 1.14 ng eq/g) for adrenal, bone marrow, eyes, heart, kidney cortex, kidney medulla, liver, lung, lymph node, muscle, pancreas, pituitary, salivary gland, skin, spleen, testes, and thymus (S-D); pigmented skin and uveal tract (Long-Evans).
c. Represents C_{max} values.
d. Terminal elimination phase was not observed.
e. Blood:tissue based on AUC_{0-t}.

AUC = Area under the concentration-versus-time curve; C_{min} = Concentration at 5 minutes; C_{max} = Peak concentration; IV = Intravenous; LLOQ = Lower limit of quantitation; LSC = Liquid scintillation counting; N = Number of animals; ND = Not determined; QWBAR = Quantitative whole body autoradiography; RPT = Report; S-D = Sprague-Dawley; $t_{1/2}$ = Apparent terminal half-life; t_{max} = Time to peak concentration.

Table excerpted from the package.

Study Title: Temsirolimus: in vitro protein binding of [14C]temsirolimus by erythrocyte partitioning

Note: this study was not reviewed. Results as reported by the sponsor are presented below.

The study was conducted in male human plasma using erythrocyte partitioning. This method was used to provide a stable matrix for temsirolimus, as previous studies had

indicated low stability of the drug in plasma of multiple species, including that of human. Erythrocyte partitioning uses whole blood or suspensions of erythrocytes. Incubations with [^{14}C]temsirolimus were performed in erythrocyte suspensions (0.45 hematocrit) prepared with diluted (0% to 10%) plasma. The unbound concentration of [^{14}C]temsirolimus in plasma was determined from the linear relationship between the ratio of the [^{14}C]temsirolimus concentration in diluted plasma to that in erythrocytes and the percentage of plasma protein in the erythrocyte suspension.

Result:

- At erythrocyte suspension [^{14}C]temsirolimus concentrations between 10 and 100 ng/mL, [^{14}C]temsirolimus was 85%-87% protein bound to male human plasma proteins.
- The capacity of [^{14}C]temsirolimus to associate with erythrocytes in suspension was exceeded at 1000 ng/mL; therefore, binding to human plasma proteins could not be determined.

Study System: In vitro
Matrix and Method: Plasma, Erythrocyte Partitioning
Radionuclide: ^{14}C
Temperature: 37°C

Report No. RPT-62965
Cross-Reference: 4.2.2.3

Species	[^{14}C]Temsirolimus Concentration (ng/mL)	% Bound to Plasma Proteins (37°C)
Humans (Males)	10	85.0
	100	87.1
	1000	ND[a]

a. Not determined due to non-linear partitioning of [^{14}C]temsirolimus between erythrocytes and diluted plasma at a [^{14}C]temsirolimus concentration of 1000 ng/mL.

ND = Not determined; RPT = Report.

Table excerpted from the package.

2.6.4.5 Metabolism

Key:

Metabolite ID	Metabolite name	Structural Characteristics
Sirolimus		Loss of the BHMP group
Temsirolimus [redacted]		
Sirolimus [redacted]		
M1	Desmethyl sirolimus	Loss of the BHMP group and demethylation (site uncharacterized)
M2	Desmethyl temsirolimus	Demethylation on C32-C8
M3	Seco-sirolimus	Loss of the BHMP group and ring opening at C25
M4	Seco-temsirolimus	Ring opening at C25
M5	Hydroxy sirolimus	Loss of the BHMP group and hydroxylation on C32-C23 region
M6	Hydroxy temsirolimus	Hydroxylation on C32-C23 region
M7	Hydroxy sirolimus	Loss of the BHMP group and hydroxylation on C32-C8 region
M8	Hydroxy temsirolimus	Hydroxylation of the piperidine ring
M9	Hydroxy sirolimus	Loss of the BHMP group and hydroxylation on the C32-C8 region
M10	Hydroxy temsirolimus	Hydroxylation at C9-C13
M11	7-O-desmethyl sirolimus	Loss of the BHMP group and demethylation of the methoxy group at the 7-position
M12	Desmethyl temsirolimus	Demethylation (site uncharacterized)
M13	41-O-desmethyl sirolimus	Loss of the BHMP group and demethylation of the methoxy group at the 41-position
M14	Desmethyl temsirolimus	Demethylation (site uncharacterized)
M15	Desmethyl sirolimus	Loss of the BHMP group and demethylation (site uncharacterized)
M16	Hydroxy sirolimus	Loss of the BHMP group and hydroxylation (site uncharacterized)
M17	Hydroxy temsirolimus	Hydroxylation of the piperidine ring
M18	Reduced seco temsirolimus	Ring opening at C25 and reduction of the C25-C30 fragment
M19	Reduced seco temsirolimus	Ring opening at C25 and reduction of the C9-C15 fragment
M20	Reduced temsirolimus	Reduction of the C9-C15 fragment
M21	Unknown polar degradation products	
M22	Unknown polar degradation products	
M23	Hydroxy reduced temsirolimus	Hydroxylation at C25-31 or C37-C44 and reduction at C25-C30

BHMP = Bis(hydroxymethyl)-propionate; M = Metabolite.

Table excerpted from the package.

RAT/MONKEY/HUMAN COMPARATIVE SUMMARY

Comparative in vivo data:

		Rats		Monkeys		Humans	
Type of Metabolite[a]	Metabolite ID	IV --GTR-34738--	Oral --GTR-38620--	IV --GTR-36527--	Oral --GTR-38606--	IV ----RPT-42535, RPT-62848----	Oral --RPT-62848--
Temsirolimus	NA	Major	Major	Major	Major	Major	Major
Sirolimus	NA		Major	X[b]	Major	Major	Major
[redacted]	NA		X		X	X	X
[redacted]	NA		X		X	X	X
Seco-temsirolimus	M4	X	X	X	X	X	
Seco-sirolimus	M3		X		X	X	
Hydroxy temsirolimus	M6, M8, M10, M17	X[c]	X	X[c]	X	X[c]	
Desmethyl temsirolimus	M2, M12, M14		X		X		
Reduced temsirolimus[d]	M18, M19, M20				X	X	
Hydroxy, reduced temsirolimus	M23						
Hydroxy sirolimus	M5, M7, M9, M16		X		X	X	Major
Desmethyl sirolimus	M1, M11, M13, M15					X	X
Reduced sirolimus						X	
Other			Temsirolimus-2CH$_2$, Seco-Temsirolimus [redacted]				

Major indicates a metabolite present > 10% relative to temsirolimus; X indicates the metabolite was observed.

a. Metabolites observed in whole blood, except where indicated.
b. Observed in plasma.
c. Only the hydroxy temsirolimus M10 metabolite was observed after IV administration in rats, monkeys, and humans.
d. Reduced temsirolimus metabolites include M18 and M19, which were characterized as reduced seco-temsirolimus.

GTR = General Technical Report; ID = Identification (M number); IV = Intravenous; NA = Not applicable.

Table excerpted from the package.

Comparative in vitro metabolic data

Cross-Reference:	4.2.2.4		4.2.2.4					
Report No.:	------RPT-54304------		----------GTR-52279----------					
Enzyme System:	Mouse Liver Microsomes		Rat Liver Microsomes		DEX-induced Rat Liver Microsomes		Human Liver Microsomes	
Concentration:	------5 and 25 µM------		---------25 µM--------		---------25 µM---------		---------25 µM---------	
Metabolite	-NADPH	+NADPH	-NADPH	+NADPH	-NADPH	+NADPH	-NADPH	+NADPH
Temsirolimus	X[a]	X	X	X	X	X	X	X
Sirolimus	X	X			X	X	X	X
[redacted]		X	X	X	X	X	X	X
					X	X	X	X
Desmethyl Sirolimus (M1/M15)						X		X
Desmethyl Temsirolimus (M2)						X		X
Seco-Sirolimus (M3)					X	X	X	X
Seco-Temsirolimus (M4)	X[a]	X	X	X	X	X	X	X
Hydroxy Sirolimus (M5)				X		X		X
Hydroxy Temsirolimus (M6)						X		X
Hydroxy Sirolimus (M7)								X
Hydroxy Temsirolimus (M8)						X		X

Report No.:	RPT-54304		GTR-32279					
Enzyme System:	Mouse Liver Microsomes		Rat Liver Microsomes		DEX-induced Rat Liver Microsomes		Human Liver Microsomes	
Concentration:	5 and 25 µM		25 µM		25 µM		25 µM	
Metabolite	-NADPH	+NADPH	-NADPH	+NADPH	+NADPH	-NADPH	+NADPH	-NADPH
Hydroxy Sirolimus (M9)						X		X
Hydroxy Temsirolimus (M10)		X				X		X
7-O-Desmethyl Sirolimus (M11)						X		X
Desmethyl Temsirolimus (M12)						X		X
41-O-Desmethyl Sirolimus (M13)						X		X
Desmethyl Temsirolimus (M14)						X		X
Desmethyl Sirolimus (M15)						X		X
Hydroxy Sirolimus (M16)						X		
Hydroxy Temsirolimus (M17)						X		
Reduced Seco-Temsirolimus (M18)		X						
Hydroxy, Reduced Temsirolimus (M23)	X	X						

Note: X indicates presence of metabolite.

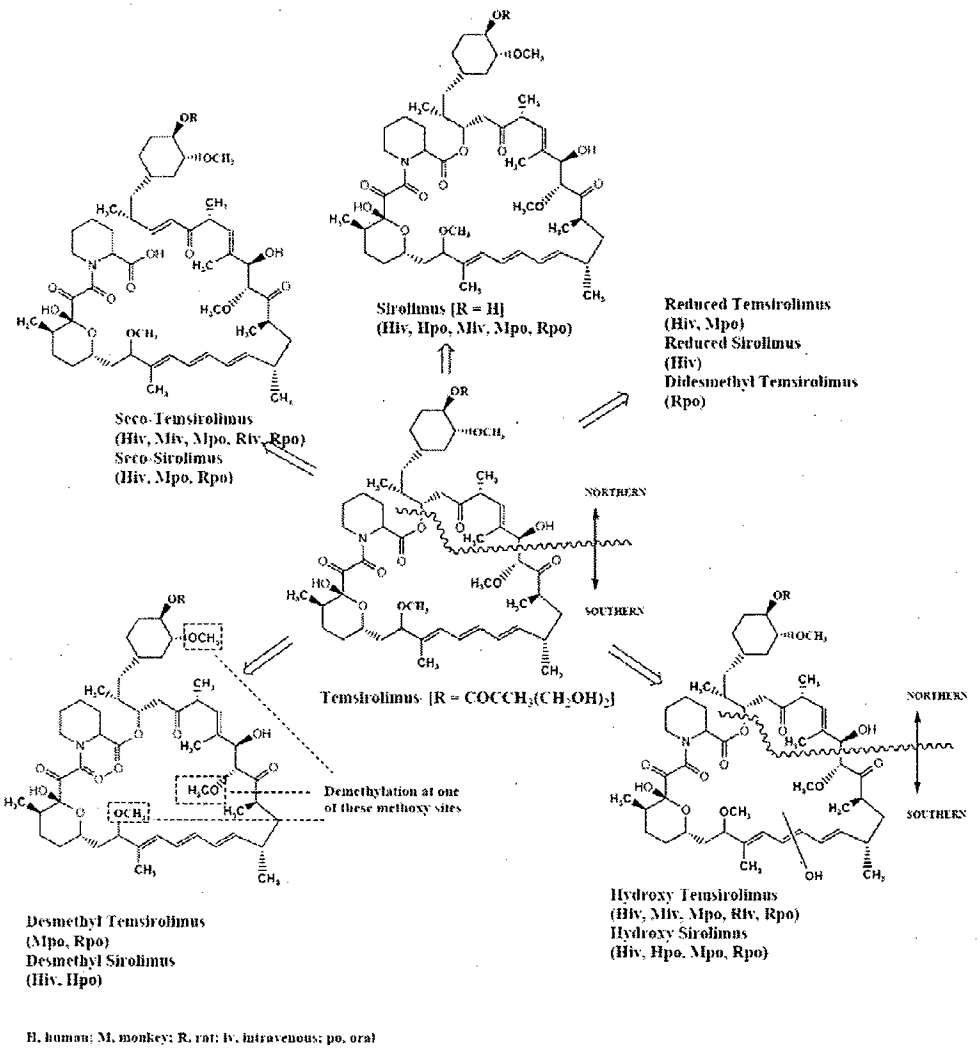
a. Also detected in control sample without liver microsomes.
DEX Dexamethasone; GTR General Technical Report; NADPH Reduced form of nicotinamide-adenine dinucleotide phosphate; RPT Report.

- After a single oral dose of ^{14}C-temsirolimus at 7.5 mg/kg to ♂ and ♀ SD rats, sirolimus represented 11%-23% of the temsirolimus peak.
- After a single oral dose of 14C-temsirolimus at 7.5 mg/kg to monkeys, sirolimus accounted for 9% to 19% of the temsirolimus peak.
- After i.v administrations to rats or monkeys, sirolimus was either not detected or it was at minimal levels.
- After i.v. administration in humans, temsirolimus was readily metabolized to sirolimus and oxidative metabolites of sirolimus. The sirolimus:temsirolimus exposure ratio (AUC) was ~2.7 after i.v. administration and 12.3 after oral administration in humans.

Conversion of temsirolimus to sirolimus (excerpted from the package):
Temsirolimus is converted to sirolimus via a presumed esterase-mediated hydrolysis of the C42 ester bond. It is postulated that binding of temsirolimus to FKBP-12, associated with formed blood elements, may affect the rates of this hydrolysis. Therefore, species differences in the sirolimus:temsirolimus ratios (high in mice and humans and low in rats and monkeys) may be affected by both species differences in hydrolytic activity and differences in the concentration of FKBP-12 in erythrocytes. The higher sirolimus:temsirolimus ratios after oral administration, compared with i.v. administration, may represent the presence of esterase activity in both the liver and GI tract increasing the conversion of temsirolimus to sirolimus after oral administration.

After i.v. administration of temsirolimus to rats and monkeys, there was little conversion of temsirolimus to sirolimus, with sirolimus:temsirolimus ratios ranging from 0.005 to 0.009 in rats and 0.05 to 0.11 in monkeys. In contrast, after i.v. administration of temsirolimus to mice and humans, sirolimus represented the predominant component in

whole blood, with sirolimus:temsirolimus ratios ranging from 1.69 to 2.54 in mice and 2.68 in humans in the renal cancer trial.



H, human; M, monkey; R, rat; iv, intravenous; po, oral

Proposed metabolic pathways of temsirolimus in rats (R), monkeys (M), and humans (H) after intravenous (iv) or oral (po) administration.
Excerpted from the package.

Study Title: CCI-779 (WAY-130779): In Vitro Metabolism in Liver Microsomes of Male CD-1 Mice

Report# RPT-54304
Testing Facility: Biotransformation/Drug Metabolism; Wyeth, Collegeville, PA

Species: ♂ CD-1 mice.

Assay: Metabolite profiles were determined by HPLC with radioactivity flow detection and the identities of the metabolites were elucidated by liquid chromatography/mass spectrometry (LC/MS) analysis.
Methods: incubations of 5 and 25 µM CCI-779 in mouse liver microsomes (1 mg/mL, 60 minute) in the presence or absence of NADPH

Results:
Following incubations of CCI-779 in mouse liver microsomes in the presence or absence of NADPH, two NADPH-dependent metabolites M10 (hydroxy CCI-779) and M18 (reduced CCI-779) were identified. In addition, three metabolites (M23, hydroxy reduced CCI-779; M4, seco CCI-779; and sirolimus) were formed via non-NADPH dependent pathways. [redacted] a degradation product and/or impurity, was also observed. Under the conditions utilized, the relative levels of the CCI-779 related components detected in incubations with NADPH were seco CCI-779 > M10 > M18 > sirolimus > [redacted] = M23.

In summary, following incubations of CCI-779 with hepatic microsomes from male mice, a total of five metabolites were characterized by LC/MS. The metabolites formed were via reduction, oxidation, ring opening and/or hydrolysis to sirolimus.

CCI-779 Related Compounds Observed by LC/MS in Incubations with Mouse Liver Microsomes

Metabolite	Retention Time (min)[a]	[M+Na]+	MW	Site of Metabolism	Metabolite Name	NADPH-dependent[b]
M23	[redacted]	1070.6	1047.6	Hydroxylation at C25-31 or C37-C44 and reduction at C25-C30	Reduced hydroxy CCI-779	No
Seco CCI-779 (M4)		1052.6	1029.6	Ring opening at C25	Seco CCI-779	No
M18		1054.6	1031.6	Reduction at C25-C30	Reduced CCI-779	Yes
M10		1068.6	1045.6	Hydroxylation at C9-C13	Hydroxy CCI-779	Yes
CCI-779		1052.6	1029.6	None	CCI-779	NA
Sirolimus		936.6	913.6	Loss of the BHMP[c] group	Sirolimus	No

a. LC/MS retention time standardized to data file PE_021004_0004
b. NADPH-dependent metabolites were observed only in the presence of NADPH, non-NADPH-dependent metabolites were observed in the presence or absence of NADPH.
c. BHMP = Bis(hydroxymethyl)-propionate
NA. Indicates not applicable

Table excerpted from the package.

Study Title: CCI-779 (WAY-130779): Biotransformation in rat and human liver microsomes, and preliminary characterization of cytochrome P450 isozyme(s) involved

Report# GTR-32279
Testing Facility: Biotransformation/Drug Metabolism; Wyeth, Princeton, NJ

Study design: the in vitro biotransformation of CCI-779 was investigated using human and rat liver microsomes. In addition, a preliminary characterization of the cytochrome (CYP) P450 isozyme(s) involved in the metabolism of CCI-779 was performed, primarily

using human lymphoblastoid cell microsomes containing different cDNA-expressed CYP isozyme.

Microsomal incubation: To evaluate inter-individual variability in the metabolism of CCI-779 in human liver microsomes, incubations were conducted with individual microsomes from the 3 subjects. These livers represented a wide range of CYP3A4 activities as indicated by the rates of testosterone-6b-hydroxylation. Each one-mL incubation in duplicate contained 25 mM CCI-779 (in 20 mL ethanol) and human liver microsomes (1 mg/mL) in 0.1M potassium/sodium phosphate buffer (pH 7.4). Incubation consisted of 10 min pre-incubation at 37°C, followed by reaction initiation by adding NADPH (1 mM). Incubation continued for an additional 20 min at 37°C and terminated by placing on ice. To generate samples for LC/MS analysis, pooled human liver microsomes (2 mg/mL) from the same three subjects were used. The incubation time was increased to 60 minutes. Incubations with control and dexamethasone-induced rat liver microsomes were performed similarly. Corresponding control incubations were performed in the absence of NADPH.

> Human liver microsomes: obtained from portions of 2 female and 1 male human livers from accident victims. All individuals were tested negative for HIV and hepatitis B. Microsomes were prepared using differentia. [redacted]
> Microsomal protein and cytochrome P450 concentrations were determined using standard methods. CYP3A4 activities were determined by the rates of testosterone-6β-hydroxylation4.
>
> Control rat liver microsomes: prepared from ♂ Sprague-D rats. Dexamethasone-induced rat liver microsomes were also prepared.
>
> Microsomes prepared from human lymphoblastoid cells containing cDNA expressed CYP isozymes were purchased from [redacted]
> They included control microsomes (without vectors) and microsomes selectively expressing CYP 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 or 3A4.

Detection assays: LC/MS and HPLC

Results:

- The majority of drug-derived products in human liver microsomes were formed by a NADPH-dependent process. In contrast, the NADPH-dependent biotransformation of CCI-779 in control rat liver microsomes was very limited.
- The marked increase in NADPH-dependent metabolism of CCI-779 in dexamethasone-induced rat liver microsomes provided an initial indication that CYP3A may be involved in the metabolism of CCI-779 in the rat.
- Using microsomes prepared from human lymphoblastoid cells containing different cDNA expressed CYP isozymes, CYP3A4 was primarily involved in the NADPH-dependent biotransformation of CCI-779. The involvement of CYP3A was further supported by the inhibitory effect of ketoconazole on CCI-779 metabolism in human liver microsomes.

- In the 3 subjects studied, the amount of cytochrome P450-dependent CCI-779 metabolites (primarily desmethyl and hydroxy) varied by more than 20-fold, whereas the amount of non-cytochrome P450-dependent drug-derived products (rapamycin, seco-CCI-779 and seco-rapamycin) did not vary significantly (<2 fold). The sponsor indicated that similar observations were previously reported for rapamycin.

SUMMARY OF THE BIOTRANSFORMATION OF CCI-779 IN HUMAN

Products	Retention time (min) RIC[a]	Retention time (min) HPLC[b]	Proposed Biotransformation Pathways
M1/M1`			Desmethyl-rapamycin
M2			Desmethyl-CCI-779 (southern)
M3			Seco-rapamycin
M4			Seco-CCI-779
M5			Hydroxy-rapamycin
M6			Hydroxy-CCI-779
M7			Hydroxy-rapamycin
M8			Hydroxy-CCI-779
M9			Hydroxy-rapamycin
M10			Hydroxy-CCI-779
M11			Desmethyl-rapamycin (7-O-)
M12			Desmethyl-CCI-779
M13			Desmethyl-rapamycin (41-O-)
M14			Desmethyl-CCI-779
M15			Desmethyl-rapamycin
Rapamycin			Rapamycin
CCI-779			CCI-779

Proposed biotransformation is based on LC/MS and LC/MS/MS analyses. Hydroxylation primarily occurred on the 'southern' fragment of CCI-779. The characterization of M11 and M13 was based on HPLC retention time comparison with the reference standards isolated previously. M9 to M12 could not be completely separated by HPLC.

a: RIC - Reconstructed ion chromatogram
b: HPLC - High performance liquid chromatogram

Study Title: CCI-779 (WAY-130779): Biotransformation in male rats after a single intravenous dose of the ^{14}C-radiolabeled drug (2.5 mg/kg)

Report# GTR-34738
Testing facility: Wyeth, Pearl River, NY
Test articles: CCI-779 (Batch OM7612, Lot 71-2923-2)
[^{14}C]CCI-779 (Lot CFQ 8054, 20 mCi/mmole, radiochemical purity of [redacted]

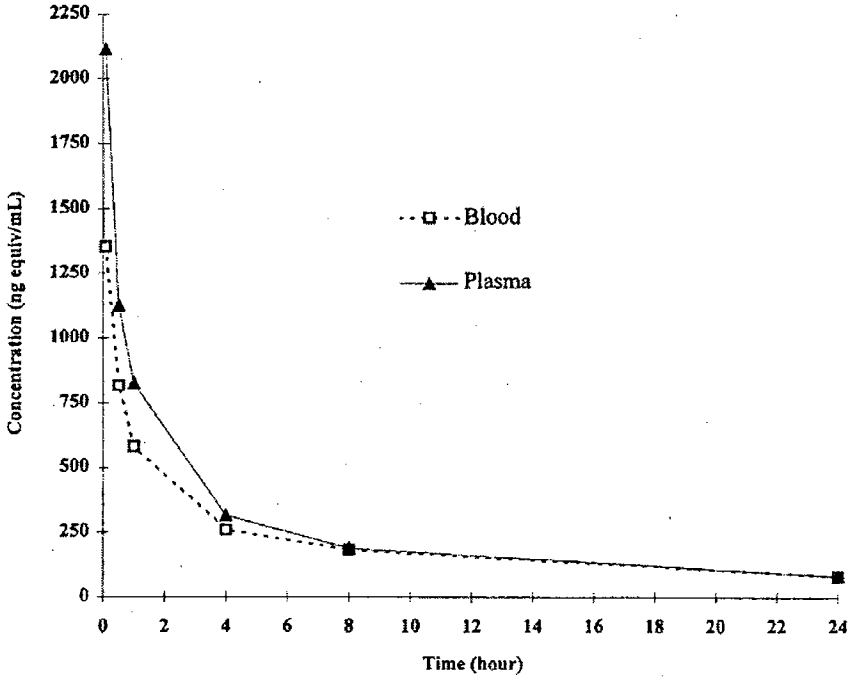
Dose: 2.5 mg/kg; single dose of CCI-779
Species: ♂ SD rats

Study design:
^{14}C-CCI-779 (in ethanol) was isotopically diluted with unlabeled CCI-779 in a diluent to obtain a drug concentration of approximately 2.5 mg/mL and 40 µCi/mL. The diluent consisted of polysorbate 80 (5% w/w) and polyethylene glycol 400 (5% w/w) in sterile water for injection (USP). The composition of the dose vehicle was ethanol (5% v/v) and diluent (95% v/v). Animals were administered intravenously via the tail vein a single 2.5 mg/kg of ^{14}C-CCI-779 (approximately 40 µCi/kg) at a volume of 1 mL/kg. The vehicle was identical to that used in a 4-cycle safety and toxicokinetic study in rats (Report gtr-32609). The dose of 2.5 mg/kg was the highest dose used in the four-cycle safety and toxicokinetic study in rats.

Blood samples (n=4 per time-point) were collected in EDTA at 5 and 30 minutes, and 1, 4, 8 and 24 hours after drug administration. Radioactivity was determined in blood and plasma by liquid scintillation counting. The metabolite profiles of CCI-779 in blood and plasma were determined by HPLC-radioactivity detection and [redacted] LC/MS following [redacted] extraction

The fecal metabolite profile of CCI-779 was determined in the 0-24 hour fecal samples. Extracts were prepared and analyzed by HPLC or LC/MS. The urinary metabolite profile of CCI-779 was determined in the 0-24 hour urine samples (this contained approximately 3% of the administered dose). Extracts were analyzed by HPLC and LC/MS.

Results:



Concentrations of radioactivity in blood and plasma

Blood to Plasma (B/P) Concentration Ratios

	Time					
Replicate	5 min	30 min	1 hour	4 hour	8 hour	24 hour
1						
2						
3						
4						
Mean	0.64	0.74	0.71	0.83	0.97	1.14
SD	0.12	0.12	0.09	0.06	0.03	0.32

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Recovery of radioactivity from blood and plasma of rats after a single dose of CCI-779 or after in vitro incubation

Percentage (%) Recovery

	Whole Blood		Plasma	
Time (hour)	Control Fortified	IV Dose	Control Fortified	IV Dose
0	90.3	102.4	92.9	99.7
0.08	NA	96.7	NA	96.3
0.25	84.2	NA	87.7	NA
0.5	76.0	90.0	77.2	94.5
1	72.2	NA	76.6	94.0
4	NA	85.9	NA	60.5
6	68.5	NA	60.1	NA
8	NA	69.0	NA	52.9
24	55.8	46.6	30.4	29.9
48	NA	NA	12.3	NA

Control fortified - Control blood and plasma of rats were incubated with ^{14}C-CCI-779 at 37°C for 48 hours. Samples were extracted by methanol/ethyl acetate at the indicated time points, and the recovery of radioactivity in extracts was determined.
NA - not applicable

Tables excerpted from the package.

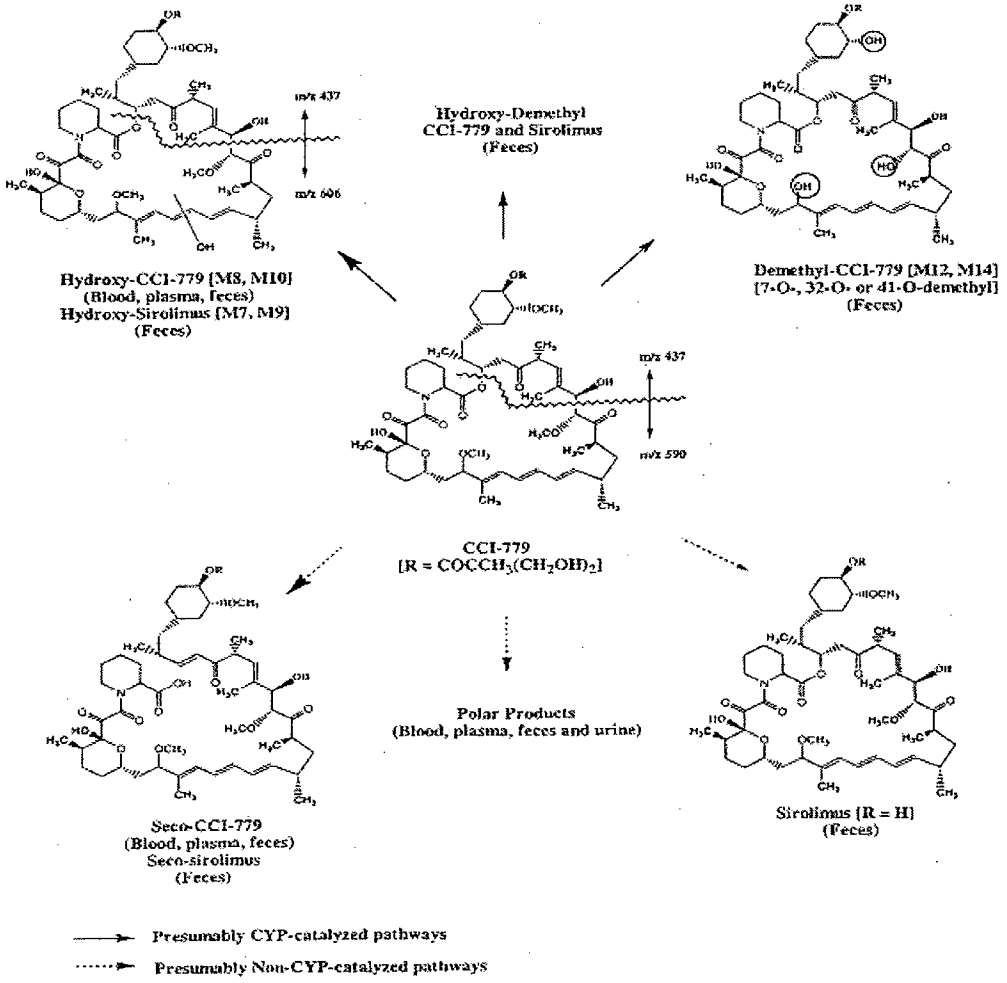
Relative abundance (%) of CCI-779 and derived products in blood and plasma extracts of rats after administration of ^{14}C-CCI-779

	Whole Blood			Plasma		
Time (hour)	Hydroxy	Seco-CCI-779	CCI-779	Hydroxy	Seco-CCI-779	CCI-779
Control Fortified	NI	2.6	97.4	NI	7.8	92.2
0.08	NI	7.8	92.2	NI	12.4	87.6
0.5	NI	13.7	86.3	NI	13.7	86.3
1	2.9	7.5	89.7	10.9	13.9	75.1
4	3.6	5.1	91.4	15.4	16.6	68.0
8	2.7	5.3	92.0	11.7	17.9	70.3
24	3.1	2.9	94.0	NI	23.5	76.5

Relative abundance was estimated by area integration of drug-derived peaks in the HPLC radiochromatograms.
NI - not integrated

- In blood, radioactivity concentrations declined from 1353 ± 342 ng parent equivalent/mL at 5 minutes to 80 ± 33 ng parent equivalent/mL at 24 hours. Corresponding radioactivity concentrations in plasma were 2113 ± 242 ng and 80 ± 58 ng parent equivalent/mL, respectively.
- Radioactivity concentrations in plasma were higher than in blood shortly after dosing and slowly increased by time. The blood to plasma (B/P) concentration ratios of radioactivity ranged from 0.64 at 5 minutes to 1.1 at 24 hours.
- The metabolite profiles of CCI-779 in blood and plasma: The recoveries of radioactivity from whole blood and plasma were >95% at 5 minutes, and decreased gradually with time to approximately 50% and 30%, respectively, at 24 hours. In addition to metabolic reactions, it is possible that degradation of CCI-779 contributed to this reduction in radioactivity, because a comparable decrease of radioactivity with time was observed in the control in vitro study, when blood and plasma samples were incubated with ^{14}C-CCI-779.
- In both blood and plasma extracts between 5 min and 24 hrs after i.v. drug administration, unchanged drug was the predominant product (86%-94% of extractable radioactivity in blood and 70%-88% in plasma). Seco-CCI-779 or M4 (up to 14% in blood and 10-24% in plasma) and hydroxyl-CCI-779 or M10 (up to 4% in blood and 10-15% in plasma) were significant drug-derived products detected.
- While feces contained about 80% of the administered dose, CCI-779 was present at relatively low levels in feces. Drug-derived products in feces were characterized as seco-CCI-779, unidentified polar products, and various oxidative metabolites of CCI-779, which included at least two demethyl, two hydroxy and several hydroxy-demethyl metabolites. The ester hydrolytic product sirolimus, seco-sirolimus and sirolimus-derived products were detected at relatively low levels in fecal homogenates. The radioactivity excreted into urine was characterized as unidentified polar products. Polar drug-derived products present

in feces and urine of rats may represent degradation products in addition to the metabolic products of CCI-779.



Proposed biotransformation of CCI-779 in rats after a single i.v. dose.
Excerpted from the package.

Study Title: CCI-779 (WAY-130779): biliary excretion and metabolite profiling of ^{14}C-WAY-130779 in male bile duct cannulated Sprague-Dawley rats following a single intravenous (2.5 mg/kg) or oral (7.5 mg/kg) administration

Report# RPT-44613
Testing facility: Wyeth Research, Collegeville, PA
Species: ♂ SD rats

Study design:
Single intravenous (2.5 mg/kg) or oral (7.5 mg/kg) doses of [^{14}C]CCI-779 were given to male bile duct-cannulated rats. Bile, urine and fecal samples were collected over a 72-hr

period, with bile samples acidified to stabilize CCI-779 and its metabolites. Total radioactivity was determined in each matrix and metabolite profiles determined for bile using HPLC with radiometric detection. Metabolites were further characterized by LC/MS.

Results

Mean % recovery of radioactivity after a single **i.v.** dose of [^{14}C]CCI-779

Bile		
Time	**Mean (%)**	**SD (%)**
0-4	34.5	14.4
4-8	14.3	2.54
8-12	10.9	2.12
12-24	10.2	1.92
24-48	5.71	1.46
48-72	3.64	0.98
0-72	**76**	**14.1**
Urine		
0-8	0.53	0.14
8-24	1.74	0.91
24-48	1.9	1.41
48-72	0.78	0.71
0-72	**4.08**	**1.9**
Feces		
0-24	2.24	1.47
24-48	3.88	2.73
48-72	0.87	0.48
0-72	**5.58**	**1.74**
Total		
TOTAL	**85.7**	**15.2**

Mean % recovery of radioactivity after a single **oral** dose of [^{14}C]CCI-779

Bile		
Time	**Mean (%)**	**SD (%)**
0-4	3.13	0.45
4-8	1.76	0.33
8-12	1.46	0.38
12-24	1.18	0.51
24-48	0.27	0.27
48-72	0.14	0.10
0-72	**7.94**	**1.50**
Urine		
0-8	0.21	0.04
8-24	1.34	0.44
24-48	1.00	0.46
48-72	0.24	0.20
0-72	**2.79**	**0.42**
Feces		
0-24	63.2	15.6
24-48	15.3	2.06
48-72	7.79	8.60
0-72	**82.4**	**25.0**
Total		
TOTAL	**93.1**	**23.4**

Distribution of radioactivity in peaks detected by radio-chromatography in bile samples after **i.v.** administration of [14C]CCI-779

Collection Period	Rat #[a]	M21	Seco-CCI-779	M18	M10	M8/M19	M17	M20	CCI-779	CCI-779-Ester
0 – 4 hr	1									
	2									
	3									
	4									
	Mean	**19.4**	**13.8**	**7.12**	**31.8**	**4.54**	**6.22**	**1.69**	**14.2**	**1.29**
	S.D.	**3.8**	**1.5**	**1.5**	**5.4**	**1.0**	**2.6**	**0.50**	**4.5**	**0.62**
4 – 8 hr	1									
	2									
	3									
	4									
	Mean	**37.2**	**14.9**	**11.4**	**23.6**	**3.89**	**4.67**	**0.75**	**3.24**	**0.34**
	S.D.	**12.8**	**1.8**	**1.2**	**7.1**	**0.37**	**1.6**	**1.5**	**6.5**	**0.68**
8 – 12 hr	1									
	2									
	3									
	4									
	Mean	**38.9**	**17.0**	**11.0**	**21.2**	**4.67**	**3.86**	**1.16**	**2.17**	**0**
	S.D.	**12.2**	**2.3**	**3.0**	**5.4**	**1.3**	**1.0**	**1.9**	**2.6**	**0**
12 – 24 hr	1									
	2									
	3									
	4									
	Mean	**35.4**	**18.4**	**13.8**	**19.2**	**4.40**	**3.66**	**1.50**	**3.54**	**0**
	S.D.	**7.4**	**5.2**	**1.4**	**5.2**	**0.73**	**0.53**	**0.40**	**1.5**	**0**
24 – 48 hr	1									
	2									
	4									
	Mean	**33.7**	**16.9**	**15.0**	**20.7**	**4.20**	**4.96**	**0.48**	**4.01**	**0**
	S.D.	**2.3**	**3.3**	**5.9**	**6.1**	**1.9**	**1.8**	**0.14**	**1.4**	**0**
48 – 72 hr	1									
	4									
	Mean[b]	**27.5**	**17.4**	**11.2**	**23.5**	**5.26**	**6.89**	**0.93**	**7.29**	**0**

a: Animal 2 died prior to the 48 hour collection and animal 3 died prior to the 72 hour collection.
b: No standard deviation was determined for this time point because there were only two values.

Table excerpted from the package.

Distribution of radioactivity in peaks detected by radio-chromatography in bile samples after **oral** administration of [^{14}C]CCI-779

Collection Period	Rat #	M21	M22	Seco-CCI-779	M18	M10	M8	M17
0 – 4 hr	5							
	6							
	7							
	8							
	Mean	**36.0**	**11.1**	**7.25**	**10.5**	**18.3**	**9.91**	**6.93**
	S.D.	**3.6**	**0.89**	**1.7**	**0.61**	**2.7**	**0.69**	**0.96**
4 – 8 hr	5							
	6							
	7							
	8							
	Mean	**49.7**	**8.70**	**6.75**	**8.19**	**14.3**	**6.27**	**6.15**
	S.D.	**8.8**	**1.67**	**1.5**	**2.7**	**3.1**	**0.87**	**2.2**
8 – 12 hr	5							
	6							
	7							
	8							
	Mean	**41.2**	**8.90**	**8.21**	**11.1**	**15.9**	**7.63**	**7.08**
	S.D.	**7.9**	**1.3**	**1.8**	**2.2**	**3.3**	**1.5**	**0.92**
12 – 24 hr	5							
	6							
	7							
	8							
	Mean	**44.6**	**7.62**	**12.6**	**13.8**	**15.4**	**5.99**	**0**
	S.D.	**6.0**	**1.5**	**4.1**	**2.9**	**2.4**	**1.5**	**0**
24 – 48 hr	5							
	6							
	7							
	8							
	Mean	**45.6**	**6.89**	**14.3**	**15.3**	**17.9**	**0**	**0**
	S.D.	**3.1**	**3.2**	**1.7**	**2.1**	**4.5**	**0**	**0**
48 – 72 hr	5							
	6							
	7							
	8							
	Mean	**32.4**	**5.73**	**17.5**	**19.6**	**24.8**	**0**	**0**
	S.D.	**19.8**	**1.9**	**7.8**	**7.9**	**10.0**	**0**	**0**

Table excerpted from the package.

- Following oral dosing, ~93% of the radioactivity was recovered over 72 hours: ~3% in urine, ~8% in bile, and ~82% in feces.
- Following i.v. dosing: ~86% of the radioactivity was recovered over 72 hours: 4% in urine, 76% in bile, and ~6% in feces.
- Based on the above, biliary excretion was the major route of excretion in i.v. administration, i.e. for absorbed radioactivity (drug and metabolites). When drug was given orally, due to low absorption, most of the radioactivity was in the feces.
- In bile, 10 radio-chromatographic peaks, representing 11 compounds, were detected following separation by HPLC. Two of these peaks (M21 and M22) were determined to be degradation products of [^{14}C]CCI-779 and were not

characterized by LC/MS. In some samples, these peaks contained more than 50% of the radioactivity in bile. Seco-CCI-779 (M4) and [redacted] also degradation products of CCI-779, were detected at up to 27.5% of the radioactivity in bile.

- The major metabolites excreted in the bile, following either i.v. or oral administration of temsirolimus, were characterized as oxidation or reduction products: 3 of the major metabolites were hydroxylated metabolites (M8, M10 and M17), 3 other (M18, M19 and M20) were reduced CCI-779 metabolites.
- Trace amounts of metabolites of sirolimus were detected in some bile samples by LC/MS, but not by radio-chromatography.

2.6.4.6 Excretion

Study Title: CCI-779: single intravenous dose mass balance study of 14C-CCI-779 in ♂ Sprague-Dawley rats

Report# GTR-32603
Testing facility: Wyeth Research; Pearl River, NY
Test article: ^{14}C-CCI-779; batch # NB L18224-44
Specific activity of ^{14}C-CCI-779 (bulk drug): 14.9 μCi/mg
Radiopurity: [redacted]
Chemical purity: [redacted]

Study design:
Animal species: 5 ♂ SD rats
12-16 weeks old, weighing 290-340 g
Dose: a single 2.5 mg/kg (~36 μCi/kg; 15 mg/m2) i.v. dose of ^{14}C-CCI-779
Bolus; 1 mL/kg
Formulation: see Table below
Sample collection: Urine and fecal samples were collected for up to 168 hrs post-dose and were analyzed by scintillation spectrometry for total radioactivity concentrations.

Results:
The recovery of radioactivity was complete (104%) after 168 hours. Urinary excretion accounted for 3.8% and fecal excretion accounted for 100% of the dose up to 168 hours following the intravenous dose. Excretion of radioactivity was rapid, with the majority (~81%) of the dose recovered within 24 hours. The high fecal recovery following intravenous administration is indicative of biliary excretion.

Species: Rats (S-D)	**Dosage (mg/kg):** 2.5
No. and Sex (M/F) of Animals: 5 M	**Analyte:** [^{14}C]Temsirolimus
Feeding Condition: Fed	**Assay:** LSC
Vehicle/Formulation: 5% polysorbate 80, 5% PEG 400, and sterile water for injection	
Method of Administration: IV	

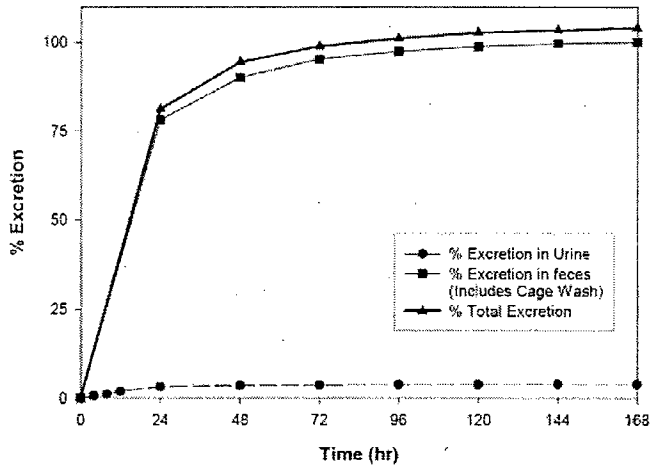
	% of Dosage		
Excretion Route:	**Urine**	**Feces**	**Cage**
Time (h)			
0-4	0.521	ND	ND
0-8	1.03	ND	ND
0-12	1.79	ND	ND
0-24	2.96	78.1	0.149
0-48	3.40	90.9	ND
0-72	3.58	95.3	0.186
0-96	3.68	97.4	ND
0-120	3.74	98.8	0.209
0-144	3.78	99.6	ND
0-168	3.81	100	0.223
Total Recovery (% of Dosage)[a]		81.2 ± 2.6	
Total Recovery (% of Dosage)[b]		104 ± 3	

a. Determined 24 hours after dosing.
b. Determined 168 hours after dosing.

GTR = General Technical Report; IV = Intravenous; LSC = Liquid scintillation counting; ND = Not determined; PEG = Polyethylene glycol.

Table excerpted from the package.

Mean Cumulative Recovery of Radioactivity Following a Single 2.5 mg/kg Intravenous Dose of ^{14}C-CCI-779 in Male Rats



Graph excerpted from the package.

Study Title: CCI-779: single ^{14}C intravenous dose (2.5 mg/kg) mass balance study in male monkeys

Report# GTR-33528
Testing facility: Wyeth Research, Pearl River, NY
Test article:

^{14}C-CCI-779:	Lot #: L18224-44
Specific Activity:	14.9 µCi/mg (ethanol solution @ 40.27 mg/mL)
Radiochemical Purity:	[redacted]
CCI-779 (unlabeled):	Lot #: OM7612
Purity:	[redacted]

Study design:

Animal species: 4 ♂ Cynomolgus monkeys
5-6 kg

Dose: single i.v. dose of 2.5 mg/kg (30 mg/m2) of ^{14}C-CCI-779
~50 µCi/monkey

Formulation: see Table below

Sample collection: urine and fecal samples were collected for up to 168 hrs after post-dose and were analyzed by scintillation spectrometry for total radioactivity.

Results:

The total recovery of the dose was near complete (~96%) at 168 hr post-dose. The majority of the radioactivity was recovered in the feces. Similar to the finding in rats, the high fecal radioactivity in i.v. dosing suggest biliary excretion. Urinary excretion was small: ~2.8% of the administered dose over the 168-hr collection period.

Unlike what was observed in rats, elimination of radioactivity was slow. At 24 hrs, recovery of radioactivity was ~34%. The majority of the dose (~80%) was eliminated by 96 hrs post-dose. Recovery of radioactivity from cage wash out was ~11% of the administered dose at 168 hrs post-dose.

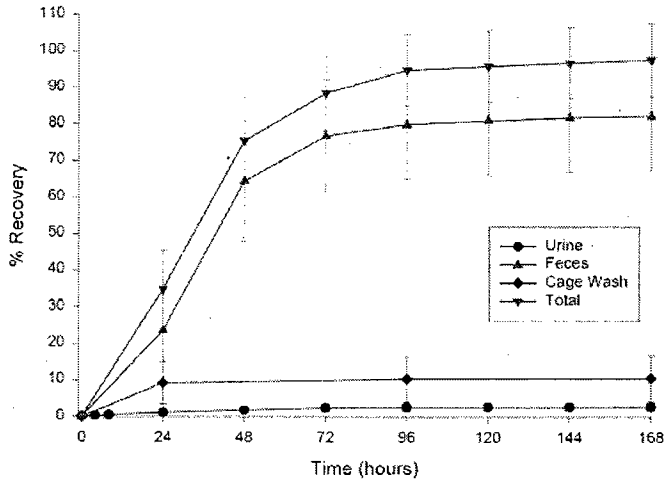
Species: Monkeys (cynomolgus)
No. and Sex (M/F) of Animals: 4 M
Feeding Condition: Fed
Vehicle/Formulation: 2.1% ethanol in 5% polysorbate 80, 5% PEG 400 and 90% water
Method of Administration: IV
Dosage (mg/kg): 2.5
Analyte: [^{14}C]Temsirolimus
Assay: LSC
Specific Activity: 14.9 µCi/mg

	% of Dosage			
Excretion Route:	**Urine**	**Feces**	**Cage**	**Total**
Time (h)				
0-4	0.32	ND	ND	ND
0-8	0.36	ND	ND	ND
0-12	0.47	ND	ND	ND
0-24	1.14	23.52	9.23	33.89
0-48	1.83	64.43	ND	75.49
0-72	2.44	76.82	ND	88.49
0-96	2.59	79.98	10.38	92.95
0-120	2.65	81.00	ND	94.03
0-144	2.71	81.87	ND	94.96
0-168	2.76	82.29	10.60	95.65

GTR = General Technical Report; IV = Intravenous; LSC = Liquid scintillation counting; ND = Not determined; PEG = Polyethylene glycol.

Table excerpted from the package.

Excretion of radioactivity in monkeys after a single dose of ^{14}C-CCI-779



Graph excerpted from the package.

2.6.4.7 Pharmacokinetic drug interactions
Drug interaction studies (interactions with letrozole, Taxol, and doxorubicin) were not reviewed.

2.6.4.8 Other Pharmacokinetic Studies
Not reviewed.

2.6.4.9 Discussion and Conclusions
See the PK/TK summary, section 2.6.4.1.

2.6.4.10 Tables and figures to include comparative TK summary
Tables have been included within the reviews.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY
Several tabulated summaries have been presented within the studies reviewed. Additional selected tables are presented below.

Table 3.17-1: AUC and Dose-Normalized AUC Values in Rats and Monkeys After Oral Administration for 4 Cycles or IV Administration for 3 Cycles

	$AUC_{0\text{-}24}$ (ng•h/mL)						Dose-Normalized[a] AUC					
	Oral Administration			IV Administration			Oral Administration			IV Administration		
Dosage (mg/kg)	0.3	1.5	7.5	0.1	0.5	2.5	0.3	1.5	7.5	0.1	0.5	2.5
Rats (GTR-38053, GTR-32609)												
Male	37.2	194	975	155	650	1445	124	129	130	1553	1301	578
Female	35.1	97.2	671	NS	460	NS	117	64.8	89.5	NS	919	NS
Monkeys (GTR-38046, GTR-32495)												
Male	814[b]	345	1286	747[c]	1863[c]	3448[c]	2713[b]	230	171	7472[c]	3726[c]	1379[c]
Female	116	313	979				387	209	131			

a. AUC values were dose normalized to 1 mg/kg.
b. One (1) male in the 0.3 mg/kg group had an exceptionally high AUC/dose value (7697) compared with the AUC/dose values (227 and 215) for the other 2 males in this dosage group.
c. Combined values for males and females.

AUC = Area under the concentration-versus-time curve; GTR = General Technical Report; IV = Intravenous; NS = Not sampled.

Identification of CYPs involved in metabolism of temsirolimus

Enzyme System: Human Liver Microsomes
Concentration: 25 µM

Inhibited CYP450 Isozyme	Isozyme Inhibitor	Concentration (µM)	% of Control Activity[a] Hydroxy Sirolimus	Hydroxy Temsirolimus	Desmethyl Sirolimus	Hydroxy Temsirolimus	Hydroxy Sirolimus	Desmethyl Temsirolimus
CYP1A2	Furafylline[b]	10	100	100	100	98.4	121	99.6
		50	97.5	101	100	97.8	100	107
CYP1A2	7,8-Benzoflavone	1	112	118	102	ND[c]	115	111
		10	110	112	88.1	ND	131	105
CYP2C9	Sulfaphenazole	10	98.7	107	92.2	104	77.8	99.0
		100	93.5	103	100	103	95.5	105
CYP2C19	S-Mephenytoin	10	108	111	104	112	114	110
		100	118	118	98.2	120	116	112
CYP2D6	Quinidine	1	103	97.1	89.4	101	103	102
		10	104	104	106	105	106	108
CYP2E1	Diethyldithio-carbamate[b]	10	77.9	88.5	103	92.8	96.9	101
		100	38.3	58.9	74.3	84.4	169	85.6
CYP3A4	Ketoconazole	1	19.3	24.3	28.9	26.8	129	59.2
		5	0	0.9	0	0	111	0
CYP3A4	Troleandomcin[b]	10	32.2	39.6	44.0	55.2	133	67.5
		50	24.3	31.1	46.9	52.8	141	59.5

Note: The metabolite designations (M numbers) used to identify metabolites in this study report do not necessarily correspond to those designations used in other metabolism studies with temisolimus and therefore, have been excluded from this table.
a. Rate of metabolite formation with inhibitor/rate of metabolite formation with no inhibitor x 100.
b. Mechanism-based inhibitor.
c. Value could not be determined because 7,8-benzoflavone peak interfered with detection of hydroxy temsirolimus.
CYP = Cytochrome P450; GTR = General Technical Report, ND = Not determined.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Information on dosing formulations:

The formulations used in nonclinical studies were modified over time. There were 3 primary vehicle formulations, 2 for i.v. administration and 1 for oral administration.

Earlier i.v. studies used a polyethylene glycol 400 (PEG)/ethanol formulation containing ethanol, polysorbate 80, PEG, and sterile water for injection. This formulation was used in the safety pharmacology i.v. cardiovascular studies in rats and monkeys, the in vivo pharmacokinetic i.v. studies, the single-dose studies in mice and rats, the 2-week dose-ranging and 4-cycle studies in rats and monkeys, the mouse micronucleus genotoxicity assay, and the drug substance impurities toxicity study in rats.

Later i.v. studies used a formulation (referred to as vitamin E formulation) containing vitamin E, ethanol, propylene glycol, polysorbate 80, PEG, sterile saline, and sterile water for injection. This formulation was used in the safety pharmacology CNS and respiratory studies in rats, the 6-month study in rats and the 9-month study in monkeys, the dose-ranging developmental study in rats, and the drug product impurities toxicity study in rats. This formulation is similar to the clinical formulation used in the phase 3 clinical trials in RCC and to be used in the marketed product for the treatment of RCC.

The majority of oral studies (e.g. reproductive toxicology) used a phosal vehicle containing phosal, ethanol, polysorbate 80, and purified water.

General toxicology:

Toxicology studies consisted of i.v. and oral studies. Because temsirolimus will be administered i.v. to patients, mainly the i.v. studies were reviewed. Since the metabolic profile of temsirolimus after i.v. administration to humans resembles those observed in rats and monkeys after oral administration of the drug, one oral toxicology study was reviewed. Based on the summary data, the 9-month oral study in monkey did not result in signs of toxicity in the animals; therefore, the 6-month oral study in rats was reviewed. Toxicities were generally comparable in the 6-month i.v. and oral dosing in rats. Hepatotoxicity was more pronounced in the oral dosing, which may be due to the oral route of administration and the first pass liver effect. Other toxicities were less evident in the oral dosing; this may be due to lower systemic exposure in oral dosing (no TK is available for the oral study).

The single- or repeat-dose studies did not fully define drug-related MTDs, as associated with life-threatening effects. Dosing was limited due to solubility issues and formulation-related toxicities.

Single dose (i.v.)

Acute toxicities of temsirolimus are unknown.

Single dose toxicology studies with 14 days observation period were conducted in mice and rats. Doses were selected based on the solubility of temsirolimus and the maximum feasible volume of administration. The studies either did not reach an MTD because of formulation problems, or the MTD appeared to be dose-volume and/or formulation related. It is difficult to separate the formulation effects from the dose volume effects at this time.

Separate single-dose toxicity studies were not conducted in monkeys. The sponsor evaluated potential toxic effects of single-dose temsirolimus on the first day of dosing in repeat-dose studies in monkeys.

Repeat-dose
i.v.:
Toxicities in repeat-dose toxicology studies in rats and monkeys included lymphoid atrophy, ↑glucose and pancreatic islet cell vacuolation, ↑cholesterol, ↑fibrinogen, GI toxicity, hypokalemia, hypophosphatemia, inflammation and alveolar macrophages in the lung, renal effects (↑BUN, ↑creatinine, tubular degeneration), coagulation effects (↑fibrinogen, ↓platelets and ↑aPTT), and effects in the male reproductive system.

The following effects were seen in rats after repeat-dosing:

- Lymphocytic/hematopoietic system: ↓lymphocytes, lymphoid atrophy (GALT, nodes, thymus), ↑RBC, ↓WBC, ↑neutrophils, ↑globulin, ↓A/G, thymic atrophy, splenic hemosiderosis and atrophy
- Metabolism/endocrine/nutrients: ↑glucose, ↑cholesterol, ↓triglycerides, pancreatic islet cell vacuolation
- Coagulation: ↓platelets, ↑aPTT, ↑fibrinogen
- ↓K, ↓Phosphorus
- ↑thyroxine (T4)
- Reproductive system: testicular tubular degeneration, ↓weight of testes and prostate, epididymal luminal cellular debris and hypospermia, ↓content of seminal vesicles
- Bone marrow: hypocellularity
- Liver: hepatocellular degeneration/necrosis, mononuclear cell infiltration
- Kidney: tubular degeneration, ↑BUN
- Heart: myocardial degeneration, ↓weight
- Lung: alveolar macrophages, inflammation
- Eye: cataract
- Pituitary: ↓weight

Monkey:

- GI: inflammation, diarrhea, bloody feces
- Lymphocytic/hematopoietic system: ↓lymphocytes, ↓WBC, ↑neutrophils and monocytes, ↑globulin, lymphoid atrophy (thymus, nodes, spleen, GALT), thymic atrophy, ↓RBC and lineages
- Metabolism/ endocrine/nutrients: ↑cholesterol, ↑glucose
- Male reproductive system: testicular tubular degeneration, small testes, immature epididymides
- Coagulation: ↑platelets, ↑fibrinogen
- Kidney: ↑creatinine

Oral:

Daily oral doses of temsirolimus given for 6 months to SD rats resulted in the following effects. Toxicities were more evident in ♂s:

- Pancreas: ↑amylase
- Liver: ↑AST, ALT, and ALP, and necrosis
- ↑Cholesterol
- Coagulation: ↑aPTT, ↑fibrinogen
- Lung: ↑incidence of alveolar macrophages
- Heart: ↑incidence of spontaneous cardiomyopathy
- Hematopoietic system: ↓reticulocytes, thymic hemorrhage

Genetic toxicology:
Four genotoxicity studies were conducted with temsirolimus and were considered negative. Those consisted of: the Ames Test, the TK+/- mouse lymphoma forward mutation assay, the chromosomal aberration study in Chinese hamster ovary (CHO) cells, and an in vivo mouse micronucleus assay.

Carcinogenicity: No study was conducted with temsirolimus. However, based on the information available on sirolimus (the active metabolite of temsirolimus present in large amounts in humans), temsirolimus should be considered a potential human carcinogen.

Based on the labeling for sirolimus:

> "Carcinogenicity studies were conducted in mice and rats. In an 86-week female mouse study at dosages of 0, 12.5, 25 and 50/6 (dosage lowered from 50 to 6 mg/kg/day at week 31 due to infection secondary to immunosuppression) there was a statistically significant increase in malignant lymphoma at all dose levels (approximately 16 to 135 times the clinical doses adjusted for body surface area) compared with controls. In a second mouse study at dosages of 0, 1, 3 and 6 mg/kg (approximately 3 to 16 times the clinical dose adjusted for body surface area), hepatocellular adenoma and carcinoma (males), were considered Rapamune related. In the 104-week rat study at dosages of 0, 0.05, 0.1, and 0.2 mg/kg/day (approximately 0.4 to 1 times the clinical dose adjusted for body surface area), there was a statistically significant increased incidence of testicular adenoma in the 0.2 mg/kg/day group."

Reproductive toxicology:
Reproductive toxicology studies, i.e. fertility/early embryonic and embryo-fetal toxicity studies, were conducted using oral temsirolimus.

The following is a summary of the fertility and early embryonic studies:
Administration of temsirolimus to ♂ rats for a period of ~70 days prior to co-habitation and 14 days during co-habitation, at doses of 0.05-0.5 mg/kg/day, resulted in ↓fertility as indicated by the fecundity index. The following effects in male reproductive organs and fertility parameters were observed:

- ↓Weight of cauda epididymis at MD (0.1 mg/kg/day or 0.6 mg/m2/day) and HD (0.5 mg/kg/day or 3 mg/m2/day)
- Small epididymis and testes at HD

- Epididymides intratubular cellular debris, and oligospermia/aspermia at HD
- Testicular seminiferous epithelium degeneration at HD
- ↓Sperm count at HD
- ↓Sperm motility at MD and HD
- ↓Fecundity index (# pregnancy: # mated) at HD

Treatment of ♀ rats with temsirolimus at doses up to 1 mg/kg or 6 mg/m2 (2 weeks prior to co-habitation through GD6) did not affect the estrous cycle, mating index, fertility index, or the conception rate. Uterine and fetal effects were evident at HD (1 mg/kg) and consisted of the following:
Uterine findings:

- ↑pre-implantation loss resulting in ↓number of implantation site per litter
- ↑embryo-fetal resorption resulting in ↑post-implantation loss and ↓number of live embryos per litter

Embryo-fetal finding:

- ↓weight

The following is a summary of the embryo-fetal toxicology studies:
Temsirolimus administered to mated rats, p.o. daily, from GD 6-17, at doses up to 0.45 mg/kg, resulted in uterine and embryo-fetal toxicities at the high-dose. The HD (0.45 mg/kg or 2.7 mg/m2) had an AUC_{0-24} of 70 ng.hr/mL, which is approximately 0.05-fold the mean AUC achieved in patients after multiple administration at the recommended dose of 25 mg i.v. Uterine and embryo-fetal toxicities consisted of:

- Uterine: ↑resorption, ↑post-implantation loss, ↓litter size.
- Embryo-fetal: ↓fetal weight, and ↓ossification of sternabrae and vertebral centra.

Temsirolimus administered to mated rabbits, p.o. daily, GD 6-18, at doses up to 0.9 mg/kg, resulted in uterine toxicities starting from the LD and embryo-fetal toxicities starting from the HMD, both at sub-therapeutic exposures. Uterine findings started from the LD (0.06 mg/kg or 0.72 mg/m2). At LD, the AUC_{0-24} was 26 ng.h/mL for CCI-779; this is approximately 0.02-fold the AUC reported in cancer patients after multiple administration of temsirolimus at the recommended human dose of 25 mg i.v. Embryo-fetal effects were most apparent at the HMD (0.6 mg/kg or 7.2 mg/m2) and HD (0.9 mg/kg or 10.8 mg/m2). At HMD, the AUC_{0-24} was 189 ng.hr/mL for CCI-779; this is approximately 0.14-fold the human AUC at the RD of 25 mg i.v.

- Uterine: ↓mean gravid uterine weight, ↑late and total resorptions, ↑post-implantation loss, ↓litter size
- Embryo-fetal: ↓fetal weight, omphalocele, fused sternebrae, bifurcated sternabrae, ↑incidence of notched ribs/ incomplete ossification of pubic bone/ incomplete ossification of frontal bone

The following information is from the labeling for Rapamune (sirolimus), which is the active metabolite of temsirolimus, present in large amounts in humans:

> "There was no effect on fertility in female rats following the administration of sirolimus at dosages up to 0.5 mg/kg (approximately 1 to 3 times the clinical

doses adjusted for body surface area). In male rats, there was no significant difference in fertility rate compared to controls at a dosage of 2 mg/kg (approximately 4 to 11 times the clinical doses adjusted for body surface area). Reductions in testicular weights and/or histological lesions (e.g., tubular atrophy and tubular giant cells) were observed in rats following dosages of 0.65 mg/kg (approximately 1 to 3 times the clinical doses adjusted for body surface area) and above and in a monkey study at 0.1 mg/kg (approximately 0.4 to 1 times the clinical doses adjusted for body surface area) and above. Sperm counts were reduced in male rats following the administration of sirolimus for 13 weeks at a dosage of 6 mg/kg (approximately 12 to 32 times the clinical doses adjusted for body surface area), but showed improvement by 3 months after dosing was stopped.
Sirolimus was embryo/feto toxic in rats at dosages of 0.1 mg/kg and above (approximately 0.2 to 0.5 the clinical doses adjusted for body surface area). Embryo/feto toxicity was manifested as mortality and reduced fetal weights (with associated delays in skeletal ossification). However, no teratogenesis was evident."

2.6.6.2 Single-dose toxicity

Study title: CCI-779: acute intravenous toxicity study in mice
Report# GTR-31708 (GLP)

Summary and results:
Due to solubility issues, the highest achievable dose in mice was 50 mg/kg (150 mg/m^2). No mortality was observed up to this level. Ethanol toxicity (an ingredient of the vehicle) was evident in all mice, but compound-related decreased motor activity and ptosis was only observed in male mice receiving 50 mg/kg (150 mg/m^2) CCI-779.

Species	Dose (mg/m^2)	Dose volume (ml/kg)	EtOH in vehicle (%)	Mortality	Clinical signs
Mouse	0	20	5	0/6	None
Mouse	150	20	5	0/6	Hypoactivity Ptosis

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dosage (mg/kg)	Dosage Volume (mL/kg)	N/ Sex/ Group	Observed Maximum Non-Lethal Dosage (mg/kg)	Approximate Lethal Dosage (mg/kg)	Noteworthy Findings
Mice/ CD-1	IV (5% ethanol in 95% diluent [5% polysorbate 80, 5% polyethylene glycol 400, and sterile water for injection])	0 50	20	3	50	ND	• No mortality. • Transient decreased motor activity and ptosis in males at 50 mg/kg.

Table excerpted from the package.

Study title: CCI-779: Acute intravenous toxicity study in rats

Key study findings: Results are inconclusive due to confounding factors: the volume of administration, e.g. 20 mL/kg may be too high for a bolus administration in rats. High volumes may cause toxic effects. Moreover, the 10% ethanol may have contributed to some of the adverse findings such as ↓motor activity and immobility.

Report no.: GTR-31709
Volume #, and page #: Module 4
Conducting laboratory and location: Wyeth Research; Chazy, NY
Date of study initiation: Sept 1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Batch/Lot # 0M7612, [redacted] pure

Methods
Doses: see Table below
Single doses followed by 2 weeks of observation.

Group	Dose		Dose volume	Number of animals	
	mg/kg	mg/m2	(mL/kg)	♂	♀
1*	100	600	20	3	3
2*	0	0	20	3	3
3*	50	300	10	3	3
4*	0	0	10	3	3
5†	0	0	20	3	2
6†	50	300	20	3	3

* Vehicle contained 10% ethanol.
† Vehicle contained 5% ethanol.

The vehicle control consisted of:
- 5% ethanol + 95% CCI diluent, or
- 10% ethanol + 90% CCI-779 diluent

The CCI diluent consisted of:
- 5% polysorbate 80 (NF), 5% polyethylene glycol 400 (NF), and sterile water for injection.

Species/strain: CD VAF (SD) rats
Number/sex/group: see Table above
Route, formulation, volume, and infusion rate: i.v. bolus, solution
Satellite groups used for toxicokinetics or recovery:
Age: ~6-7 weeks old
Weight: 194-281 g (♂s) and 144-200 g (♀s)

Observations and times:
Mortality and clinical signs: intermittently during the 0-4 hrs and 4-24 hrs post-dose, a minimum of twice daily from Day 2 through Day 14, and once on Day 15
Body weights: prior to dosing and on Days 7 and 14
Food consumption: not done
Ophthalmoscopy: not done

EKG: not done
Hematology: not done
Clinical chemistry: not done
Urinalysis: not done
Gross pathology: Thoracic and abdominal viscera
Organ weights: not done
Histopathology: not done
Toxicokinetics: not done

Results

Mortality:

- Deaths in the animals that were administered the formulations containing 10% alcohol occurred within ~10 min post-dose.
- Deaths in 1 ♂ and 2 ♀s that were administered 50 mg/kg in the formulation that contained 5% alcohol occurred within 10-15 min post-dose
- 1 ♂ that received 50 mg/kg of the drug in 5% alcohol was found dead the day after dosing.

Mortality Results

Dosage (mg/kg)	Alcohol Concentration (%)	Dose Volume (mL/kg)	Mortality (Number Dead/ Total Number) Male	Female
0 (Group II)	10	20	1/3	0/3
0 (Group IV)	10	10	0/3	0/3
0 (Group V)	5	20	0/3	0/2
50 (Group III)	10	10	1/3	1/3
50 (Group VI)	5	20	2/3	2/3
100 (Group I)	10	20	3/3	3/3

Table excerpted from the package.

Clinical signs:

Clinical signs in rats administered either 0, 50, or 100 mg/kg in 10% alcohol included:

- ↓motor activity, immobility, ptosis, ataxia, dyspnea, red pigmentation around or foamy discharge from the nose and/or mouth, red pigmentation around eye(s), convulsions and/or low carriage.
- Signs had generally subsided in surviving rats, within 4 hrs post-dose with the exception of red pigmentation around the nose/mouth and/or eyes that was seen on days 6 to 7 of ♂s given 50 mg/kg.

Clinical signs in rats administered 50 mg/kg in 5% alcohol included:

- ↓motor activity, ptosis, ataxia, low carriage, convulsions, myoclonus, red foamy discharge from the nose and mouth, and/or dyspnea.
- All signs had subsided within 4 hrs with the exception of ↓motor activity that was observed during the 4-24 hr observation period.

Clinical signs in rats administered vehicle control containing 5% alcohol:

- Ataxia, ↓motor activity, and/or low carriage during the 0-4 hrs observation period.

Body weights: ↓BW gain (Day 1-14) for the 50 mg/kg groups in ♂ animals. No effect in ♀s.
No D1-14 weight gain data is available for the 100 mg/kg group due to death.

Gross pathology:
Many rats that were found dead after being given 50 or 100 mg/kg of CCI-779 had congested, mottled, or dark red lungs. Incidence of congested, mottled, or dark red lungs varied from 33% to 67%, with the highest incidence occurring in the HD ♂s (100 mg/kg of CCI-779). The sponsor indicated that these findings were likely agonal in nature and caused by pooling of blood in the lungs when the rats died.

Conclusions:
Results are inconclusive due to confounding factors: the volume of administration, e.g. 20 mL/kg, is generally considered too high for a bolus administration. High volumes may cause toxic effects such as lung lesions. Moreover, the 10% ethanol may have contributed to some of the adverse findings such as ↓motor activity and immobility. Below is the summary of the study.

Single doses of 50 or 100 mg/kg temsirolimus were administered to SD rats in formulations that contained 5% or 10% ethanol, at dose volumes of 10 or 20 mL/kg. Animals were observed for 14 days. Deaths were evident in the temsirolimus-treated groups. Most deaths occurred shortly after dose administration and were at least partially attributed to the presence of high levels of alcohol in the dosing formulation. High volume of administration may have also contributed to the toxicities/mortalities observed.

Gross pathology findings included congested, mottled, or dark red lungs, with the highest incidence in the 100 mg/kg ♂s.

Study title: CCI-779: Acute intravenous toxicity study in rats (addendum 1)

Key study findings: No temsirolimus-related finding was observed. The study did not reach an MTD. Formulation-related toxicities did not allow higher dose administration. Formulation-related ataxia was reported in the vehicle-control (10 mL/kg) group. Formulation and/or dose-volume-associated toxicities were seen in the 20 mL/kg control group and consisted of: ataxia, convulsion, dyspnea, red discharge around the nose/mouth, hematuria, and swollen head.

Report no.: GTR-31897
Volume #, and page #: Module 4
Conducting laboratory and location: Wyeth Research, NY
Date of study initiation: Oct 1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Batch/Lot# 0M7612, [redacted] pure

Methods

Doses: 25 mg/kg (150 mg/m2) single dose, with 14-day observation period
Vehicle: 5% ethanol and 95% diluent (5% polysorbate 80, 5% PEG 400, and water for injection)

- 10 mL/kg
- 20 mL/kg

DOSAGE GROUP	DOSAGE[a] (mg/kg)	CONCENTRATION[a] (mg/mL)	DOSE VOLUME (mL/kg)	ANIMAL NUMBERS MALE	FEMALE
I	25	2.5	10	1-3	4-6
II	0	0	20	7-9	10-12
III	0	0	10	13-15	16-18

a: Based on an active moiety of 100%.

Table excerpted from the package.

Species/strain: CD VAF (SD) rats
Number/sex/group: 3/sex/group
Route, formulation, volume, and infusion rate: i.v., solution, 10 mL/kg
Satellite groups used for toxicokinetics or recovery: none
Age: ~6 weeks old
Weight: 192-228 g (♂s) and 148-176 g (♀s)

Observations and times:
Mortality and clinical signs: intermittently during the 0-4 hrs and 4-24 hrs post-dose periods, a minimum of twice daily from Day 2 through Day 14, and once on Day 15.
Body weights: prior to dosing and on study days 7 and 14
Food consumption: not done
Ophthalmoscopy: not done
EKG: not done
Hematology: not done
Clinical chemistry: not done
Urinalysis: not done
Gross pathology: A visceral examination was performed on all rats, including one that was found dead during the study.
Organ weights: not done
Histopathology: not done
Toxicokinetics: not done

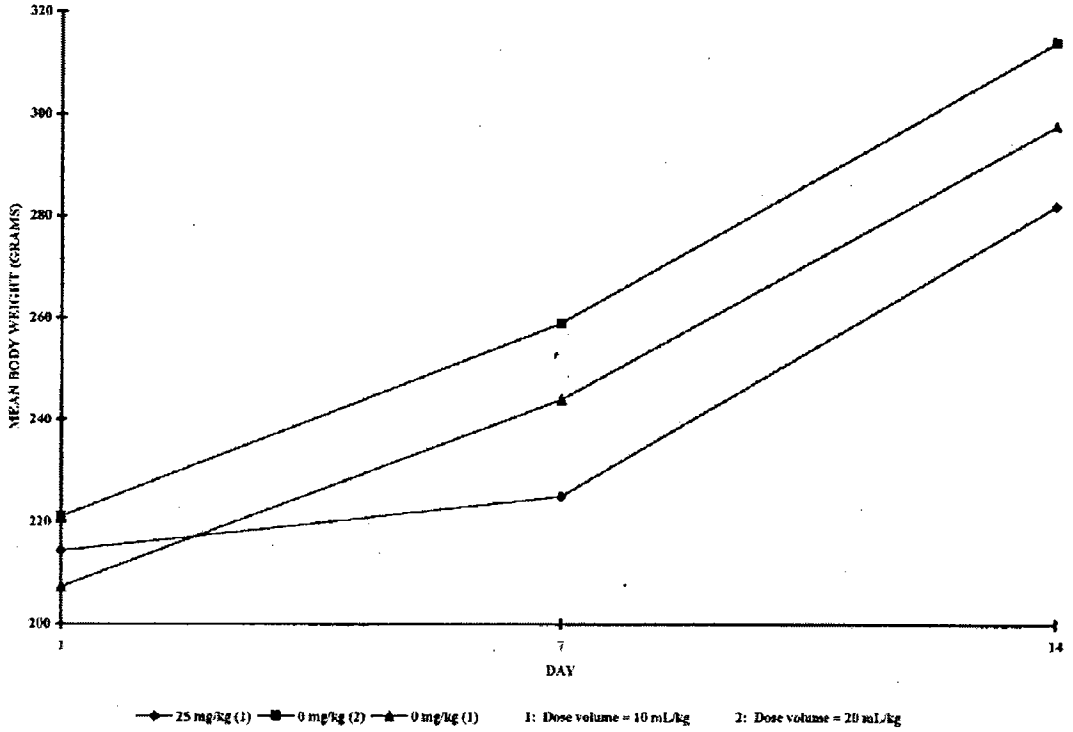
Results
Mortality: 1/3 ♂s given the control at a dose volume of 20 mL/kg died within 7 min of dosing.

Clinical signs:

Group	Clinical signs
Control; 20 mL/kg	Ataxia, low carriage, hematuria, swollen area on the head, dyspnea, convulsions, and red foamy discharge from the nose and/or mouth: immediately after dosing
Control; 10 mL/kg	Ataxia : observed at 0-4 hrs observation period

CCI-779 (25 mg/kg)	None

Body weights: Days 1-7 BW gain was reduced in temsirolimus-treated group.



Graph excerpted from the package.

Gross pathology: No gross lesions
One male control rat (Group II) was found dead. The cause of death is unknown.

Conclusions:
Single dose of 25 mg/kg (10 mL/kg) temsirolimus was administered i.v. to SD rats. Animals were observed for 14 days. Two control groups were administered the vehicle at dose volumes of 10 mL/kg or 20 mL/kg. No temsirolimus-related finding was observed. The study did not reach an MTD. Formulation-related toxicities did not allow higher dose administrations. Formulation-related ataxia was reported in the vehicle-control (10 mL/kg) group. The 20 mL/kg control group resulted in 1/3 death in ♂s and clinical observations of: ataxia, low carriage, hematuria, swollen area on the head, dyspnea, convulsions, and red foamy discharge from the nose and/or mouth, immediately after dosing. These toxicities appear to be related to both the formulation and to the high volume of administration.

2.6.6.3 Repeat-dose toxicity

The following is a tabulated summary of studies: GTR-30878, GTR-30341, GTR-31183, GTR-30340, and GTR-31575, reviewed as part of the original IND. All studies were i.v.

Species	Schedule	Doses (mg/m^2)	EtOH in vehicle (%)	Findings
SD Rat (non-GLP)	d x 5	0, 0.6 4.5, 30	*2	Hypoactivity, ↓Body weight ↑RBC, ↓PLT, ↓WBC ↑BUN, ↓Alk Phos, ↑Globulin, ↓A/G ratio, ↓K+, ↓Pi
SD Rat (non-GLP)	d x 14	0, 0.6 1.5, 6.0 15	*5	No clinical signs ↑RBC, ↓PLT, ↓WBC, ↑Neutrophils, ↑Fibrinogen ↑Glc, ↑BUN, ↓Alk Phos, ↑Chol, ↓Triglyc, ↓K+, ↓Pi Thymus: Atrophy Pancreas: Islet cell vacuolization Testes: Tubule degeneration Liver: Hepatocellular degeneration Kidney: Tubule degeneration Heart: Myocardial degeneration Lung: Alveolar macrophages, inflammation
Rat (GLP)	d x 5 of every 2 Weeks x 4 cycles	0, 0.6 3.0, 15	*5	No clinical signs ↑RBC, ↓PLT, ↓WBC, ↑Neutrophils, ↑Fibrinogen ↑Glc, ↑Chol, ↓Triglyc, ↓Pi Eyes: Cataracts Thymus: Atrophy Lymph node: Atrophy Testes: Tubule degeneration Liver: Hepatocellular degeneration Epididymis: Hypospermia Ovaries: ↓weight Lung: Alveolar macrophages, inflammation
Monkey (non-GLP)	d x 14	0, 1.2 3, 12 30	*5	Diarrhea ↓Lymphocytes, ↑Fibrinogen Thymus: Atrophy Lymph node: Atrophy
Monkey (GLP)	d x 5 of every 2 weeks x 4 cycles	0, 1.2 6, 30	*5	Diarrhea ↓WBC, ↑PLT, ↓Lymphocytes, ↑Fibrinogen ↑Creatinine, ↑Globulin, Lymphoid atrophy (thymus, nodes, spleen) Testes: Tubule degeneration GI: Inflamed cecal mucosa

Doses were given as mg/kg.
No drug-relate mortality was observed in any study.
* 2% ethanol in 98% diluent or 5% ethanol in 95% diluent. The diluent consisted of: 5% polysorbate 80, 5% polyethylene glycol 400, and water for injection

Study title: CCI-779: 26-week (one dose per week) intravenous toxicity study in rats with a 13-week recovery

Key study findings: lymphoid atrophy; hyperglycemia with associated cataracts, clinical pathology changes and hepatocellular necrosis; testicular tubular degeneration; and increased numbers of pulmonary alveolar macrophages.

Report no.: RPT-43567
Volume #: Module 4
Conducting laboratory and location: Drug Safety Wyeth Research
641 Ridge Road
Chazy, NY 12921

Date of study initiation: Sept 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Lot/Batch number MA9611
Purity 10 Apr 2000 release:
Total impurities [redacted]
Largest single impurity [redacted]
27 Mar 2001 release:
Total impurities [redacted]
Largest single impurity [redacted]

Methods

Doses: 0.1, 0.5, and 2.5 mg/kg/week
0.6, 3, 15 mg/m2/week

	Dosage Group	Dosage[a] (mg/kg/week)	Concentration[b] (mg/mL)	Number/Sex	Animal Numbers	
1	Vehicle-Control	0	0	25[d]	1-25	26-50
2	Low	0.1	0.1	15	51-65	66-80
3	Middle	0.5	0.5	15	81-95	96-110
4	High	2.5	2.5	25[d]	111-135	136-160
5	Low[c]	0.1	0.1	24	161-184	185-208
6	Middle[c]	0.5	0.5	24	209-232	233-256
7	High[c]	2.5	2.5	24	257-280	281-304

a: Dosages were administered based on the active moiety of the test article
b: Concentrations of the dosing formulation were based on delivery of a constant volume of 1 mL/kg.
c: Satellite group for toxicokinetic analysis
d: Beginning study day 177, the following animals were allowed a 13 week recovery period: Group 1 males: 16-25 and females: 41-50; and Group 4 males 126-135 and females 136, 151-158, and 160. Animals 18, 43, and 129 were electively euthanized or an accidental death occurred during recovery week 10, 13 and 12, respectively.

Table excerpted from the package.
Note: The dose of 2.5 mg/kg was said to be the maximum dose that could be administered due to formulation limitations, including the solubility of the compound and the level of excipients that could be administered without confounding results.

Vehicle: vitamin E, ethanol, propylene glycol, polysorbate 80, PEG, sterile saline, and sterile water for injection (referred to as vitamin E formulation).

Species/strain: CD VAF rats
Number/sex/group: see Table above
Route, formulation, volume, and infusion rate: i.v. bolus (tail), 1 mL/kg

Satellite groups used for toxicokinetics or recovery: see Table above
Age: ~7 weeks at initiation
Weight: 193-259 g for ♂s; 151-187 g for ♀s at initiation
Formulation analysis: The stock formulations were stable for at least 23 days and the dosing formulations were stable for up to 9 days. Content of the stock formulations ranged from [redacted] of the claimed concentration. Content of the dosing formulations ranged from [redacted] of the claimed concentration. One 0.5 mg/mL sample initially assayed at [redacted] the retention sample assayed within limits. The pH of the dosing formulations ranged from 6.0 to 8.0.

Observations and times:
Mortality: twice daily starting
Clinical signs: once daily
Body weights: once per week
Food consumption: once per week
Ophthalmoscopy: Once pretest and during weeks 12, 25, and 38 (recovery animals only). Biomicroscopy and indirect ophthalmoscopy were performed after the application of tropicamide onto each eye.
EKG: not done
Hematology: Weeks 13, 25, 39 (recovery animals only); retro-orbital sinus
Clinical chemistry: Weeks 13, 25, 39 (recovery animals only); retro-orbital sinus
Urinalysis: not done
Gross pathology: at necropsy
Examination of the external surface of the body, orifices, the cranial, thoracic, and abdominal cavities and their contents, and organs and tissues.
Organ weights:

Adrenal Glands	Pituitary Gland
Brain	Prostate
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Ovaries	Thyroid/Parathyroid Glands

Histopathology: Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )
Samples of the organs and tissues listed below were fixed in 10% neutral buffered formalin. Testes were fixed in Bouin's solution.

Adrenal Glands (cortex and medulla)	Macroscopic Lesions
Aorta (thoracic)	Mammary Gland
Bone and Joint (femoral-tibial joint)	Ovaries
Bone Marrow (sternum)	Pancreas
Brain	Peripheral Nerve (sciatic)
Cecum	Pituitary Gland
Colon	Prostate Gland
Duodenum	Salivary Gland (submandibular)
Epididymides	Seminal Vesicles
Esophagus	Skeletal Muscle (thigh)
Eyes	Skin
Gut Associated Lymphoid Tissue	Spinal Cord (cervical and lumbar)
Harderian Gland	Spleen
Heart	Stomach (squamous and glandular)
Ileum	Testes
Injection Site (tail)	Thymus
Jejunum	Thyroid/Parathyroid Glands
Kidneys	Tongue
Liver	Trachea
Lungs	Urinary Bladder
Lymph Nodes (mandibular and mesenteric)	Uterus
	Vagina

Bone marrow smears were prepared from each animal at necropsy, except animals that were found dead. Bone marrow smears were not evaluated.

Examined microscopically (H & E stain): all fixed organs and tissues from the vehicle-control group, HD group (Group 4), and unscheduled sacrifices, as well as eyes, epididymides, gut associated lymphoid tissue, heart, liver, lungs, mandibular and mesenteric lymph nodes, prostate, testes, thymus, seminal vesicles, spleen, uterus and macroscopic lesions from LD and MD (Groups 2 and 3).

Ovaries, pancreas and skin from LD and MD were processed but not examined.
Grading of severity: scale of 1 to 5, corresponding to slight, mild, moderate, marked, or severe changes.

Toxicokinetics:
TK parameters of CCI-779 and sirolimus (active metabolite) were evaluated.

- Blood sample collection: from 3 animals/sex/groups 5-7 on study Days 1-3 (retro orbital sinus), and Days 176-178 (from abdominal aorta or caudal vena cava).
- Collected time: 0 (predose) hours, 5 and 30 minutes, and 1.5, 4, 12, 24, and 48 hours.
- Following blood sampling on Days 1-3, all females received a 2 mL subcutaneous injection of Lactated Ringers Solution.

Results

Mortality: 7 unscheduled deaths occurred during the study: 2 from the control group, 2 from the LD group, 1 from the MD group, and 2 from the HD group. Deaths do not appear to be drug-related since they were observed in all groups. A toxic effect of the excipients could not be excluded.

Cause of death: lower urinary tract disease, trauma, decubital ulcer, neoplasia, or bleeding. There was one undetermined death in one recovery animal.

Major findings in unscheduled sacrifices:

- Two rats (MD and HD): lower urinary tract infection with hematuria. Gross pathology showed pelvic dilatation of the kidneys and discoloration or distension of the urinary bladder. Histopathology also showed pelvic dilatation and hemorrhage or dilatation of urinary bladder.
- One LD animal: trauma. This rat presented with gross finding of fracture of the cervical vertebrae and histopathological finding of hemorrhage around the cervical vertebrae and within the spinal canal.
- One HD rat: rales and hematuria. Cause of death was unclear. This animal died during the recovery period.
- One LD rat: death was not drug-related. According to the sponsor, animal escaped from the restrainer while in the water bath and burned its feet.
- One control rat: neoplasia (histocytic sarcoma)
- One control rat: died during the retro-orbital bleeding. There was no macroscopic or microscopic correlate.

Clinical signs:
Mainly at MD and HD:

- Red pigmentation around the nose and/or mouth
- Red pigmentation around the eyes

HD:

- Opacities, which were also seen by ophthalmoscopic exam

MD (1 ♂) and HD (1 ♂):

- Lameness (mainly of limb) for short periods of time during the study.

Body weights: ↓BW gain in all dose groups. Changes were more pronounced in ♂s. Effects were reversible.

Changes in the BW gain as compared to control on Week 25 (end of treatment)

	♂*	♀*
LD	↓9%	↓7%
MD	↓23%	↓10%
HD	↓29%	↓17%

* All changes are statistically significant.

Food consumption: ↓mainly in MD and HD ♂s, during the treatment period.

Ophthalmoscopy: Cortical cataract formation which increased in incidence and severity from Week 12 to Week 25 (non-progressive post-dose, but non-reversible)

Week 12

- Cortical cataracts at HD: 13/25 ♂s (52%) and 1/25 ♀s (4%)
  The lens opacities were usually bilateral and consisted in the presence of anterior suture cataracts, incipient anterior cortical opacities, and/or posterior polar cataracts.

Week 25

- The severity of lens opacities was increased in several of the previously affected animals. However, examination of the fundus was still feasible.
- The incidence of cortical cataracts increased at HD: 16/24 ♂s (67%) and 2/25 ♀s (8%). In addition, early cataracts were observed at MD (3/14 ♂s; 21%).

Week 38 (end of the recovery period)

- Cataracts still present in all affected animals
- There were no new cases of cataract

EKG: not done

Hematology: see below- effects were reversible.

↓Basophils and monocytes were observed in ♂s on Week 13. Since findings were inconclusive (i.e. not observed on Week 25 and were non-dose dependent) they are not listed below.

	Neutrophils					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑19%	↑19%	↑28%	—	↑21%	↑13%
Week 25	↑30%	↑42%	↑36%	—	↑33%	↑29%

—: no change/ no toxicologically significant change

	Lymphocytes					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↓13%	↓11%	—	—	↓17%
Week 25	—	—	↓9%	—	—	↓24%

—: no change/ no toxicologically significant change

Coagulation:

	aPTT (sec)					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑9% (↑4)	↑6% (↑2.7)	↑7% (↑3)	—	↑8% (↑3.5)	↑12% (↑5)
Week 25	—	—	—	—	↑8% (↑1.7)	↑8% (↑1.7)

—: no change/ no toxicologically significant change
Numbers in parenthesis are absolute changes (sec) from control.

	Fibrinogen					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑12%	↑13%	↑17%	—	—	↑11%
Week 25	↑7%	↑13%	↑17%	—	—	↑11%

—: no change/ no toxicologically significant change

Clinical chemistry:
Non-toxicologic and non-dose-dependent increases (e.g. up to 60%) were reported for AST, ALT and ALP on Weeks13 and 25.

	Glucose					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑25%	↑15%	—	—	—
Week 25	—	↑40%	↑40%	—	—	—
W 39 (rec)	No data	No data	↑35%	No data	No data	—

—: no change/ no toxicologically significant change
rec: recovery data

	BUN					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑11%	—	—	—
Week 25	—		↑14%	—	—	↑6%
W 39 (rec)	No data	No data	—	No data	No data	—

—: no change/ no toxicologically significant change
rec: recovery data

	Cholesterol					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑23%	↑40%	↑67%	↑16%	↑30%	↑30%
Week 25	↑40%	↑59%	↑70%	↑11%	↑37%	↑37%
W 39 (rec)	No data	No data	—	No data	No data	—

—: no change/ no toxicologically significant change
rec: recovery data

	Triglycerides					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↓45%	↓45%	↓59%	—	—	↓39%

Week 25	↓32%	↓35	↓52%	—	—	↓47%
W 39 (rec)	No data	No data	↓20%	No data	No data	↓40%*

—: no change/ no toxicologically significant change.
* The value for the control animals appears to be too high.
rec: recovery data

	K					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↓3.5%	↓11%	↓6%	—	↓3.8%	↓5%
Week 25	↓3%	↓10%	↓9%	—	↓4%	↓4.5%
W 39 (rec)	No data	No data	↓2%	No data	No data	↓2%

—: no change/ no toxicologically significant change.
rec: recovery data

	Phosphorus					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↓10%	↓8%	↓11%	—	↓6%	↓3%
Week 25	↓8%	↓15%	↓11%	—	↓6%	↓6%
W 39 (rec)	No data	No data	↓2%	No data	No data	↓11%

—: no change/ no toxicologically significant change.
rec: recovery data

	T4 (Thyroxine)					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	—	↑12%	↑25%	↑33%
Week 25	—	—	—	↑12%	↑42%	↑47%
W 39 (rec)	No data	No data	—	No data	No data	—

—: no change/ no toxicologically significant change.
rec: recovery data

Urinalysis: not done

Gross pathology: for information on unscheduled deaths, see under "mortality".

Main sacrifice

	Male Dosage (mg/kg/week)				Female Dosage (mg/kg/week)			
Lesion	0	0.1	0.5	2.5	0	0.1	0.5	2.5
Number Examined	15	13	14	14	15	15	15	15
Testes								
Small	0	0	0	12	-	-	-	-
	(0)	(0)	(0)	(86)				
Thymus								
Small	0	0	0	1	0	1	0	3
	(0)	(0)	(0)	(7)	(0)	(7)	(0)	(20)

(): % Incidence

Recovery sacrifice

	Male	
	Dosage (mg/kg/week)	
Lesion	0	2.5
Number Examined	9	9
Testes		
Small	0	3
	(0)	(33)

(): % Incidence

Table excerpted from the package.

Organ weights:

Statistically significant changes relative to brain weight- Main sacrifice

Pituitary						Prostate					
♂			♀			♂			♀		
LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
↑13%	↓27%	↓28%	↓27%	↓29%	↓37%	—	↓14%	↓17% (↓15%)	NA	NA	NA

Spleen						Testes					
♂			♀			♂			♀		
LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
↓22%	↓23%	↓19%	—	—	—	—	—	↓40% (↓30%)	NA	NA	NA

Thymus						Heart					
♂			♀			♂			♀		
LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
—	↓18%	↓27% (↓33%)	—	—	↓12%	—	↓11%	↓10%	—	—	—

Number of animals in each group: LD= 13, MD=14, HD=14

Numbers in () represent noteworthy recovery data. Recovery data are available for the HD only.

NA: not applicable; —: no change/ no remarkable change.

Histopathology:

Main Sacrifice

		♂				♀			
		C	**LD**	**MD**	**HD**	**C**	**LD**	**MD**	**HD**
Bone marrow	N=	15	13	14	14	15	0	0	15
	hypocellularity	4	2	6	12	2	NA	NA	2
GALT	N=	15	10	11	14	15	12	11	15
	Lymphoid atrophy	0	0	8	5	3	6	8	4
Mandibular node	N=	15	12	14	14	15	15	15	15
	Lymphoid atrophy	1	0	9	13	2	0	5	12
Mesenteric node	N=	15	13	14	14	14	15	14	15
	Lymphoid atrophy	4	8	11	13	6	12	9	14
	Pigmented	0	0	0	0	0	14	13	10

	macrophage infitrate								
Thymus	N=	15	13	14	14	15	15	15	15
	Lymphoid atrophy	0	0	11	11	2	2	3	7
Eyes	N=	15	13	14	14	15	15	15	15
	Cataract	0	0	2	3	0	0	0	0
	*Retro-orbital trauma	3	0	0	5	6	0	1	8
	Degeneration	0	0	0	1	0	0	0	0
Heart	N=	15	13	14	14	15	15	15	15
	Cardiomyopathy (spontaneous)	8	10	14	14	5	8	9	6
Liver	N=	15	13	14	14	15	15	15	15
	Mononuclear cell inflamm.	7	9	9	5	5	11	8	10
	Necrosis	0	4	7	5	0	2	2	6
	Fatty change	1	1	4	2	2	0	0	5
Lung	N=	15	13	14	14	15	15	15	15
	Alveolar macrophages	5	13	14	14	3	13	12	14
Spleen	N=	15	13	14	14	15	15	15	15
	Hemosiderosis	2	12	13	11	10	15	15	13
Lumbar cord	N=	15	0	0	14	15	0	0	15
	Axonal degeneration/ necrosis	0	0	0	1	0	0	0	0

NA: not applicable.
* It should be noted that blood collection for clinical pathology was via retro-orbital sinus. The right eye was used for almost all animals, with few exceptions. The left eye was reserved for tracking ophthalmologic findings.

Reproductive system- main sacrifice

	Male Dosage (mg/kg/week)			
Lesion	0	0.1	0.5	2.5
Testes[a]	15	13	14	14
Tubular Degeneration	0	1	0	14
Epididymides[a]	15	13	14	14
Luminal Cellular Debris	0	1	0	13
Hypospermia	0	1	0	14
Prostate[a]	15	13	14	14
Atrophy	0	0	14	14
Seminal Vesicle	15	13	14	14
Decreased Content	0	10	11	11

a: Number examined

Table excerpted from the package.

	Female			
	Dosage (mg/kg/week)			
Lesion	0	0.1	0.5	2.5
Uterus[a]	15	15	15	15
Atrophy	0	4	7	10

a: Number examined

Table excerpted from the package.

The following were still remarkable at recovery necropsy (HD):

- ♂ reproductive system: epididymal luminal cellular debris (1/9) and hypospermia (4/9); testes tubular degeneration (4/9); prostate atrophy (4/9)
- Eye: cataract (1/9 ♂s)
- Lung: alveolar macrophages (7/9 ♂s and 2/10 ♀s)
- Spleen: hemosiderosis (8/9 ♂s and 10/10 ♂s); this was also seen with high incidence in the control animals
- Liver: mononuclear cell infiltration (5/9 ♂s and 9/10 ♀s); this was also seen with high incidence in control animals
- Ovaries: follicular cyst (4/10 ♀s)
- Mesenteric lymph node: pigmented macrophage infiltrate (9/9 ♂s and 8/10 ♀s); this was also seen with high incidence in control ♀s
- Lumbar cord: axonal degeneration (1/9 ♂)

The following are findings at recovery sacrifice, which had incidence in control animals (in at least one gender) comparable to that in the treatment group(s).

	Male		Female	
	Dosage (mg/kg/week)		Dosage (mg/kg/week)	
Lesion	0	2.5	0	2.5
Lung[a]	9	9	9	10
Alveolar Macrophages	0	7	1	2
Mesenteric Node[a]	9	9	9	10
Pigmented Macrophage Infiltrate	0	9	9	8
Spleen[a]	9	9	9	10
Hemosiderosis	3	8	9	10
Liver[a]	9	9	9	10
Mononuclear Cell Inflammation	6	5	6	9

a: number examined.

Excerpted from the package.

Toxicokinetics: provided in a separate report

- Exposures to temsirolimus were generally higher in ♂s than ♀s.
- The half-life of temsirolimus was long, being 18-33 hrs during Week 1 and 37-55 hrs during Week 26.
- Repeated dosing resulted in accumulation of temsirolimus as indicated by increased temsirolimus exposures on Week 26 when compared to Week 1 and the increased half-life on Week 26.

Species: Rats
No. and Sex: 24/sex/group
Feeding Condition: Ad libitum
Matrix: Whole blood
Assay: LC/MS/MS
Analyte: Temsirolimus and sirolimus

Cross-Reference: 4.2.3.2
Report No.: RPT-48596
Route of Administration: Intravenous
Dosing Schedule: Once weekly for 6 months
Sampling Schedule: Weeks 1 and 26, day of first and 26th doses.
Vehicle/Formulation: Ethanol, vitamin E, and propylene glycol in sterile saline and diluent (5% polysorbate 80, 5% polyethylene glycol 400, and sterile water for injection).

Pharmacokinetic Parameters (Mean ± SE)

Sampling Point	Temsirolimus (mg/kg)	Sex	N Time point	Analyte	C_{5min} (ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (h)	$AUC_{0-\tau}$ (ng•h/mL)	AUC_{0-48} (ng•h/mL)	AUC_{0-168}/ Dosage
Week 1 (first dose)	0.1	M	3	Temsirolimus	52.7 ± 2.7	NA	27.8[a]	343	257 ± 14	NA
		F	2-3	Temsirolimus	37.3 ± 8.9	NA	33.1[a]	265	185 ± 11	NA
	0.5	M	3	Temsirolimus	144 ± 5	NA	20.9[a]	1158	941 ± 56	NA
		F	3	Temsirolimus	85.4 ± 18.2	NA	22.2[a]	950	767 ± 34	NA
	2.5	M	3	Temsirolimus	700 ± 82	NA	18.2[a]	2304	2053 ± 92	NA
		F	3	Temsirolimus	1232 ± 139	NA	20.2[a]	2113	1865 ± 51	NA
	0.1	M	3	Sirolimus	NA	BQL	ND[b]	BQL	BQL	NA[c]
		F	3	Sirolimus	NA	BQL	ND[b]	BQL	BQL	NA[c]
	0.5	M	3	Sirolimus	NA	BQL	ND[b]	BQL	BQL	NA[c]
		F	3	Sirolimus	NA	BQL	ND[b]	BQL	BQL	NA[c]
	2.5	M	3	Sirolimus	NA	1.75 ± 0.39	ND[b]	66.7	28.8 ± 2.5	NA[c]
		F	2-3	Sirolimus	NA	2.16 ± 0.14	ND[b]	22.9	18.9 ± 1.9	NA[c]

Report No.: 4.2.3.2; RPT-48596

Sampling Day	Temsirolimus (mg/kg)	Sex	N Time point	Analyte	C_{5min} (ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_{0-48} (ng•h/mL)	AUC_{0-168} (ng•h/mL)	AUC_{0-168}/ Dosage
Week 26 (26^{th} dose)	0.1	M	3	Temsirolimus	44.0 ± 5.0	NA	48.9	NA	591 ± 201	5915 ± 2009
		F	3	Temsirolimus	57.0 ± 4.5	NA	ND[d]	NA	328 ± 29	3277 ± 294[e]
	0.5	M	3	Temsirolimus	394 ± 128	NA	55.2	NA	1376 ± 95	2751 ± 190[f]
		F	3	Temsirolimus	267 ± 72	NA	47.3	NA	730 ± 40	1460 ± 80
	2.5	M	3	Temsirolimus	1987 ± 265	NA	40.3	NA	3397 ± 133	1359 ± 53[f]
		F	3	Temsirolimus	1441 ± 202	NA	37.2	NA	2106 ± 164	842 ± 66[f]
	0.1	M	3	Sirolimus	NA	BQL	ND[b]	BQL	NA	NA[c]
		F	3	Sirolimus	NA	BQL	ND[b]	BQL	NA	NA[c]
	0.5	M	3	Sirolimus	NA	0.75 ± 0.13	ND[b]	6.38 ± 2.39	NA	NA[c]
		F	3	Sirolimus	NA	BQL	ND[b]	BQL	NA	NA[c]
	2.5	M	3	Sirolimus	NA	4.93 ± 0.89	ND[b]	29.7 ± 2.4	NA	NA[c]
		F	3	Sirolimus	NA	3.06 ± 0.50	ND[b]	16.8 ± 1.6	NA	NA[c]

a. Values should be interpreted with caution because the sampling period was too short (48 hours) to adequately characterize the terminal phase reflected by such a long half-life.
b. Not determined because sirolimus is a metabolite of temsirolimus and the terminal rate constant would be influenced by continued production of sirolimus from temsirolimus metabolism.
c. Sirolimus was not administered to the animals.
d. Not determined because the blood concentration-time profiles did not support the definition of the terminal phase by at least 3 data points.
e. Significantly different from corresponding value in males; $p \leq 0.05$.
f. Significantly different from corresponding value at 0.1 mg/kg; $p < 0.05$.

AUC = Area under the concentration-versus-time curve; BQL = Below the quantitation limit; C_{5min} = Concentration at 5 minutes after dosing; C_{max} = Peak concentration; IV = Intravenous; LC/MS/MS = Liquid chromatography-tandem mass spectrometry; N = Number of animals; NA = Not applicable; ND = Not determined; RPT = Report; SE = Standard error; $t_{1/2}$ = Apparent terminal half-life.

Summary of the study:

CD rats were dosed i.v., weekly with 0.1, 0.5, or 2.5 mg/kg (0.6, 3, or 15 mg/m2) of temsirolimus, for 26 weeks. Additional groups of animals from control and HD were allowed 13 weeks of recovery period.

A total of 7 unscheduled deaths occurred during this study: 2 control rats, 2 LD, 1 MD, and 2 HD rats. It is not clear whether any of these deaths were drug-related. Major findings in 1 MD and 2 HD rats consisted of urinary tract infection with hematuria which corresponded to pelvic dilation and hemorrhage/dilation of urinary bladder by histopathology in 1 MD and 1 HD animals, and hematuria and rales in 1 MD animal.

Clinical signs of toxicity included opacities which correlated with ophthalmascopic findings. Cortical cataract, which appeared to be secondary to hyperglycemia, increased in severity and incidence from Week 12 to Week 25, and was irreversible. Cataracts

were more pronounced in ♂s as was hyperglycemia. Of note, although hyperglycemia was not reported for ♀ rats, cataract was observed in small number of ♀s.

↑Neutrophils, ↓lymphocytes, ↑aPTT, ↑fibrinogen, ↑cholesterol, ↓triglycerides, ↓potassium, and ↓phosphorus observed in this study were more pronounced in ♂ rats. ↑glucose was reported for ♂s and ↑T4 for ♀ rats. Hematology and clinical chemistry parameters were generally reversible.

Organ weight showed ↓size of pituitary, spleen, thymus, heart, testes, and prostate. Histopathology findings included lymphoid atrophy, bone marrow hypocellularity, macrophage infiltrate in multiple tissues, increased incidence of cardiomyopathy, necrosis and fatty change in the liver, spleen hemosiderosis, and cataract. Most findings were reversible. Follicular cyst of ovary was observed at the end of the recovery period only.

Study title: CCI-779: 26-week oral (gavage) toxicity study in rats

Key study findings: daily oral doses of temsirolimus given for 6 months to SD rats resulted in the following effects. Toxicities were more evident in ♂s:

- Pancreas: ↑amylase
- Liver: ↑AST, ALT, and ALP, and necrosis
- ↑Cholesterol
- Coagulation: ↑aPTT, ↑fibrinogen
- Lung: ↑incidence of alveolar macrophages
- Heart: ↑incidence of spontaneous cardiomyopathy
- Hematopoietic system: ↓reticulocytes, thymic hemorrhage

Report no.: RPT-47743
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]
Date of study initiation: Feb 2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Lot# RA0622; [redacted] pure

Methods

Doses: 0.03, 0.1, 0.3 mg/kg/day
0.18, 0.6, 1.8 mg/m^2/day
Once daily for 6 months

Group	No. of Animals Male	Female	Dose Level[a] (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)[c]	20	20	0	0
2 (Low)	20	20	0.03	0.01
3 (Mid)	20	20	0.1	0.033
4 (High)	20	20	0.3	0.1

a Dose levels were based on the use at value of the test article [redacted] and adjusted for strength.
b Dose volume was 3 mL/kg.
c The control animals received a 90% dilution of the stock carrier solution with reverse osmosis water.

Table excerpted from the package.

The stock carrier components used in the dosing preparations: 7% (v/v) ethanol 200 proof, USP; 2% (w/v) polysorbate 80, NF; and Phosal 50 PG

Formulation analysis: some dosing formulations were out of the ± [redacted] accepted range; e.g. the stock solution of 0.1 mg/mL had a drug concentration of [redacted] on Week 19. Since the stock solutions were prepared every 2 weeks and dosing was for 6 months, this low concentration may not substantially affect the results.

The stock solutions and the dose preparations ranged from [redacted] of theoretical for the 0.01, 0.33, 0.1, 0.3 and 1 mg/ml concentrations, respectively.

Species/strain: Sprague-Dawley rats
Number/sex/group (main study): 20/sex/group
Route, formulation, volume: oral gavage, suspension, 3 mL/kg
Satellite groups (TK or recovery): No recovery group
Age: 43-49 days
Weight: ♂s: 183-247
♀s: 140-204

Observations and times:
Mortality: twice daily
Clinical signs: daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: pre-test and during Week 26
EKG: not done
Hematology and coagulation: during Weeks 13 and 26; from 10 animals/sex/group
Clinical chemistry: during Weeks 13 and 26; from 10 animals/sex/group
Urinalysis: during Weeks 13 and 26; from 10 animals/sex/group
Gross pathology: at necropsy; at the end of 6-month treatment
Organ weights:

adrenal (2)
brain
heart
kidney (2)
liver
ovary (2)
pituitary
prostate
spleen
testis (2)
thymus
thyroid (2) with parathyroid
uterus

Histopathology: Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )

The following tissues from each animal were collected and preserved in 10% neutral-buffered formalin, unless otherwise specified. Stain: H&E

adrenal (2)
aorta
brain
cecum
colon
duodenum
epididymis [longitudinal sections through head, body, and tail (2)]
esophagus
eye [preserved in Davidson's fixative (2)]
femur with bone marrow (articular surface of the distal end)
gut-associated lymphoid tissue
Harderian gland
heart
ileum
jejunum
kidney (2)
liver
lung (with mainstem bronchi)
lymph nodes [mandibular and mesenteric (2)]
macroscopic lesions
mammary gland
ovary (2)
pancreas
pituitary gland
prostate
rib with bone marrow
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)
skin
spinal cord (cervical and lumbar)
spleen
stomach
testis [preserved in Bouin's fixative (2)]
thymus
thyroid (2) with parathyroid
tongue
trachea
urinary bladder
uterus
vagina (longitudinal section through cervix)

Bone marrow smears from the femur of each animal were prepared, stained with Wright's stain, and retained for possible examination.

Examined:

- All tissues from animals in groups 1 and 4
- All animals in groups 1-4 that died at an unscheduled interval
- All macroscopic lesions from all animals in groups 1-4
- In addition, the heart, liver, and lung in ♂s and the lung in ♀s in groups 2 and 3 were examined microscopically.

Toxicokinetics: not done

Results

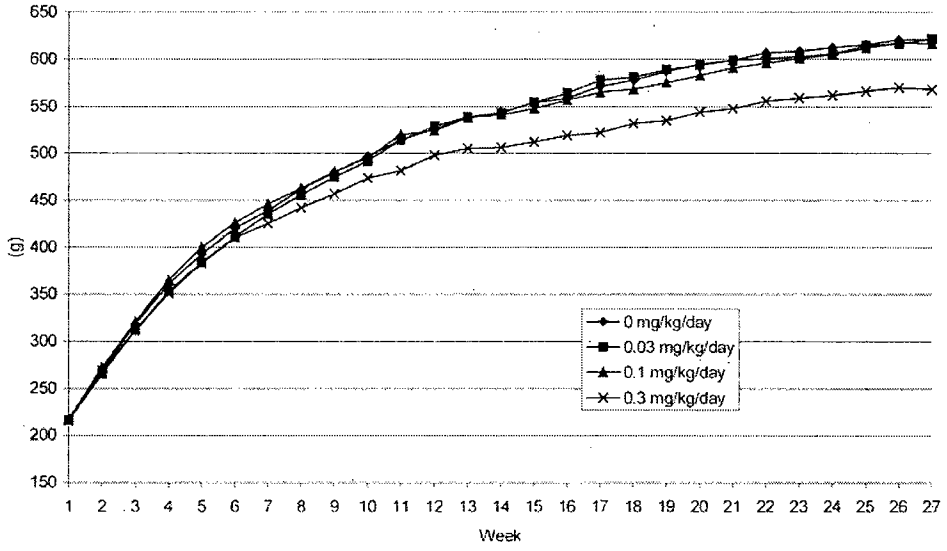
Mortality:

- One control ♀: blood collection–related
- One LD ♂: cause of death not determined

Deaths	Findings
1 control ♀ (Day 89)	• Death was said to be blood collection related, since the animal died shortly after blood sampling. • Gross pathology: dark red fluid in the GI tract and lacerations in the tongue. • Histopathology: signs of autolysis present in the GI tract; mild focal lymphohistiocytic inflammation in lung; slight multifocal lymphohistiocytic inflammation in liver; slight multifocal hemosiderosis in spleen; and moderate unilateral uterine dilatation.
1 LD ♂ (Day 65)	• Gross pathology: diffusely reddened thymus. • Histopathology: moderate multifocal hemorrhage in thymus; mild focal spontaneous cardiomyopathy; mild focal lymphohistiocytic inflammation of the lung; slight focal lymphohistiocytic inflammation in the liver

Clinical signs: no drug-related effect

Body weights: ↓BW gain in HD ♂s and ♀s were not toxicologically significant.



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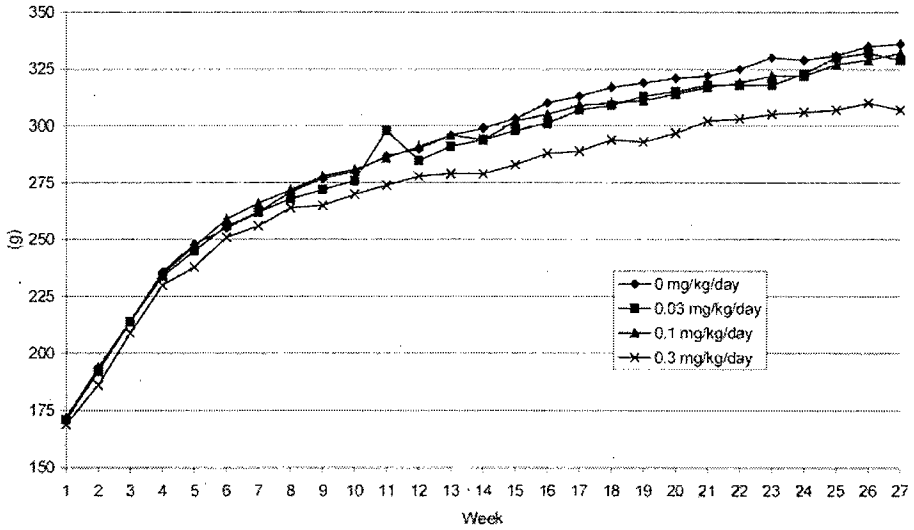


Graphs excerpted from the package.

Food consumption: no drug-related effect

Ophthalmoscopy: no drug-related effect

EKG: not done

Hematology: changes were generally not toxicologically significant

	Reticulocytes					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	—	—	—	—
Week 26	—	—	—	↓13%	↓16%	↓26%

	MPV					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	—	—	—	↓15%*
Week 26	—	—	—	—	—	↓11%*

—: no change/ no toxicologically significant change.
* Statistically significant.

Coagulation:

	aPTT (sec)					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑18% (4)	—	—	—	—
Week 26	—	↑22% (6)	↑11%* (2)	↑23% (4)	↑18% (3)	↑29% (5)

—: no change/ no toxicologically significant change
Numbers in parenthesis are absolute changes (sec) from control.

	Fibrinogen					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑18%*	↑32%*	—	↑8%	↑22%*
Week 26	↑20%	↑17%	↑20%	—	↑20%	↑27%*

—: no change/ no toxicologically significant change.
* Statistically significant.

Clinical chemistry:

	Cholesterol					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑18%	↑40%*	↑69%*	—	↑17%	↑15%
Week 26	↑20%	↑25%	↑60%*	—	↑22%	↑14%

—: no change/ no toxicologically significant change.
* Statistically significant.

	AST					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	—	—	—	—
Week 26	—	—	↑2-fold†	—	—	—

—: no change/ no toxicologically significant change.
* Statistically significant.
† Large standard deviation.

	ALT					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑54%*	—	—	—
Week 26	—	—	↑3-fold*†	—	—	—

—: no change/ no toxicologically significant change.
* Statistically significant.
† Large standard deviation.

	ALP					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑25%	—	—	—
Week 26	—	—	↑64%	—	—	—

—: no change/ no toxicologically significant change.
* Statistically significant.

	Amylase					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑42%*	↑50%*	—	—	—
Week 26	—	↑14%	↑27%*	—	—	—

—: no change/ no toxicologically significant change.
* Statistically significant.

Urinalysis: not done

Gross pathology: for unscheduled sacrifices look under mortality
No clear drug-related finding.

Organ weights:
Changes relative to brain at the end of the 6-month treatment period.

Pituitary						Spleen					
♂			♀			♂			♀		
LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
—	↓12%	↓17%*	—	—	↓28%*	↓9%	↓9%	↓15%*	—	—	—

Prostate						Testes					
♂			♀			♂			♀		
LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
—	↑10%	↑7%	NA	NA	NA	—	—	↑6%*	NA	NA	NA

Thymus					
♂			♀		
LD	MD	HD	LD	MD	HD
—	—	—	↑10%*	↑20%*	↑30%*

* Statistically significant.
Number of animals in each group: LD ♂= 19, LD ♀= 20, MD ♂ or ♀= 20, HD ♂ or ♀= 20 (except for the thymus in ♂s; N=19)
NA: not applicable; —: no change/ no remarkable change.

Histopathology:

GROUP INCIDENCES (WITH AVERAGE SEVERITIES) OF CCI-779-RELATED MICROSCOPIC OBSERVATIONS AT FINAL NECROPSY

	Male Dosage (mg/kg/day)				Female Dosage (mg/kg/day)			
FINDING	0	0.03	0.1	0.3	0	0.03	0.1	0.3
Lung[a]	20	19	20	20	19	20	20	20
Alveolar Macrophages	6	13	15	15	2	8	16	11
	(0.4)	(0.8)	(1.1)	(1.4)	(0.2)	(0.6)	(1.1)	(0.9)
Heart[a]	20	19	20	20	19	0	0	20
Spontaneous Rat Cardiomyopathy	9	15	19	19	10	0	0	12
	(0.6)	(1.0)	(1.4)	(1.4)	(0.7)	(0.0)	(0.0)	(1.0)
Liver[a]								
Necrosis	20	19	20	20	19	0	0	20
	1	1	6	6	0	0	0	1
	(0.1)	(0.1)	(0.4)	(0.6)	(0.0)	(0.0)	(0.0)	(0.1)

a: Number examined
( ): Average severity (0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Animals Affected -- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	20	19	20	20	19	20	20	20
THYMUS	Number examined:	20	1	1	20	19	1	0	20
HEMORRHAGE		1	1	1	11	8	1	0	10
UTERUS	Number examined:					19	0	0	20
DILATATION						1	0	0	5

Tables excerpted from the package.

Toxicokinetics: not reported/ not done

Conclusions:
SD rats were dosed with temsirolimus p.o. daily for 6 months. There were no recovery groups. Doses were 0.18, 0.6, and 1.8 mg/m2/day.

Doses did not reach clear MTD, associated with life-threatening effects. However, toxicological responses were observed . The 2 deaths in the study were in the control and the LD group. Although the cause of deaths were not fully determined, formulation might have contributed since some of the findings, i.e. histocytic inflammation of liver and lung, were similar in both animals. Toxicities were generally more pronounced in ♂s. Since no TK data is available, it is not clear whether the difference is exposure related.

Hematology changes consisted mainly of ↓reticulocytes on Week 26. Coagulative changes consisted of ↑fibrinogen and small increases in the aPTT. Hepatotoxicity was evident in MD and HD ♂s as indicated by ↑AST, ALT, and ALP (at HD) at the end of the 6-month treatment period and liver necrosis at MD and HD. ↑Cholesterol was seen at all dose levels in ♂s and at MD and HD in ♀s, on both Weeks 13 and 26. Pancreatic toxicity (↑amylase) was evident in ♂s on both Weeks 13 and 26.

Histopathology findings consisted of the following:

- Pulmonary alveolar macrophages in ♂s and ♀s. This effect was observed with lower incidence in control animals. In addition to the drug, the formulation (i.e. components of the excipients) may contribute to this finding.
- Increased incidence and severity of spontaneous rat cardiomyopathy in ♂s at all dose levels
- Increased incidence of hepatic necrosis, mainly in ♂s at MD and HD
- Thymic hemorrhage, mainly in ♂s. Thymic hemorrhage was also reported in the LD ♂s that died during the study.
- Dilatation of uterus. This was also reported in the control animal that died during the study.

Study title: 39-week (one dose per week) intravenous toxicity study in monkeys with a 13-week recovery

Key study findings: All doses were well tolerated; an MTD was not achieved. The following drug-induced toxicities were evident:

- Lymphocytic system: lymphoid atrophy in thymus, lymph nodes, and GALT
- Male reproductive toxicities: small testes, immature epididymides, testicular tubular degeneration
- GI toxicity: fecal alterations, bloody feces, inflammation
- ↓RBC and lineages, ↑WBC, neutrophils, and monocytes
- ↑Cholesterol, ↑glucose
- ↑Fibrinogen

Report no.: RPT-43566
Volume #, and page #: Module 4
Conducting laboratory and location: Wyeth Research
641 Ridge Road
Chazy, NY 12921

Date of study initiation: August 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: CCI-779,
Lot/Batch # MA9611

10 Apr 2000 release:
Total impurities [redacted]
Largest single impurity

27 Mar 2001 release:
Total impurities [redacted]
Largest single impurity

Lot/Batch # RA0622

6 Feb 2001 release:
Total impurities
Largest single impurity [redacted]

Methods

Doses: 0.1, 0.5, 2.5 mg/kg/week
0.12, 6, 30 mg/m2/week
Weekly administration for a total of 39 doses (39 weeks)

Dosage Group	Dosage[a] (mg/kg/week)	Concentration[b] (mg/mL)	Animal Numbers[c] Male	Female
1 Vehicle-Control	0	0	1-7	8-14
2 Low	0.1	0.1	15-21	22-28
3 Middle	0.5	0.5	29-35	36-42
4 High	2.5	2.5	43-49	50-56

a: Based on the active moiety and adjusted for strength.
b: Dose volume of 1 mL/kg was administered.
c: Beginning the day after dosing in week 39, the last three animals per sex per group began a 13 week recovery.

Formulation analysis: the stock formulations ranged from [redacted] % of the claimed concentrations and the dosing formulations ranged from [redacted] of the claimed concentrations.
The dose of 2.5 mg/kg was the maximum dose that could be administered due to formulation limitations, including the solubility of the compound and the level of excipients that can be practically administered without confounding results.

Vehicle: ethanol, vitamin E, propylene glycol and diluent (5% polysorbate 80, 5% PEG 400 and water for inection)

Species/strain: Cynomolgus monkeys
Number/sex/group: 7/sex/group (main)
3/sex/group (recovery)
Route, formulation, volume, and infusion rate: i.v., bolus, 1 mL/kg
Age: 1.8-5.6 years old at dose initiation
Weight: 3 to 5.6 kg (♂s); 2.7-4 kg (♀s) at initiation

Observations and times:
Mortality: twice daily
Clinical signs: once daily
Detailed clinical examination: once weekly
Body weights: once weekly
Food consumption: estimated daily
Ophthalmoscopy: pre-test, during Week 38 (treatment period), and during Week 51 (recovery period). Biomicroscopy, and direct and indirect ophthalmoscopy was performed after the application of tropicamide.
EKG: not done
Hematology: pretest and weeks 13, 26, 37, and 52 (recovery)

Clinical chemistry: pretest and weeks 13, 26, 37, and 52 (recovery)
Urinalysis: pretest and weeks 37, and 52 (recovery)
Gross pathology: at necropsy: external surface of the body, all orifices, the cranial, thoracic, and abdominal cavities and their contents and organs and tissues.
Organ weights:

Adrenal Glands	Ovaries
Brain	Pituitary Gland
Heart	Prostate
Kidneys	Testes
Liver	Thyroid/Parathyroid Glands

Histopathology: Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )

Bone marrow smears were prepared but not evaluated.

Adrenal Glands (cortex and medulla)	Mammary Gland
Aorta (thoracic)	Ovaries
Bone and Joint (distal femur)	Pancreas
Bone Marrow (rib)	Peripheral Nerve (sciatic)
Brain	Pituitary Gland
Cecum	Prostate Gland
Colon	Salivary Gland (submandibular)
Duodenum	Seminal Vesicles
Epididymides	Skeletal Muscle (thigh)
Esophagus	Skin
Eyes	Spinal Cord (cervical and lumbar)
Gall Bladder	Spleen
Gut Associated Lymphoid Tissue	Stomach (cardiac, fundic, and
Heart	pyloric)
Injection Sites(left fore leg, right fore leg,	Testes
left hind leg, right hind leg)	Thymus
Ileum	Thyroid/Parathyroid Glands
Jejunum	Tongue
Kidneys	Trachea
Liver	Urinary Bladder
Lungs	Uterus
Lymph Nodes (mandibular and mesenteric)	Vagina
Macroscopic Lesions	

Samples were fixed in 10% NBF, except for testes which were fixed in Bouin's solution

Toxicokinetics:
Blood samples were collected from all animals after the 1st and 38th dose at the following time-points: pre-dose, and ~5 min, 30 min, 1.5, 4, 12, 24, 48 and 72 hrs post-dose.

Results

Mortality: 2 unscheduled deaths

Deaths		Findings
1 LD ♀	Week26	• Thin • Soft feces • Erosions, cysts, mixed cell inflammation in the cecal or colonic mucosa • Lymphoid atrophy in thymus and mandibular lymph node
1 HD ♀	Week 15	• Thin • Liquid/bloody feces • Lymphoid atrophy in the thymus, mesenteric lymph node, and gut associated lymphoid tissue (GALT) • Week 13 and 14 hematology showed ↓13-16% in RBC, hemoglobin, and hematocrit and ↑34% (compared to pre-test) in fibrinogen.

Clinical signs:

- Fecal alterations: at all dose levels in ♂s and ♀s
- Bloody feces: mainly at HD (♂s and ♀s)
- Rash on limbs and trunk: mainly at MD and HD (♂s and ♀s)
- Injection site reactions (lesion and swelling): all groups

Body weights: ↓BW gain at MD (♂s) and HD (♂s and ♀s)

Food consumption: occasionally reduced at HD

Ophthalmoscopy: no drug-related finding

EKG: not done

Hematology: changes were reversible, unless otherwise indicated.

	RBC					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↓12%	—	—	↓12%
Week 26	—	—	↓5%	—	—	↓11%
Week 37	—	—	—	—	—	↓11%

—: no change/ no toxicologically significant change

	Hemoglobin					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↓8%	↓15%	—	↓6%	↓8%
Week 26	—	↓5%	↓11%	—	↓6%	↓9%
Week 37	—	↓5%	↓9%	—	↓4%	↓9%

—: no change/ no toxicologically significant change

	Hematocrit					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↓14%	—	—	↓8%
Week 26	—	—	↓8%	—	—	↓8%
Week 37	—	—	↓8%	—	—	↓8%

—: no change/ no toxicologically significant change

	WBC					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑12%	↑10%	↑26%	—	↑20%	↑20%
Week 26	↑15%	↑10%	↑23%	↑8%	↑20%	↑25%
Week 37	—	—	*↑20%	↑17%	↑40%	↑57%

—: no change/ no toxicologically significant change.
* ↑28% at the end of the recovery period (Week 52).

	Neutrophils					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑42%	↑20%	↑45%	↑40%
Week 26	—	—	↑30%	↑40%	↑63%	↑85%
Week 37	—	—	*↑13%	↑10%	↑70%	↑130%

—: no change/ no toxicologically significant change.
* ↑58% at the end of the recovery period (week 52).

	Monocytes					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑45%	—	—	—
Week 26	↑24%	↑38%	↑42%	—	—	—
Week 37	—	—	↑10%	—	—	↑10%

—: no change/ no toxicologically significant change

	Fibrinogen					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑40%	↑86%	↑36%	↑28%	↑42%
Week 26	—	↑26%	↑46%	↑29%	↑20%	↑45%
Week 37	↑15%	↑30%	↑45%	—	↑19%	↑48%

—: no change/ no toxicologically significant change

Clinical chemistry: Changes were small and reversible.
In addition to the changes reported below, ↓ALT and ↓ALP was also noted.

	†Glucose					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↑15%	↑22%	↑25%	↑10%	↑20%	↑28%
Week 26	↑23%	↑25%	↑22%	—	↑9%	↑9%
Week 37	—	↑16%	↑16%	↑16%	↑13%	↑20%

—: no change/ no toxicologically significant change
† Parameter was also high at pre-test.

	Albumin					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↓10%	—	—	—
Week 26	—	—	↓10%	—	—	—
Week 37	—	—	↓10%	—	—	↓9%

—: no change/ no toxicologically significant change

	Globulin					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	—	↑7%	—	↑5%	↑6%
Week 26	—	—	↑10%	—	↑8%	↑5%
Week 37	—	—	↑14%	—	—	↑8%

—: no change/ no toxicologically significant change

	A/G					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↓6%	↓9%	↓16%	↓6%	↓6%	↓6%
Week 26	↓8%	↓11%	↓18%	↓8%	↓7%	↓8%
Week 37	↓10%	↓10%	↓19%	—	↓5%	↓16%

—: no change/ no toxicologically significant change

	Cholesterol					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	—	↑20%	↑20%	—	—	—
Week 26	↑14%	↑22%	↑28%	—	—	—
Week 37	↑17%	↑20%	↑23%	—	—	—

—: no change/ no toxicologically significant change

	Phosphorus					
	♂			♀		
	LD	MD	HD	LD	MD	HD
Week 13	↓8%	↓10%	↓20%	—	—	—
Week 26	↓7%	↓10%	↓17%	—	—	—
Week 37	↓8%	↓10%	↓17%	—	—	—

—: no change/ no toxicologically significant change

Urinalysis: No clear drug-related changes

Gross pathology: ↓testes in main sacrifice

	Control	LD	MD	HD
N	4	4	4	4
Small testes	1	1	2	2

Increased incidence of macroscopically small testes was associated with microscopic testicular tubular degeneration in 1 HD ♂. Small testes in the remaining CCI-779-treated animals (3 animals) was associated with immaturity of the seminiferous tubules.

Organ weights: ↑ adrenals, ↓testes, ↓pituitary, ↓ovaries.

Main sacrifice

		Male					Female				
	Dosage (mg/kg/week)	N[a]	G[b]	% REF[c]	% REF (TBW)[d,e]	% REF (BNW)[f]	N	G	% REF	% REF (TBW)	% REF (BNW)
Adrenals	0	4	0.406	REF	REF	REF	4	0.415	REF	REF	REF
	0.1	4	0.510	125	143[h]	136	3	0.468	113	117	116
	0.5	4	0.489	120	140[h]	136	4	0.440	106	106	102
	2.5	4	0.524	129[g]	149[h]	133	3	0.487	118	143	128
Testes	0	4	15.0	REF	REF	REF	-	-	-	-	-
	0.1	4	13.5	90	101	94	-	-	-	-	-
	0.5	4	9.5	63	77	73	-	-	-	-	-
	2.5	4	4.6	31	37	31	-	-	-	-	-
Brain	0	4	74.4	REF	REF	-	4	65.8	REF	REF	-
	0.1	4	68.1	92	104	-	3	64.0	97	99	-
	0.5	4	65.4	88	103	-	4	68.3	104	104	-
	2.5	4	71.6	96	110	-	3	61.7	94	110	-
TBW	0	4	5.03	REF	-	-	4	3.68	REF	-	-
	0.1	4	4.38	87	-	-	3	3.60	98	-	-
	0.5	4	4.25	85	-	-	4	3.75	102	-	-
	2.5	4	4.33	86	-	-	3	3.17	86	-	-

a: Number of animals
b: Mean absolute weight in grams for organ weights, kilograms for body weight.
c: % of reference group (controls)
d: Terminal body weight
e: % of body weight values for adrenals are multiplied by 100.
f: Brain weight
g: Mean absolute or relative weights statistically significant (Trend $p \leq 0.05$).
h: Mean absolute or relative weights statistically significant (Trend and Pairwise $p \leq 0.05$).

Recovery sacrifice

		Male						Female			
	Dosage (mg/kg/week)	N[a]	G[b]	% REF[c]	% REF (TBW)[d,e]	% REF (BNW)[f]	N	G	% REF	% REF (TBW)	% REF (BNW)
Adrenals	0	3	0.429	REF	REF	REF	3	0.440	REF	REF	REF
	0.1	3	0.453	106	98	99	3	0.462	105	106	105
	0.5	3	0.462	108	105	104	3	0.495	113	122	110
	2.5	3	0.607	141[g]	129[g]	142[g]	3	0.480	109	119	105
Brain	0	3	70.5	REF	REF	-	3	62.8	REF	REF	-
	0.1	3	75.1	107	99	-	3	61.1	97	101	-
	0.5	3	73.1	104	101	-	3	62.8	100	110	-
	2.5	3	70.3	100	93	-	3	63.9	102	112	-
TBW	0	3	4.30	REF	-	-	3	3.67	REF	-	-
	0.1	3	4.63	108	-	-	3	3.63	99	-	-
	0.5	3	4.40	102	-	-	3	3.37	92	-	-
	2.5	3	4.83	112	-	-	3	3.37	92	-	-

a: Number of animals
b: Mean absolute weight in grams for organ weights, kilograms for body weight.
c: % of reference group (controls)
d: Terminal body weight
e: % of body weight values for adrenals are multiplied by 100.
f: Brain weight
g: Mean absolute or relative weights statistically significant (Trend $p \leq 0.05$).

Tables excerpted from the package.

In addition to the above, the following changes in the organ weight were observed:

		♂: **Main sacrifice**		
		absolute	Relative to BW	Relative to brain weight
Pituitary	HD	↓15%	↓23%	↓16%
		♂: **recovery sacrifice**		
Kidneys	HD	↑6%	*↑25%	↑13%
		♀: **Main sacrifice**		
Ovaries	MD	↓22%	↓25%	↓25%
	HD	↓23%	↓18%	↓21%
Pituitary	HD	↓38%	↓29%	↓49%

* Statistically significant.

Histopathology: See Table below; changes were generally reversible.
The following effects are not presented in Table below.

- Injection site reactions were observed in all groups.
- Inflammation/mononuclear cell infiltration was observed in several organs (e.g. kidney, liver, lung, GI tract, pancreas, salivary gland, and vagina), in all groups and may be due to the formulation.

Controls from group(s): 1		-- Animals Affected --							
	Animal sex:	-- Males --				-- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	4	4	4	4	4	3	4	3
LUNG	Number examined:	4	4	4	4	4	3	4	3
MONONUCLEAR CELL INFILTRATE		2	0	2	1	1	1	1	0
FIBROSIS		1	1	0	0	0	0	1	0
HISTIOCYTIC INFLAMMATION		0	0	0	1	0	0	0	0
FIBRINOUS INFLAMMATION		0	0	1	0	0	0	0	0
HEMORRHAGE		0	0	1	0	0	0	0	0
ALVEOLAR HISTIOCYTOSIS		0	0	0	0	0	0	0	0

Main Sacrifice

		♂				♀			
		C	**LD**	**MD**	**HD**	**C**	**LD**	**MD**	**HD**
Testis	N=	3	4	3	4	NA	NA	NA	NA
	Tubular degeneration	0	0	0	1	NA	NA	NA	NA
	Immature	1	1	1	2	NA	NA	NA	NA
GALT	N=	4	4	4	4	4	3	4	3
	Lymphoid atrophy	0	0	2	2	0	0	3	2
Mandibular node	N=	4	4	4	4	4	3	4	3
	Lymphoid atrophy	0	0	0	2	0	1	3	3
	Hemosiderosis	0	0	0	0	0	0	0	1
Mesenteric node	N=	4	4	4	4	4	3	4	3
	Lymphoid atrophy	1	0	1	4	0	1	2	1
	Hemosiderosis	1	2	3	3	3	3	3	3
Thymus	N=	4	4	4	4	4	3	4	3
	Lymphoid atrophy	1	1	1	3	1	0	2	3
Adrenal cortex	N=	4	4	4	4	4	3	4	3
	Hypertrophy	0	0	0	1	0	0	0	0
Cecum	N=	4	4	4	4	4	3	4	3
	Hemorrhage	1	0	0	1	0	0	0	0
	Edema	0	0	0	1	0	0	0	0
Epididymides	N=	4	4	4	4	NA	NA	NA	NA
	Immature	2	1	2	4	NA	NA	NA	NA

NA: not applicable.

Toxicokinetics:
TK data were reported separately (Report# RPT-48391)
Assay: LC/MS/MS

- Exposures (AUC) to temsirolimus were higher in ♂s than ♀s; with the exception of Week 1 HD data
- Exposures to temsirolimus increased with increasing doses; increases were less than dose-proportional
- Repeated weekly dosing did result in increased AUCs, as AUCs on Weeks 1 and 38 were comparable. However, the half-life of temsirolimus increased after repeated dosing: 21-27 hrs on Week 1 and 36-48 hrs on Week 38.

Assay: LC/MS/MS
Analyte: Temsirolimus and sirolimus

Sampling Day: Days of first and 38th doses
Vehicle/Formulation: Ethanol, vitamin E, and propylene glycol in sterile saline and diluent (5% polysorbate 80, 5% polyethylene glycol 400, and sterile water for injection).

Pharmacokinetic Parameters (Mean ± SD)

Sampling Day	Temsirolimus (mg/kg)	Sex	N	Analyte	C_{5min} (ng/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)	AUC_{0-72}[a] (ng•h/mL)	AUC^{b}/ Dosage
Week 1	0.1	M	7	Temsirolimus	354 ± 32	NA	NA	26.6 ± 2.0	1437 ± 186	1288 ± 161	14,373 ± 1858[c,d,e]
		F	7	Temsirolimus	321 ± 70	NA	NA	24.6 ± 1.7	1029 ± 155	947 ± 144	10,286 ± 1554[c,d,f]
	0.5	M	7	Temsirolimus	411 ± 112	NA	NA	22.1 ± 1.4	3767 ± 630	3455 ± 594	7534 ± 1260[d,e]
		F	7	Temsirolimus	453 ± 88	NA	NA	21.0 ± 1.3	2886 ± 570	2689 ± 537	5772 ± 1139[d,e,f]
	2.5	M	7	Temsirolimus	1007 ± 194	NA	NA	21.6 ± 1.8	5908 ± 659	5478 ± 563	2363 ± 264[e]
		F	7	Temsirolimus	1584 ± 489	NA	NA	22.0 ± 1.7	6705 ± 731	6284 ± 677	2682 ± 293[e]
	0.1	M	7	Sirolimus	NA	2.57 ± 0.51	2.2 ± 1.2	NA	NA	70.8 ± 10.7	NA
		F	7	Sirolimus	NA	2.19 ± 1.75	2.7 ± 1.7	NA	NA	50.2 ± 14.1	NA
	0.5	M	7	Sirolimus	NA	8.50 ± 1.81	1.1 ± 1.3	NA	NA	306 ± 132	NA
		F	7	Sirolimus	NA	8.03 ± 3.16	0.6 ± 0.4	NA	NA	218 ± 39	NA
	2.5	M	7	Sirolimus	NA	19.1 ± 4.7	4.0 ± 0.0	NA	NA	741 ± 227	NA
		F	7	Sirolimus	NA	21.6 ± 5.3	5.8 ± 4.4	NA	NA	749 ± 233	NA

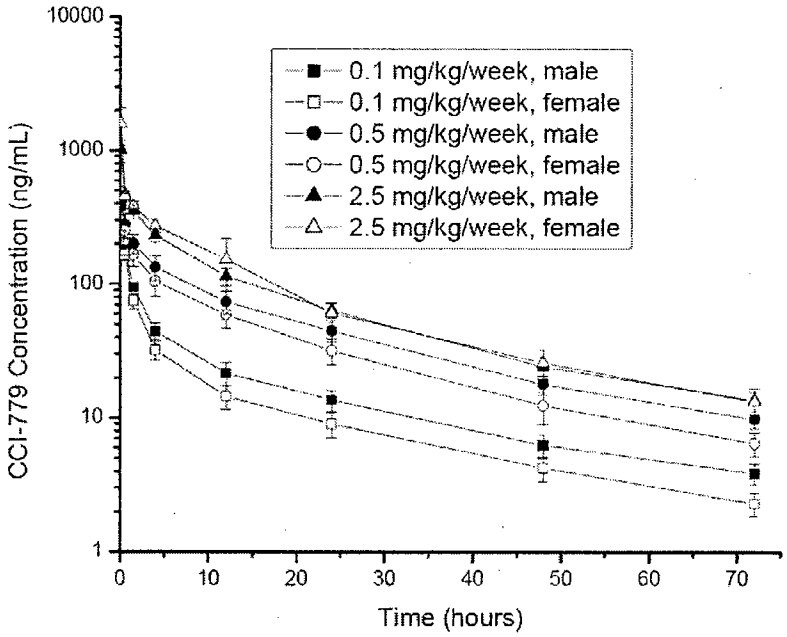
Sampling Day	Temsirolimus (mg/kg)	Sex	N	Analyte	C_{5min} (ng/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng•h/mL)	AUC^{a} (ng•h/mL)	AUC^{b}/ Dosage
Week 38	0.1	M	7	Temsirolimus	308 ± 32	NA	NA	47.7 ± 7.2	NA	1244 ± 183	12,444 ± 1828[c,d]
		F	6	Temsirolimus	324 ± 13	NA	NA	41.7 ± 3.2	NA	1016 ± 117	10,160 ± 1170[c,d,f]
	0.5	M	7	Temsirolimus	482 ± 93	NA	NA	43.3 ± 6.3	NA	3215 ± 364	6429 ± 728[d]
		F	7	Temsirolimus	476 ± 80	NA	NA	37.9 ± 3.6	NA	2373 ± 515	4747 ± 1051[d,f]
	2.5	M	7	Temsirolimus	2112 ± 1109	NA	NA	41.5 ± 5.4	NA	6621 ± 764	2648 ± 306
		F	6	Temsirolimus	1429 ± 526	NA	NA	35.5 ± 3.1	NA	4904 ± 981	1962 ± 392[f]
	0.1	M	7	Sirolimus	NA	2.12 ± 0.41	1.7 ± 1.1	NA	NA	56.2 ± 12.0	NA
		F	6	Sirolimus	NA	2.03 ± 1.12	2.2 ± 1.5	NA	NA	51.7 ± 16.2	NA
	0.5	M	7	Sirolimus	NA	6.28 ± 1.45	4.5 ± 8.7	NA	NA	227 ± 73	NA
		F	7	Sirolimus	NA	5.36 ± 1.79	0.6 ± 0.4	NA	NA	135 ± 47	NA
	2.5	M	7	Sirolimus	NA	19.5 ± 2.3	2.4 ± 2.0	NA	NA	606 ± 205	NA
		F	6	Sirolimus	NA	18.0 ± 3.5	1.7 ± 1.8	NA	NA	515 ± 139	NA

a. For temsirolimus, AUC_{0-72} for week 1 or AUC_{0-168} for week 38. For sirolimus, AUC_{0-72} for weeks 1 and 38.
b. $AUC_{0-\infty}$ for week 1 or AUC_{0-168} for week 38.
c. Significantly different from mid-dosage group, same sex, same week; $p \leq 0.05$.
d. Significantly different from high-dosage group, same sex, same week; $p \leq 0.05$.
e. Significantly different from week 38, same dosage, same sex; $p \leq 0.05$.
f. Significantly different from males, same dosage, same week; $p \leq 0.05$.

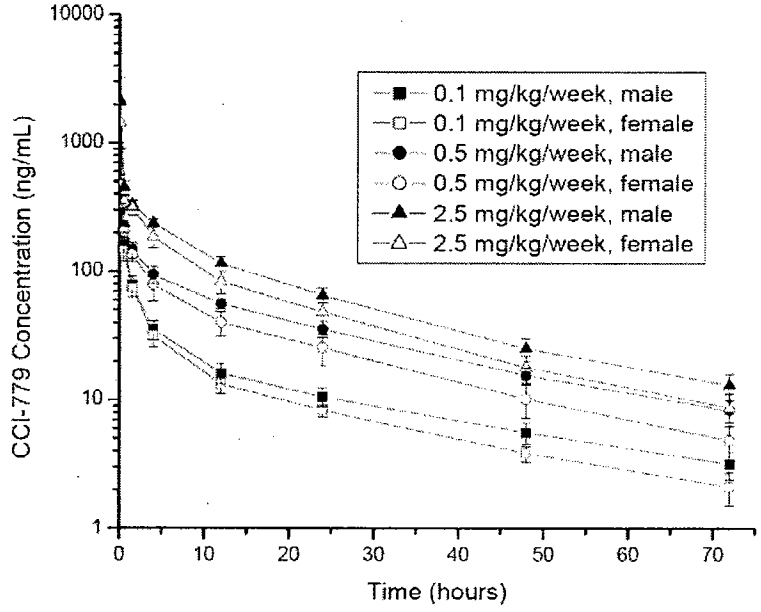
AUC = Area under the concentration-versus-time curve; C_{5min} = Concentration at 5 minutes after dosing; C_{max} = Peak concentration; IV = Intravenous; LC/MS/MS = Liquid chromatography-tandem mass spectrometry; N = Number of animals; NA = Not applicable; ND = Not determined; RPT = Report; SD = Standard deviation; $t_{1/2}$ = Apparent terminal half-life; t_{max} = Time to peak concentration.

Week 1

Appears This Way
On Original



Week 38



Conclusions:

Temsirolimus was administered to monkeys, i.v., once weekly for 9 months. In general drug-induced changes were small; clear MTD, as associated with life-threatening effects, was not achieved. According to the sponsor, the highest dose that could be achieved was 2.5 mg/kg. This was due to the solubility of the compound and the level of excipients that could be administered without confounding results.

Two unscheduled deaths occurred in this study: 1 LD ♀ on Week 26 and 1 HD ♀ on Week 15. Both animals appeared thin and had fecal alterations (the HD animal had bloody feces). Lymphoid atrophy was seen in both animals. Erosions, cysts and mixed cell inflammation in the cecal or colonic mucosa were reported for the LD monkey. ↓RBC and lineages, and ↑fibrinogen were reported for the HD animal.

Clinical signs in the surviving animals consisted of fecal alterations, blood in feces, rash, and injection site reactions. Clinical pathology findings included: ↓RBC, hemoglobin, and hematocrit, ↑WBC, neutrophils, monocytes, and globulin, ↑fibrinogen, ↑cholesterol, ↑glucose, ↓albumin.

Temsirolimus-induced lymphoid atrophy was observed in the lymph nodes, GALT, and thymus. Effects in the male reproductive system included, small testes with tubular degeneration and immature epididymides. Of note, inflammation was observed in multiple organs (lung, kidney, GI tract, liver, pancreas, etc) in all groups, including the control, and may be related to the formulation. Organ weights showed the following changes in the weights: ↑adrenals, ↓testes, ↓pituitary, and ↓ovaries.

2.6.6.4 Genetic toxicology

Study title: Mutagenicity Test with CCI-779 in the Salmonella – Escherichia coli/Mammalian-Microsome Reverse Mutation Assay With a Confirmatory Assay

Key findings: Under the conditions of the bacterial reverse mutation assay (Ames Test), temsirolimus was not mutagenic in the E.coli and salmonella strains tested, in the presence or absence of metabolic activation mix (S9).

Study no.: [redacted] # 18559-0-409R
Report # GTR-30767

Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]

Date of study initiation: 05/02/1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted] pure
WAY-130779 (CCI-779)
Lot# 0M7612 TIM# 9700006

Methods
Agar plate incorporation technique

Tester strains:

- Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537

- E.coli strain WP2uvrA

In the presence or absence of Aroclor-treated rat liver microsome activation system (S9)

Tester strains genotype:

Histidine Mutation			Additional Mutations		
*his*G46	*his*C3076	*his*D3052	LPS	Repair	R Factor
TA1535	TA1537		*rfa*	*uvr*B	-
TA100		TA98	*rfa*	*uvr*B	+R

Table excerpted from the package.

WP2uvrA: mutation in the tryptophan operon, uvrA DNA repair deficiency.

Concentrations used in definitive study: 33.3, 100, 333, 1000, 3330, and 5000 µg per plate

Basis of dose selection: The concentrations were selected based on the results of a dose range finding study using tester strains TA100 and WP2uvrA and 10 concentrations of test article ranging from 6.36 to 4770 µg per plate, in the presence or absence of S9 mix. Since no cytotoxicity was observed in the dose range finding study, the highest dose level of test article used in the mutagenicity assay was 5000 µg per plate, as per Guidance S2A.

Negative controls: tester strains with or without S9 controls; vehicle (DMSO) control

Vehicle controls were plated for all tester strains, in the presence and absence of S9 mix. The vehicle control was plated, using a 50 µl aliquot of vehicle (equal to the maximum aliquot of test article dilution plated), along with a 100 µl aliquot of the appropriate tester strain and a 500 µl aliquot of S9 mix (when necessary).

Positive controls:

Tester Strain	S9 Mix	Positive Control	Conc per plate
TA98	+	2-aminoanthracene	2.5 µg
TA98	-	2-nitrofluorene	1.0 µg
TA100	+	2-aminoanthracene	2.5 µg
TA100	-	sodium azide	2.0 µg
TA1535	+	2-aminoanthracene	2.5 µg
TA1535	-	sodium azide	2.0 µg
TA1537	+	2-aminoanthracene	2.5 µg
TA1537	-	ICR-191	2.0 µg
WP2*uvr*A	+	2-aminoanthracene	25.0 µg
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1.0 µg

Table excerpted from the package.

Incubation and sampling times: incubated for 48 ± 8 hours, at 37 ± 2°C

Revertant counting method:

- Vehicle controls and all plates containing test article: counted manually.

- Positive controls: counted by automated colony counter.

Criteria for positive response:

- Tester Strains TA98, TA100. and WP2uvrA
  For a test article to be considered positive, it had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

- Tester Strains TA1535 and TA1537
  For a test article to be considered positive, it had to produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Deviation from the protocol:
The test article was checked for cytotoxicity up to 4770 µg per plate in the dose range finding study instead of 5000 µg per plate as specified in the protocol. This deviation had no impact on the study, as the test article was subsequently tested up to 5000 µg per plate in the mutagenicity assay.

Results

Study validity:

- Mutations in the tester strains (rfa and pKM101) and mean number of spontaneous revertants were confirmed.
  The acceptable ranges for the mean vehicle controls were as follows:

TA98	8 - 60
TA100	60 - 240
TA1535	4 - 45
TA1537	2 - 25

  The acceptable range for the WP2uvrA mean vehicle controls was: 5 to 40 revertants per plate.
- Triplicate plates were tested at each concentration level for all strains
- A vehicle control (DMSO) and positive controls (2-aminoanthracene, 2-nitrofluorene, sodium azide, ICR-191, and 4-nitroquinoline-N-oxide) were also tested
- Positive control values:
  Positive Control Values in the Absence of S9 Mix: the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.
  Positive Control Values in the Presence of S9 Mix (S9 Mix Integrity): the mean value of the positive control for a respective tester strain in the presence of the S9

mix exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.

- Cytotoxicity: A minimum of three non-toxic doses were required to evaluate assay data. The criteria were met.

Study outcome:

Assay # 1

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colony Counts (Mean ± SD) TA 98	TA100[a]	TA1535	TA1537	WP2 *uvrA*
Without Activation	DMSO (vehicle control)	–	15 ± 2	NA	12 ± 4	5 ± 3	11 ± 3
	Temsirolimus	33.3	12 ± 2	NA	10 ± 2	3 ± 1	12 ± 4
		100	10 ± 3	NA	11 ± 2	2 ± 2	16 ± 2
		333	11 ± 2	NA	9 ± 3	7 ± 1	14 ± 7
		1000	8 ± 2	NA	11 ± 3	3 ± 3	14 ± 4
		3330	8 ± 2	NA	11 ± 3	4 ± 2	9 ± 6
		5000	7 ± 1	NA	7 ± 2	3 ± 0	8 ± 1
	2-Nitrofluorene	1	116 ± 34	NA	NA	NA	NA
	Sodium azide	2	NA	NA	348 ± 37	NA	NA
	ICR-191	2	NA	NA	NA	282 ± 12	NA
	4-Nitroquinoline-N-oxide	1	NA	NA	NA	NA	217 ± 25

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colony Counts (Mean ± SD) TA98	TA100[a]	TA1535	TA1537	WP2 *uvrA*
With Activation	DMSO (vehicle control)	–	18 ± 4	NA	10 ± 2	11 ± 2	12 ± 3
	Temsirolimus	33.3	27 ± 7	NA	13 ± 4	8 ± 4	13 ± 9
		100	27 ± 2	NA	10 ± 5	7 ± 1	19 ± 5
		333	21 ± 4	NA	11 ± 4	10 ± 2	18 ± 4
		1000	22 ± 3	NA	14 ± 2	6 ± 2	21 ± 3
		3330	24 ± 7	NA	11 ± 1	9 ± 1	10 ± 2
		5000	20 ± 7	NA	9 ± 3	8 ± 2	12 ± 2
	2-Aminoanthracene	2.5	896 ± 57	NA	105 ± 4	171 ± 15	NA
		25	NA	NA	NA	NA	288 ± 28

Assay # 2

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colony Counts (Mean ± SD) TA98	TA100[a]	TA1535	TA1537	WP2 *uvrA*
Without Activation	DMSO (vehicle control)	–	13 ± 3	11[b] ± 4	12 ± 4	8 ± 2	10 ± 6
	Temsirolimus	33.3	12 ± 1	NC[c]	11 ± 4	7 ± 2	12 ± 3
		100	19 ± 4	NC[c]	12 ± 2	6 ± 2	12 ± 2
		333	15 ± 4	NC[c]	13 ± 4	7 ± 3	11 ± 4
		1000	13 ± 5	NC[c]	9 ± 3	5 ± 1	9 ± 4
		3330	21 ± 4	NC[c]	10 ± 3	3 ± 3	9 ± 3
		5000	13 ± 4	NC[c]	9 ± 1	5 ± 3	9 ± 1
	2-Nitrofluorene	1	154 ± 20	NA	NA	NA	NA
	Sodium azide	2	NA	NC[c]	503 ± 4	NA	NA
	ICR-191	2	NA	NA	NA	167 ± 16	NA
	4-Nitroquinoline-N-oxide	1	NA	NA	NA	NA	315 ± 39
With Activation	DMSO (vehicle control)	–	25 ± 5	12[b] ± 2	11 ± 3	7 ± 2	10 ± 2
	Temsirolimus	33.3	24 ± 4	NC[c]	11 ± 4	13 ± 4	10 ± 3
		100	29 ± 1	NC[c]	8 ± 2	6 ± 3	10 ± 3
		333	24 ± 2	NC[c]	11 ± 2	7 ± 4	11 ± 3
		1000	20 ± 2	NC[c]	3 ± 3	7 ± 2	9 ± 3
		3330	19 ± 6	NC[c]	11 ± 2	9 ± 3	8 ± 3
		5000	17 ± 3	NC[c]	11 ± 4	10 ± 6	10 ± 3
	2-Aminoanthracene	2.5	884 ± 119	NC[c]	96 ± 10	170 ± 10	NA
		25	NA	NA	NA	NA	236 ± 74

Assay # 3

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colony Counts (Mean ± SD) TA100	TA100
Without Activation	DMSO (vehicle control)	–	94 ± 12	89 ± 25
	Temsirolimus	33.3	77 ± 12	61 ± 16
		100	82 ± 8	78 ± 8
		333	78 ± 8	75 ± 6
		1000	79 ± 14	86 ± 7
		3330	83 ± 7	78 ± 11
		5000	76 ± 5	79 ± 6
	Sodium azide	2	696 ± 30	526 ± 27
With Activation	DMSO (vehicle control)	–	92 ± 14	81 ± 13
	Temsirolimus	33.3	95 ± 4	87 ± 8
		100	91 ± 4	90 ± 16
		333	89 ± 17	95 ± 4
		1000	91 ± 21	93 ± 7
		3330	104 ± 8	78 ± 35
		5000	86 ± 4	89 ± 8
	2-Aminoanthracene	2.5	1083 ± 118	568 ± 145

a. In the initial assays (experiments 18559-B1 and 18559-C1), TA100 was plated but no data were generated due to contamination or unacceptable vehicle control value. Additional assays were conducted with TA100.
b. Unacceptable vehicle control value.
c. Not counted due to unacceptable vehicle control value.

NA: not applicable; NC: not counted.
Tables excerpted from the package.

- No clear cytotoxicity was observed, as effect was not reproducible and/or dose dependent.
- The positive controls induced satisfactory mutagenic responses.
- Moderate precipitates were formed at the 2 highest concentrations of temsirolimus: 3330 and 5000 µg/plate. At the highest concentration (5000 µg/plate), the precipitates formed by the test article obscured an accurate count of the background bacterial lawn. Manual count was done for the background lawn for the 2 high concentrations.
- In the first assay, TA100 strain was contaminated. In the second (confirmatory) assay, the count for vehicle control was outside of the accepted range, hence plates were not counted. Additional assays were performed to assess mutagenicity in the TA100 strain.

Conclusion:
Under the conditions of the bacterial reverse mutation assay (Ames Test), temsirolimus was not mutagenic in the E.coli and salmonella strains tested, in the presence or absence of metabolic activation mix (S9). The 2 highest concentration of the test article (~3000 and 5000 µg/plate) resulted in moderate precipitation, which interfered with accurate measurement of the background bacterial lawn at 5000 µg/plate.

Of note, the positive control for the +S9 system was 2-aminoanthracene, when salmonella strains were used. It is recommended that when 2-aminoanthracene is used as the indicator of the efficacy of the S9 system, the S9 be characterized with a mutagen that requires activation by microsomal enzymes, e.g [redacted]

Study title: Mutagenicity test on CCI-779 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with a confirmatory assay with multiple harvests

Key findings: Temsirolimus is considered negative in the CHO chromosome aberration assay.

[redacted] **Study no.**: 18559-0-437 [redacted]
Report no: GTR-30694
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]

Date of study initiation: May 2, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: WAY-130779 (CCI-779), Lot # OM7612, TIM# 9700006, [redacted] pure

Methods

Cell line: CHO
This cell line has an average cycle time of 12-14 hrs with a modal chromosome number of 21.

Concentrations used in definitive study: see Table below

Design of the study:

	Treatment time (hr)	Colcemid (time after initiation of treatment)	Harvest/ Fixation (time after initiation of treatment)	Concentrations of test article (µg/mL)
Initial Trial				
-S9	17.8	18	20	0.313, 0.625, 1.25, 2.5, 5, 10, 20, 40
+S9	3	18	20	5, 10, 20, 30, 40, 80, 160
Confirmatory trial				
-S9	17.8	18	20	1.25, 2.5, 5, 10, 20, 30, 40
+S9	3	18	20	10, 20, 30, 40, 80, 160
-S9	41.8	42	44	0.625, 1.25, 2.5, 5, 10, 20, 30, 40
+S9	3	42	44	10, 20, 30, 40, 80, 120, 160

- In each assay cells were treated with Colcemid for 2 hours prior to harvest to fix cells in metaphase for evaluation of chromosomal aberrations.
- Two cultures were used at each concentration.
- Mitotic index was analyzed by analyzing the number of metaphases present in 1000 consecutive cells.
- Slides were prepared using harvested cultures. The slides were stained with 5% Giemsa solution for the analysis of mitotic indices and chromosomal aberrations.

Basis of dose selection: dose range-finding study

Negative controls: cell line with or without S9 controls; DMSO (solvent) control

Positive controls:

- Mitomycin C (MMC) dissolved in water at concentrations of 0.08 and 0.10 μg/ml as the positive control in the non-activated (-S9) study.
- Cyclophosphamide (CP) dissolved in water at concentrations of 5 and 10 μg/ml as the positive control in the S9 activated (+S9) study.

Incubation and sampling times: The study consisted of 2 independent trials with and without rat liver activation mix S9 (trial 1, ~20-hr harvest; trial 2, ~20 and ~44-hr harvests).

Analysis of aberrations/ positive response:
The percentage of cells with any aberrations, the percentage of cells with more than one aberration and any evidence for increasing amounts of damage with increasing dose, ie, a positive dose response were used to evaluate whether a positive response had occurred. Polyploidy and endoreduplication were also evaluated.

Assay Acceptance Criteria
An assay was considered acceptable for evaluation of test results only if all of the following criteria were satisfied:

- Acceptable controls: the negative (untreated) and the vehicle control cultures must contain less than ~5% cells with aberrations. The positive control result must be significantly higher ($p < 0.01$) than the vehicle controls.
- Acceptable high-concentration: if the aberration results were negative and there was no significant reduction (approximately ≥50%) in confluence, or mitotic index, the assay must include the highest applicable dose (approximately 5 mg/mL) or a dose exceeding the solubility limit in culture medium. Testing would be conducted at insoluble dose levels when a well-dispersed suspension in culture medium was obtained that did not settle rapidly.
- Acceptable number of doses: the assay must include at least three analyzable dose levels.
- Acceptable dose-related response: there should be a clear evidence for a dose-response.

Results

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

- No. of replicated cultures at each concentration: 2
- No. of independent assays: 2
- One hundred cells from each replicate culture at 4 concentrations of CCI-779 and from the negative, solvent, and positive control cultures were analyzed for chromosomal aberrations.

- At least 25 cells per culture were analyzed from those cultures with >25% of the cells having chromosomal aberrations.
- Acceptance criteria were met.

Culture confluency/ mitotic index (-S9 system)
Initial Trial:

- 40 µg/ml: no cell monolayers; floating dead cells and debris
- 20 µg/mL: unhealthy ce11 monolayers 70% reduction in the cell monolayer confluence; floating dead cells and debris. ↓mitotic index of 33%.
- 10 µg/mL: unhealthy cell monolayers, ~15% reduction in the cell monolayer confluence; reduction in the number of visible mitotic cells. ↓mitotic index of 33%.
- 5 µg/mL: slightly unhealthy cell monolayers and a slight reduction in the number of visible mitotic cells. ↓mitotic index of 42%.
- 2.5 µg/mL: a slight reduction in the number of visible mitotic cells

Chromosomal aberrations were analyzed from the cultures at the following concentrations: 2.50, 5, 10, and 20 µg/ml.

Confirmatory Trial (20-hr assay):

- 30 and 40 µg/mL: <5% cell monolayer confluence, dead cell monolayers, floating dead cells and debris, no visible mitotic cells. ↓mitotic index of 100%.
- 20 µg/mL: ↓~55% in the cell monolayer confluence, unhealthy cell monolayers, severe reduction in the number of visible mitotic cells ↓mitotic index of 96%.
- 10 µg/mL: ↓~15% in the cell monolayer confluence, slight reduction in the number of visible mitotic cells. ↓mitotic index of 84%
- 5 µg/mL: slight reduction in the number of visible mitotic cells. ↓mitotic index of 80%.
- 2.5 µg/mL: ↓mitotic index of 57%.
- 1.25 µg/mL: ↓mitotic index of 24%.

Chromosomal aberrations were analyzed from the cultures at 1.25, 2.50, 5, and 10 µg/ml.

Confirmatory Trial (44-hr assay):

- 30 and 40 µg/ml: <5% cell monolayer confluence dead cell monolayers, no visible mitotic cells
- 20 µg/m: ↓~85% in the cell monolayer confluence, very unhealthy cell monolayers, floating dead cells and debris, a severe reduction in the number of visible mitotic cells. ↓mitotic index of 93%.
- 10 µg/mL: ↓~15% in the cell monolayer confluence, slightly unhealthy cell monolayers, reduction in the number of visible mitotic cells. ↓mitotic index of 51%.
- 5 and 2.5 µg/mL: slightly unhealthy cell monolayers, slight reduction in the number of visible mitotic cells. ↓mitotic index of 7% and 18%, respectively.

- 1.25 µg/mL: slight reduction in the number of visible mitotic cells. ↓mitotic index of 20%.
- 0.625: ↓mitotic index of 9%.

Chromosomal aberrations were analyzed from cultures at test article concentrations of 1.25, 2.5, 5, and 10 µg/ml.

Culture confluency/ mitotic index (+S9 system)
Criteria for selection of the appropriate concentrations were similar to that for the non-activated system and will not be presented.

Study outcome:

- A precipitate was observed at test article concentrations of 166 and 499 µg/mL in the range finding study.

				Assay No. 1[a]				
Metabolic Activation	Test Article	Concentration	Treatment Time (h)	% Cells with Aberrations	% Cells with > 1 Aberration	% Polyploid Cells	% Endo-Reduplicated Cells	Mitotic Index
Without	McCoy's 5a	-	17.8	0.5	0.0	1.0	0.0	5.1
Activation	DMSO (VC)	10.0 µL/mL	17.8	0.5	0.0	1.0	0.0	5.3
	Temsirolimus	2.50 µg/mL	17.8	1.0	0.0	0.5	0.0	3.9
		5.00 µg/mL	17.8	2.5	0.0	2.6	1.0	1.9
		10.0 µg/mL	17.8	1.0	0.0	1.5	0.5	2.2
		20.0µg/mL	17.8	1.0	0.5	2.5	0.0	2.2
	Mitomycin C	0.100 µg/mL	17.8	40.0**	6.0**	1.5	0.0	-
With Activation	McCoy's 5a	-	3	1.0	0.0	2.0	1.5	8.4
	DMSO (VC)	10.0 µL/mL	3	1.0	0.0	1.0	0.5	8.7
	Temsirolimus	20.0 0µg/mL	3	2.0	0.5	0.5	2.5	6.2
		30.0 µg/mL	3	0.0	0.0	1.5	9.0**	4.8
		40.0 µg/mL	3	5.0	1.0	0.5	4.5	5.3
		80.0 µg/mL	3	1.0	0.0	1.5	8.5**	10.1
	Cyclophosphamide	5.00 µg/mL	3	60.0**	22.0**	1.5	0.5	-

				Assay No. 2[b]				
Metabolic Activation	Test Article	Concentration	Treatment Time (h)	% Cells with Aberrations	% Cells with > 1 Aberration	% Polyploid Cells	% Endo-Reduplicated Cells	Mitotic Index
Without	McCoy's 5a	-	17.8	0.0	0.0	2.0	0.0	7.7
Activation	DMSO (VC)	10.0 µL/mL	17.8	0.5	0.0	0.5	0.0	5.1
	Temsirolimus	1.25 µg/mL	17.8	1.0	0.0	0.0	0.0	3.9
		2.50 µg/mL	17.8	0.5	0.0	1.7	0.6	2.2
		5.00 µg/mL	17.8	0.5	0.0	1.7	1.7	1.0
		10.0 µg/mL	17.8	1.0	0.0	16.0**	1.1	0.8
	Mitomycin C	0.100 µg/mL	17.8	28.1**	10.5**	2.5	0.0	-
With Activation	McCoy's 5a	-	3	0.0	0.0	2.0	1.0	5.8
	DMSO (VC)	10.0 µL/mL	3	0.5	0.0	2.0	0.0	8.7
	Temsirolimus	30.0 µg/mL	3	0.0	0.0	1.5	2.5	5.7
		40.0 µg/mL	3	0.5	0.0	0.5	2.0	5.4
		80.0 µg/mL	3	0.0	0.0	3.0	3.0	4.9
		160 µg/mL	3	4.5	0.0	10.5**	1.0	2.5
	Cyclophosphamide	5.00 µg/mL	3	56.0**	24.0**	1.5	0.0	-

				Assay No. 2				
Metabolic Activation	Test Article	Concentration	Treatment Time (h)	% Cells with Aberrations	% Cells with > 1 Aberration	% Polyploid Cells	% Endo-Reduplicated Cells	Mitotic Index
Without	McCoy's 5a	-	41.8	0.5	0.0	0.0	0.0	6.6
Activation	DMSO (VC)	10.0 µL/mL	41.8	1.0	0.0	0.5	0.0	4.5
	Temsirolimus	1.25 µg/mL	41.8	0.5	0.0	1.0	0.0	3.6
		2.50 µg/mL	41.8	1.5	0.0	1.0	0.0	3.7
		5.00 µg/mL	41.8	0.5	0.0	3.0	0.0	4.2
		10.0 µg/mL	41.8	2.5	0.0	3.0	0.0	2.2
With Activation	McCoy's 5a	-	3	0.0	0.0	0.0	0.0	6.5
	DMSO (VC)	10.0 µL/mL	3	0.5	0.0	1.0	0.0	4.5
	Temsirolimus	40.0 µg/mL	3	2.0	0.0	0.5	0.0	5.1
		80.0 µg/mL	3	1.5	0.0	1.0	0.0	5.2
		120 µg/mL	3	1.5	0.0	2.5	1.0	6.7
		160 µg/mL	3	2.0	0.5	1.0	0.0	5.1

**p ≤ 0.01 compared with the vehicle control (statistical significance was determined by Fisher's Exact test).

a. Concentrations of 0.313, 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg/mL were tested without activation and concentrations of 5.00, 10.0, 20.0, 30.0, 40.0, 80.0, and 160 µg/mL were tested with activation. Only the concentrations listed in the table were analyzed for chromosome aberrations.
b. Concentrations of 1.25, 2.50, 5.00, 10.0, 20.0, and 30.0 µg/mL were tested without activation and concentrations of 5.00, 10.0, 20.0, 30.0, 40.0, 80.0, and 160 µg/mL were tested with activation. Only the concentrations listed in the table were analyzed for chromosome aberrations.
c. Concentrations of 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg/mL were tested without activation and concentrations of 30.0, 40.0, 80.0, 120, and 160 µg/mL were tested with activation. Only the concentrations listed in the table were analyzed for chromosome aberrations.

CHO = Chinese hamster ovary; DMSO = Dimethylsulfoxide; GLP = Good Laboratory Practices; GTR = General Technical Report; VC = Vehicle control.

Endoreduplication was defined by the sponsor as "a failure of chromosomes to separate, resulting in a 4n cell."

Conclusion:

Temsirolimus is considered negative in the CHO chromosome aberration assay. ↑chromosomal aberration (statistically significant) was observed with temsirolimus in the presence of S9 metabolic activation mix in the 3-hr treatment (Trial 1). Chromosomal aberration was characterized as endoreduplication. Since the harvest time of 44 was negative, and since the observations in the two-20 hour assays with and without metabolic activation did not agree, the significance of this observation is uncertain.

Trial 1:

-S9: no statistically significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

+S9: ↑(100%-500%) of endoreduplicated cells were observed at the 3 highest concentrations (30, 40, and 80 µg/mL) of the test article. Increases were statistically significant at 30 and 80 µg/mL. Based on the mitotic index and confluency, cultures at the concentrations of 30 and 40 µg/mL were not considered very unhealthy (e.g. 40 µg/mL: slightly unhealthy cell monolayers and a slight reduction in the number of visible mitotic cells, ↓mitotic index of 39%). Therefore, chromosomal aberration appears to be a true effect of the test article.

Trial 2 (20-hr assay):

-S9: statistically significant increase in the number of cells with polyploidy was reported for cultures at 10 µg/ml of test article (16% for test article vs 2% for control; ↑7-fold). Cultures at this concentration had ↓15% of confluency and ↓mitotic index of 84%.

+S9: statistically significant increases in the number of cells with polyploidy was reported for cultures at 160 µg/ml of test article (↑4-fold vs control). This resulted in a

statistically significant increase in the number of cells with aberrations. Cultures with this concentration of the test article were reported to have very unhealthy cells, with ↓85% in confluency and ↓52% in mitotic. The aberration may be secondary to the unhealthy conditions of the cells/extreme cytotoxicity.

Trial 2 (44-hr assay):
-S9/ +S9: No statistically significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed at the concentrations analyzed.

Study title: Mutagenicity test on CCI-779 in the L5178 TK +/- mouse lymphoma forward mutation assay with a confirmatory assay

Key findings: Under the conditions tested, temsirolimus was not mutagenic in the TK+/- mouse lymphoma forward mutation assay.

Report no.: GTR-30972

Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]

Date of study initiation: May 2, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779
Lot # OM7612, TIM: 9700006, [redacted] pure
Solvent: DMSO
Test article was insoluble in DMSO at concentrations > 250 µg/mL

Methods

Cell line: mouse lymphoma L5178 cell

Doses used in definitive study: see Table below, under "study design"

Basis of dose selection: range finding study
Note: due to the formation of precipitate at > 250 µg/mL of the test article the highest concentration used in the range finding study was 500 µg/mL

In the range finding assay, cells were exposed to the test article for 4 hrs in the presence or absence of rat liver S9 metabolic activation. Concentrations of the test article were from 0.985 to 500 µg/ml.

- Precipitate was observed at 250 µg/ml and 500 µg/ml.
- -S9: CCI-779 was noncytotoxic to weakly cytotoxic from 0.985 µg/ml to 15.7 µg/ml and highly cytotoxic at 31.3 µg/ml. Higher concentrations were lethal.

- +S9: CCI-779 was noncytotoxic to weakly cytotoxic from 0.985 µg/ml to 31.3 µg/ml. Higher concentrations were lethal.

Negative controls: cell line with or without S9 controls; DMSO (vehicle) control

Positive controls:

- Methyl methanesulfonate (MMS): -S9; used at 5 nl/ml and 10 nl/ml.
- Methylcholanthrene (MCA): +S9 system; used at 2 µg/ml and 4 µg/ml as a positive control for assays performed with S9 metabolic activation.

Incubation and sampling times: 4 hrs exposure to test article

Study design/ definitive study:
Two independent trials were conducted, each + or – S9 (Aroclor 1254-treated rat liver).

System	Concentrations of CCI-779 (µg/mL)
-S9	Trial 1: 7.5, 10, 15, 20, 25, 30 Trial 2: 10, 15, 20, 25, 30, 40
+S9	Trial 1: 7.5, 10, 15, 20, 25, 30 Trial 2: 10, 15, 20, 25, 30, 40, 50

- Concentrations were selected to include non-toxic to highly toxic conditions.
- Exposure to test article was ~4 hrs.
- Cells were grown for 2 days post-treatment, to allow for recovery, and expression of mutants. Then selected by TFT (selection of mutants) and counted (cloning efficiency)
- One culture was used for each test article concentration
- The mutant frequency: the ratio of the total number of mutant colonies divided by the total number of cells seeded.
- Measurement of toxicity/percent relative growth: the relative suspension growth of cells over the 2-day expression period x the relative cloning efficiency at the time of selection
- Cloning efficiency of the vehicle and positive controls: total number of viable colonies/600 (total # of cells seeded) x 100.

Assay acceptance criteria (selected criteria):

- The average absolute cloning efficiency of the vehicle controls should be between 60% and 130%.
- Assays for the vehicle controls should not be outside of the normal range of background frequencies.
- The minimum value for the average suspension growth of the vehicle controls for two days is an 8.0 fold increase from the original cell numbers.
- The minimum acceptable mutant frequency induced by MMS (-S9) and MCA should be met (200×100^{-6}).
- For test articles with weak or no mutagenic activity, an assay should include concentrations that reduce the relative growth to 10% to 20% of the average

vehicle controls or reach the maximum recommended concentration (5000 µg/mL).

- A mutant frequency obtained for the test article will be considered acceptable for evaluation only if the relative cloning efficiency exceeds 30%. Thus, among the set of three cloning efficiency dishes, at least 180 colonies must be obtained.
- Mutant frequencies are normally derived from sets of three dishes for both the mutant colony count and the viable colony count. In order to allow for contamination losses, an acceptable mutant frequency was allowed to be calculated from a minimum of two dishes per set.

Assay evaluation criteria (selected criteria):

- The minimum criterion considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that is ≥ 2 times the concurrent background frequency. The background frequency is defined as the average mutant frequency of the vehicle control cultures. However, in order to reach a conclusion on test article-related mutagenicity, the following criteria should also be met:
  - Effect should be dose-related or toxicity-related.
  - Effect should be confirmed in a second assay.
  - Treatments that induce less than 10% relative growth are not used as sufficient evidence for mutagenicity.
- A test article is evaluated as non-mutagenic in a single assay only if the minimum increase in mutant frequency is not observed for a range of applied concentrations that extends to toxicity causing 10% to 20% relative growth or, in the case of relatively nontoxic materials, a range of applied concentrations extending to the maximum of 5000 pg/ml on to at least twice the solubility limit in medium.

Results

Study validity:

- Two independent trials were performed.
- Criteria for positive and negative assays were met.
- Adequate cytotoxicity/relative growth was observed at the highest concentrations in each trial.

Study outcome:

			Assay No. 1				
Metabolic Activation	Test Article	Concentration	Suspension Growth	Total Mutant Colonies	CE	Relative Growth[a] (%)	Mutant Frequency[b] In 10^{-6} Units
Without Activation	DMSO (vehicle control)	1%	20.8 (100% a)	203	93.4 (100% a)	100	73.6
	Methyl methanesulfonate	5 nL/mL	15.9	964	50.8	41.6	632.1[c]
		10 nL/mL	7.5	559	9.3	3.6	1996.4[c]
			% of VC		% of VC		
	Temsirolimus	7.50 µg/mL	50.4	184	105.8	53.3	62.1
		10.0 µg/mL	60.1	137	81.9	49.2	59.7
		15.0 µg/mL	40.3	159	71.7	28.9	79.1
		20.0 µg/mL	27.5	257	92.6	25.5	99.0
		25.0 µg/mL	20.6	188	99.2	20.5	67.6
		30.0 µg/mL	8.2	275	82.8	6.8	118.5

			Assay No. 1 (Cont'd)				
Metabolic Activation	Test Article	Concentration	Suspension Growth	Total Mutant Colonies	CE	Relative Growth[a] (%)	Mutant Frequency[b] In 10^{-6} Units
With Activation	DMSO (vehicle control)	1%	11.2 (100%)	222	107.1 (100%)	100	69.8
	Methylcholanthrene	2 µg/mL	8.6	813	52.3	37.5	517.8[d]
		4 µg/mL	2.8	815	23.7	5.5	1145.1[d]
			% of VC		% of VC		
	Temsirolimus	7.50 µg/mL	78.4	212	82.0	64.3	80.5
		10.0 µg/mL	59.7	253	91.2	54.4	86.3
		15.0 µg/mL	65.0	174	71.4	46.4	75.8
		20.0 µg/mL	42.0	176	72.7	30.5	75.4
		25.0 µg/mL	21.1	215	66.6	14.1	100.5
		30.0 µg/mL	20.8	212	88.2	18.4	74.8
			Assay No. 2				
Without Activation	DMSO (vehicle control)	1%	11.1 (100%)	153	78.1 (100%)	100	66.2
	Methyl methanesulfonate	5 nL/mL	15.8	562	55.7	101.5	336.5[c]
		10 nL/mL	9.9	790	54.3	62.0	484.7[c]
			% of VC		% of VC		
	Temsirolimus	10.0 µg/mL	53.3	112	92.6	49.4	51.6
		15.0 µg/mL	75.7	119	105.6	79.9	48.1
		20.0 µg/mL	58.3	117	94.8	55.5	52.7
		25.0 µg/mL	55.0	162	102.9	56.6	67.2
		30.0 µg/mL	64.4	114	77.5	49.9	62.8
		40.0 µg/mL	47.2	124	81.5	38.5	64.9

			Assay No. 2 (cont'd)				
Metabolic Activation	Test Article	Concentration	Suspension Growth	Total Mutant Colonies	CE	Relative Growth[a] (%)	Mutant Frequency[b] (x 10^{-6})
With Activation	DMSO (vehicle control)	1%	11.0 (100%)	267	78.5 (100%)	100	119.1
	Methylcholanthrene	2 µg/mL	7.9	924	43.0	39.3	716.3[f]
		4 µg/mL	4.9	995	40.8	23.2	812.2[f]
			% of VC		% of VC		
	Temsirolimus	10.0 µg/mL	89.5	166	94.1	75.8	74.9
		15.0 µg/mL	67.8	155	76.2	51.7	86.4
		20.0 µg/mL	64.6	124	76.9	49.7	68.5
		25.0 µg/mL	58.3	114	69.0	40.2	70.2
		30.0 µg/mL	58.7	141	70.7	41.5	84.7
		40.0 µg/mL	22.7	178	72.2	16.4	104.7
		50.0 µg/mL	12.4	295	84.5	10.5	148.2

a. Relative growth = (Relative suspension growth x relative cloning efficiency)/100.
b. Mutant Frequency = (Total mutant colonies/Total viable colonies) x 2 x 10^{-4}. Expressed as units of 10^{-6}.
c. Exceeds criterion of 147.2 x 10^{-6} for a positive response.
d. Exceeds criterion of 139.6 x 10^{-6} for a positive response.
e. Exceeds criterion of 132.4 x 10^{-6} for a positive response.
f. Exceeds criterion of 238.1 x 10^{-6} for a positive response.

CE = Cloning Efficiency (Total viable colony count/number of cells seeded x 100); DMSO = Dimethylsulfoxide; GLP = Good Laboratory Practice; GTR = General Technical Report; VC = Vehicle control.

Tables excerpted from the package.

Conclusions:

Under the conditions tested, temsirolimus was not mutagenic in the TK +/- mouse lymphoma forward mutation assay.

-S9 system: a slight (18%) increase in the mutation frequency, reported in the first trial for the –S9 system, was seen at the highest CCI-779 concentration (30 µg/mL) which resulted in high toxicity (7% relative growth). In addition, this effect was not observed in the second trial at that concentration.

+S9 system: a small (50%) increase in the mutation frequency reported was seen at the highest concentration of CCI-779 (50 µg/mL) which resulted in 10% relative growth.

Study title: Mutagenicity Test on CCI-779 in the In Vivo Mouse Micronucleus Assay

Key findings: Temsirolimus was negative in the in vivo micronucleus assay.

Report no.: GTR-30826

Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]

Date of study initiation: May 2, 1997
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779
Lot # 0M7612, TIM: 9700006

Methods
An assessment of micronuclei formation in bone marrow polychromatic erythrocytes of CD-1 mice

Species: CD-1 mice
8 weeks

Doses used in definitive study: 25, 50 and 100 mg/kg (i.v. dosing; single dose)

Basis of dose selection: preliminary study at CCI-779 doses of 2, 4, 10, 40, 80 and 100 mg/kg. MTD was defined as ~100 mg/kg. 100 mg/kg was also the maximum feasible dose based on the formulation.

Negative controls: CCI-779 diluent (polysorbate80, polyethylene glycol 400, and water)

Positive controls: cyclophosphamide

Study Design:
Single dose treatment

- Ten animals (5 ♂s and 5 ♀s) were randomly assigned to each dose level/harvest time-point group.
- The animals dosed with the test article and the vehicle control were euthanized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow. The animals dosed with the positive control were euthanized approximately 24 hours after dosing for extraction of the bone marrow.
- Five animals/sex/dose were sacrificed at each harvest timepoint. Bone marrow was extracted from the hind limb bones each animal and slides were prepared and stained. One thousand polychromatic erythrocytes (PCEs) were examined from each animal for the presence of micronuclei. The number of normochromatic erythrocytes (NCEs) and PCEs in at least 1000 erythrocytes was also determined.

Target Treatment (mg/kg)	Route of Admini-stration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint 24 Hour M	F	48 Hour M	F	72 Hour M	F
CCI-779								
25.0	IV	40	5	5	6*	5	5	6*
50.0	IV	40	5	5	5	5	5	5
100	IV	40	5	5	5	5	5	5
Vehicle Control, CCI-779 diluent	IV	40	5	5	5	5	5	5
Positive Control, Cyclophosphamide, 80.0	PO	10	5	5	-	-	-	-

* An additional animal was dosed to replace those that died during or shortly after dosing.

Table excerpted from the package.

Results

Study outcome:

- Exposure to CCI-779 was confirmed by increases in the PCE:NCE ratio in bone marrow and signs of toxicity. Clinical signs of toxicity consisted of hypoactivity, labored breathing, and postration.
- One HD ♀ died 20 hrs post-dose.
- A statistically non-significant increase in the percentage of micronucleated PCEs was observed in HD ♂s at 72 hrs harvest.

Time After Dosing (hours)	Target Dose Level (mg/kg)	Observations
CCI-779		
≈2.5	25.0	Male # 2383[2] died during dosing. Female # 2422[3] died immediately after dosing. These animals were replaced. Males[2,3] hypoactive, # 2371[2] also squinted eyes. Remaining animals normal.
	50.0	Normal.
	100	Males[2,3] slightly hypoactive, except # 2355[2] normal, # 2324[3] hypoactive, squinted eyes. Females[2] & #s 2430[1], 2464[3] slightly hypoactive. Female # 2429[3] hypoactive, squinted eyes, # 2444[3] squinted eyes, prostrate, labored breathing. Remaining animals normal.
≈3.6	25.0	Males[2], except # 2371[2], slightly hypoactive. Male # 2371[2] squinted eyes, hypoactive. Remaining animals normal.
	50.0	Normal.
	100	Male #s 2349[2], 2370[2], female # 2464[3] slightly hypoactive. Male # 2372[2], female # 2430[1] slightly hypoactive, squinted eyes. Male # 2324[3], female # 2429[3] hypoactive, squinted eyes. Female # 2444[3] squinted eyes, prostrate, labored breathing.

[1,2,3] 24, 48 or 72 hour harvest timepoint, respectively.

Table excerpted from the package.

Test Article	Dosage (mg/kg)	No. of Animals	Harvest Time	----------% Micronucleated PCEs---------- Mean of 1000/Animal ± SE Males	Females	Total	--------Ratio PCE:NCE-------- Mean ± SE Males	Females
Vehicle	--	5M, 5F	24 hours	0.10 ± 0.03	0.24 ± 0.10	0.17 ± 0.06	0.68 ± 0.03	0.68 ± 0.09
		5M, 5F	48 hours	0.02 ± 0.02	0.18 ± 0.11	0.10 ± 0.06	0.80 ± 0.06	0.84 ± 0.05
		5M, 5F	72 hours	0.06 ± 0.02	0.04 ± 0.04	0.05 ± 0.02	0.78 ± 0.05	0.62 ± 0.12
Cyclophosphamide	80.0	5M, 5F	24 hours	3.42 ± 0.58**	2.88 ± 0.32**	3.15 ± 0.33**	0.73 ± 0.07	0.57 ± 0.02

Test Article	Dosage (mg/kg)	No. of Animals	Harvest Time	----------% Micronucleated PCEs---------- Mean of 1000/Animal ± SE Males	Females	Total	--------Ratio PCE:NCE-------- Mean ± SE Males	Females
Temsirolimus	25.0	5M, 5F	24 hours	0.08 ± 0.02	0.24 ± 0.07	0.16 ± 0.05	0.84 ± 0.06	0.71 ± 0.05
		5M, 5F	48 hours	0.14 ± 0.06	0.14 ± 0.06	0.14 ± 0.04	0.70 ± 0.05	0.72 ± 0.04
		5M, 5F	72 hours	0.12 ± 0.06	0.02 ± 0.02	0.07 ± 0.03	0.84 ± 0.05	0.69 ± 0.07
	50.0	5M, 5F	24 hours	0.10 ± 0.04	0.08 ± 0.08	0.09 ± 0.04	0.80 ± 0.03	0.77 ± 0.07
		5M, 5F	48 hours	0.12 ± 0.04	0.16 ± 0.07	0.14 ± 0.04	0.64 ± 0.05	0.81 ± 0.06
		5M, 5F	72 hours	0.10 ± 0.03	0.02 ± 0.02	0.06 ± 0.02	0.78 ± 0.02	0.58 ± 0.07
	100	5M, 5F	24 hours	0.10 ± 0.05	0.16 ± 0.02	0.13 ± 0.03	0.78 ± 0.08	0.64 ± 0.10
		5M, 5F	48 hours	0.16 ± 0.05	0.14 ± 0.02	0.15 ± 0.03	0.67 ± 0.07	0.67 ± 0.04
		5M, 5F	72 hours	0.26 ± 0.07	0.03 ± 0.03	0.16 ± 0.06	0.55 ± 0.07*	0.72 ± 0.03

Statistical significance was achieved by analysis of variance or Dunnett's t-test.
* Significantly different from the vehicle control, $p \leq 0.05$. ** Significantly different from the vehicle control, $p \leq 0.01$.

GLP = Good Laboratory Practice; GTR = General Technical Report; NCE = Normochromatic erythrocyte; PCE = Polychromatic erythrocyte; SE = Standard error.

Conclusions:
The micronucleus test is considered negative. A non-statistically significant increase in the micronucleated PCE was observed in HD ♂s at the 72-hr harvest. The significance of this finding is uncertain.

2.6.6.5 Carcinogenicity

no study was conducted with temsirolimus. However, based on the information available on sirolimus (the active metabolite of temsirolimus present in large amounts in humans), temsirolimus should be considered a potential human carcinogen.

Based on the labeling for sirolimus:

> "Carcinogenicity studies were conducted in mice and rats. In an 86-week female mouse study at dosages of 0, 12.5, 25 and 50/6 (dosage lowered from 50 to 6 mg/kg/day at week 31 due to infection secondary to immunosuppression) there was a statistically significant increase in malignant lymphoma at all dose levels (approximately 16 to 135 times the clinical doses adjusted for body surface area) compared with controls. In a second mouse study at dosages of 0, 1, 3 and 6 mg/kg (approximately 3 to 16 times the clinical dose adjusted for body surface area), hepatocellular adenoma and carcinoma (males), were considered Rapamune related. In the 104-week rat study at dosages of 0, 0.05, 0.1, and 0.2 mg/kg/day (approximately 0.4 to 1 times the clinical dose adjusted for body surface area), there was a statistically significant increased incidence of testicular adenoma in the 0.2 mg/kg/day group."

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: CCI-779: oral (gavage) fertility study in male rats

Key study findings: Administration of temsirolimus to male rats for a period of ~70 days prior to co-habitation and 14 days during co-habitation resulted in ↓fertility as indicated by the fecundity index. The following effects in male reproductive organs and fertility parameters were observed:

- ↓Weight of cauda epididymis at MD and HD
- Small epididymis and testes at HD
- Epididymides intratubular cellular debris, and oligospermia/aspermia at HD
- Testicular seminiferous epithelium degeneration at HD
- ↓Sperm count at HD
- ↓Sperm motility at MD and HD
- ↓Fecundity index (# pregnancy: # mated) at HD

Study no.: RPT-46442
Volume #, and page #: module 4
Conducting laboratory and location: [redacted]
Date of study initiation: March 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Batch/Lot # RA0622; [redacted] pure

Methods

Doses: 0.05, 0.1, 0.5 mg/kg/day x 70 days
0.3, 0.6, 3 mg/m2/day x 70
Administered prior to cohabitation with untreated ♀s, during cohabitation, and until necropsy
Vehicle: water, polysorbate 80, phosal 50 PG, and 0.7% ethanol

Group No./ Identification	Dose Level (mg/kg/day)	Concentration[a] (mg/mL)
1 Vehicle control	0.00	0.00
2 CCI-779	0.05	0.01
3 CCI-779	0.10	0.02
4 CCI-779	0.50	0.10

[a] Dose Volume: 5 mL/kg/day

Table excerpted from the package.

Formulation analysis: samples had concentrations of the test article within the accepted range of ±10% of the nominal values. The following exceptions were reported:

- The 0.2 and 0.02 mg/mL MD stock and dose formulations during Week 6 were~76-77% of the nominal conc.

- The 1 and 0.01 mg/mL LD stock and dose formulations during Week 8 were ~158-167% of the nominal conc.
- The 1 and 0.1 mg/mL HD stock and dose formulations during Week 14 were ~79-82% of the nominal conc.

Species/strain: SD rats
Age: 45-71 days at initiation
Number/sex/group: 25/group
Route, formulation, volume: oral gavage, suspension, 5 mL/kg
Satellite groups used for toxicokinetics: not done/not reported
Study design:

- Only ♂s were dosed.
- ♂s were dosed for 10 weeks prior to co-habitation through 2 weeks after mating period.
- Day of C-section: GD14

Parameters and endpoints evaluated:
♂: mortality, clinical signs, BW, food consumption, male reproductive assessments:

- Spermatozoa motility and count
- Histopathology of testes, epididymides, seminal vesicles, and prostate
- Organ weight of reproductive organs: epididymides, prostate, seminal vesicles, testes
- Mating performance: mean days to mating and mating index (% ♂s that mated)
- Fecundity and fertility index

$$\text{Mating index (\%)} = \frac{\text{No. of males mating}}{\text{No. of males placed for mating}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. of males producing a pregnancy}}{\text{No. of males placed for mating}} \times 100$$

$$\text{Fecundity index (\%)} = \frac{\text{No. of males producing a pregnancy}}{\text{No. of copulating males}} \times 100$$

♀: mortality, clinical signs, BW, food consumption.

- Uterine content. On GD14, the mated ♀s were sacrificed and the uterine content were examined for status of implantations
- Corpora lutea count.

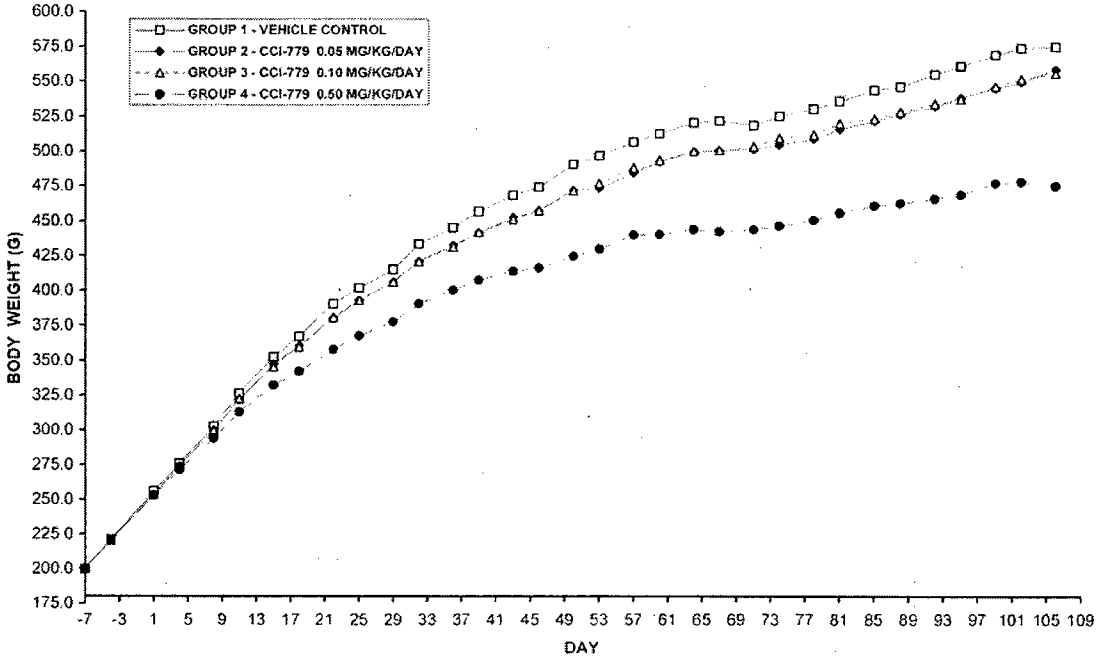
Results
Toxicokinetics: no data

Mortality: no test article-related mortality

Clinical signs: no test article-related effect

Body weight:
♂:

- ↓14% at MD on Day 71 (pre-mating)
- ↓17% at HD on Day 102 (co-habitation)



Graph excerpted from the package.

Food consumption: no toxicologically significant effect

Fertility parameters:
Findings in ♀s:

- There was no toxicologically significant effect on uterine parameters for females that were pregnant: minimally ↓mean # of corpora lutea and implantations were observed at the HD

Findings in ♂s:

- Organ weight: ↓weight of cauda epididymis at MD (↓8%) and HD (↓18%, statistically significant)
- Gross pathology: small epididymis (1/25 HD) and small testes (1/25 HD)
- Histopathology:
  - Epididymides: intratubular cellular debris (1/15 HD), oligospermia/aspermia (1/25 HD)
  - Testes: seminiferous epithelium degeneration (2/25 HD)
- Sperm evaluation: ↓spermatozoa (per gram of epididymis) at HD (~↓19%); ↓motility at MD (↓9%) and HD (↓16%, statistically significant)
- ↓Fecundity index at HD (↓8%)

	# placed for mating		# mating	# pregnant ♀s	Mating index	Fertility index	Fecundity index
	♂	♀					
Control	25	25	24	23	96%	92% (23/25)	95.8% (23/24)
LD	25	25	25	25	100%	100% (25/25)	100% (25/25)
MD	25	25	25	24	100%	96% (24/25)	96% (24/25)
HD	24	24	24	21	100%	87.5% (21/24)	87.5% (21/24)

Dosage (mg/kg)	—0 (Control)—	———0.05———	———0.1———	———0.5———
Treated F_0 Males				
Number Evaluated	25	25	25	25
Number Died or Sacrificed Moribund	0	1[a]	0	2[b]
Clinical Observations	–	–	–	–
Necropsy Observations				
Organ Weights				
Cauda Epididymis	0.279 g	-3	-8	-18**
Macroscopic Pathology				
Epididymides				
Small	0	0	0	1
Testes				
Small	0	0	0	1
Number Evaluated	25	25	25	25
Histopathology				
Epididymides				
Intratubular cellular debris	0	0	0	1
Oligo/aspermia	0	0	0	1
Testes				
Seminiferous epithelium degeneration	0	0	0	2
Sperm Evaluation				
Spermatozoa/gram (10^6)	601.61	609.38	605.50	487.22
Motility (%)	76.8	74.1	69.3	64.5*
Body Weight (%)[c]				
Day 1 (Premating)	256.0 g	-1	-1	-1
Day 71 (Premating)	518.6 g	-3	-3	-14**
Day 102 (Cohabitation)	573.8 g	-4	-4	-17**
Food Consumption (%)	–	–	–	–
Mean Time to Mating (days)	3.3	2.8	2.4	2.6
Mating Index (% of males that mated)	96	100	100	100
Fecundity Index (% of mated males that produced a pregnancy)	96	100	96	88

Dosage (mg/kg)	—0 (Control)—	———0.05———	———0.1———	———0.5———
Untreated F_0 Females				
Number of Pregnant Females	23	25	24	21
Mean Gestation Weight (%)	–	–	–	–
Mean Gestation Food Consumption (%)	–	–	–	–
Uterine Contents				
Mean No. Corpora Lutea	18.3	18.0	17.5	17.3
Mean No. of Implantations	16.7	16.2	16.5	15.8
Mean % Pre-implantation Loss	8.3	9.2	5.8	8.6
Litters from Untreated F_0 Females				
Number of Litters Evaluated	23	25	24	21
Mean No. Live Conceptuses	15.7	15.4	15.5	15.1
Mean No. Early Resorptions	1.0	0.8	1.0	0.6
Mean No. Dead Embryos	0	0	0	0
Mean % Post-plantation Loss	5.9	5.1	6.0	3.8

** $p \leq 0.01$ (statistical significance was determined by Dunnett's test).
– No noteworthy finding.

a. Cause of death undetermined; not considered to be compound related.
b. Euthanized because of fractured tibia and deteriorating condition attributed to gavage accident; not considered to be compound related.
c. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

GD = Gestation day; GLP = Good Laboratory Practice; NA = Not applicable; RPT = Report.

Table excerpted from the package.

Conclusions:
SD ♂ rats were dosed with temsirolimus at 0.05, 0.1, and 0.5 mg/kg/day. Administration was 10 weeks prior to co-habitation through 2 weeks after mating period with untreated ♀s, and until the day prior to necropsy. Males were sacrificed approximately 2 weeks after the mating period. Females were sacrificed on GD14. No toxicologically significant effect was observed in ♀s.

In ♂ rats, fertility parameters were affected at oral doses ≥ 0.6 mg/m2/day (approximately 0.3 fold the human recommended intravenous dose on the mg/m2/week basis). Of note, based on the summary of absorption studies, the oral bioavailability was low and estimated to be only 5% in rats, in 4-cycle toxicity studies. The following effects in reproductive organs and fertility parameters were observed:

- ↓Weight of cauda epididymis at MD and HD
- Small epididymis and testes at HD
- Epididymides intratubular cellular debris, and oligospermia/aspermia at HD
- Testicular seminiferous epithelium degeneration at HD
- ↓Sperm count at HD
- ↓Sperm motility at MD and HD
- ↓Fecundity index at HD

Since TK parameters are not available, a comparison to human based on exposure levels cannot be made. The above comparison was made based on the weekly dose, adjusted to the body surface area.

Study title: CCI-779: oral gavage fertility study in female rats

Key study findings: Treatment of ♀ rats with temsirolimus at doses up to 1 mg/kg or 6 mg/m2 (2 weeks prior to co-habitation through GD6) did not affect the estrous cycle, mating index, fertility index, or the conception rate. Uterine and fetal effects were evident at HD and consisted of the following:
Uterine findings:

- ↑pre-implantation loss resulting in ↓number of implantation site per litter
- ↑embryo-fetal resorption resulting in ↑post-implantation loss and ↓number of live embryos per litter

Embryo-fetal finding:

- ↓weight

Report no.: RPT-45217
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]
Date of study initiation: February 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Batch/Lot# RA0622, [redacted] pure

Methods

Doses: 0.1, 0.3, 1 mg/kg/day
0.6, 1.8, 6 mg/m2/day
Vehicle: 0.7% ethanol, 0.2% polysorbate 80, 9.1% phosal 50 PG, water

Group No./ Identification	Dose Level (mg/kg/day)	Concentration[a] (mg/mL)
1 Vehicle control	0.00	0.00
2 CCI-779	0.10	0.01
3 CCI-779	0.30	0.03
4 CCI-779	1.00	0.10

[a] Dose Volume: 10 mL/kg/day

Table excerpted from the package.

Formulation analysis: concentrations of CCI-779 were within the accepted range of ± [redacted], with the following exception: 0.1 and 0.01 mg/mL LD stock and dose formulations for use during Week 1 of the study were [redacted] of the nominal (initial samples) or [redacted] of the nominal concentrations.

Species/strain: SD rats
Initial age: 60-65 days
Number/sex/group: 25/group
Route, formulation, volume: oral gavage, suspension, 10 mL/kg
Satellite groups used for toxicokinetics: not done/not reported
Study design:

- ♂s were untreated.
- Females were treated 14 days prior to co-habitation through GD6
- C-section: GD20

Parameters and endpoints evaluated: mortality, clinical signs, BW, food consumption, estrous cycle, gravid uterus weight, ovarian/uterine data (status of implantation, number of corpora lutea, and number of live fetuses), fertility parameters (mating index, fertility index, and conception rate). Fetuses were evaluated for: external findings, sex, and weight. Placentas were examined for gross appearance.

$$\text{Mating index (\%)} = \frac{\text{No of females copulating}}{\text{No of females cohabited with males}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. of pregnant females}}{\text{No. of females placed for mating}} \times 100$$

$$\text{Conception rate (\%)} = \frac{\text{No. of pregnant females}}{\text{No. of copulating females}} \times 100$$

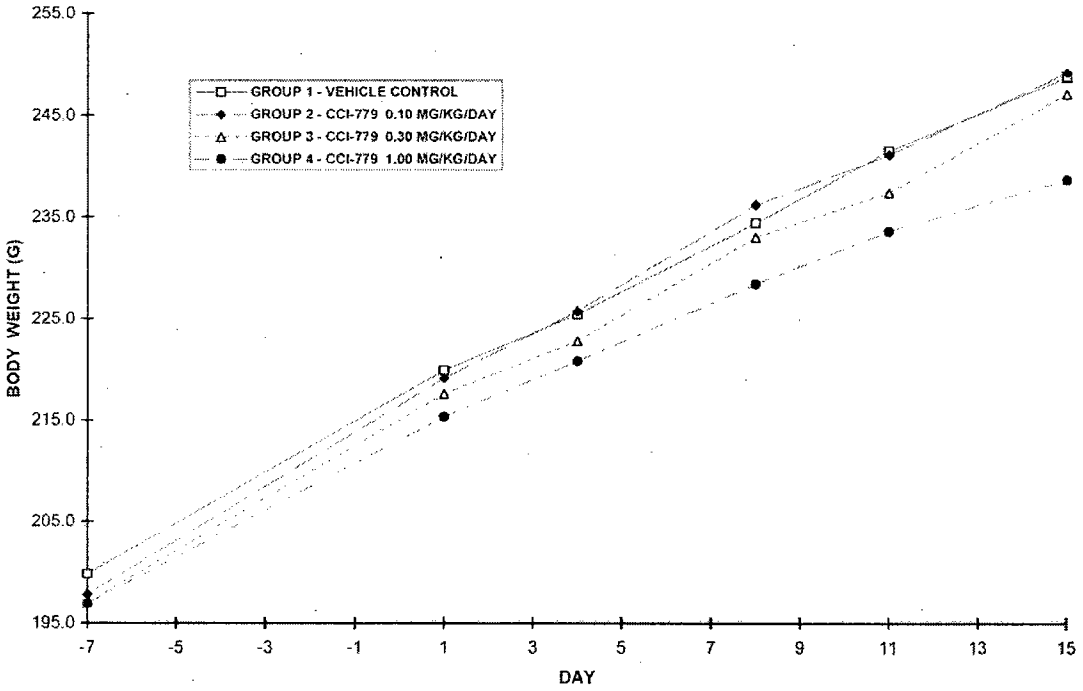
Results

Mortality: none

Clinical signs: no drug-related effect

Body weight:

- ↓BW gain of 19% at HD during the 2-week pre-mating period.
- ↓BW or BW gain during the gestation period (GD 0-20) was mainly secondary to the ↓gravid weight of the uterus. When corrected for the uterus weight, changes were minimal.



Graph excerpted from the package.

Food consumption: no drug-related effect

Toxicokinetics: not done/not reported

Necropsy: no clear drug-related effect.

Fertility and reproductive parameters:
No effect was observed for estrous cycle, mating index, fertility index, or conception rate.

Uterine findings:

- HD: ↑pre-implantation loss (↑29%) contributed to the ↓number of implantation site per litter (↓8%)

- HD: ↑embryo-fetal resorption (↑~2.5-fold) resulted in ↑post-implantation loss (↑~2.5-fold) and ↓number of live embryos per litter (↓24%)
- LD: some effects (↓# of implantation sites, corpora lutea, and live embryos) were observed at the LD; however, since the MD did not show the same effects, it is not clear whether these findings were incidental or due to dosing errors.

Embryo-fetal finding:

- HD: ↓ weight (↓~13% for ♂ and ♀ fetuses combined)

	#placed for mating		# mating	Mean day to mating	#s pregnant	Mating index	Fertility index	Conception rate
	♂	♀						
C	25	25	25	22	24	100%	96%	96%
LD	25	25	25	24	25	100%	100%	100%
MD	25	25	25	22	25	100%	100%	100%
HD	25	25	25	21	25	100%	100%	100%

Dosage (mg/kg)	----0 (Control)----	--------0.1---------	--------0.3--------	--------1---------
Number of Females	25	25	25	25
Treated F_0 Females				
Number Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	--	--	--	--
Necropsy Observations	--	--	--	--
Premating Body Weight (%)[a]				
Premating Day 15	248.8 g	0	-1	-4
Premating Body-Weight Gain (%)[a]				
Day 1 to 15	29.0 g	3	1	-19*
Gestation Body Weight (%)[b]				
GD 20	402.8 g	-5	-3	-9**
GD 20 (corrected for gravid uterine weight)	319.2 g	-3	-3	-5
Gestation Body-Weight Gain (%)[b]				
GD 0 to 20 (corrected for gravid uterine weight)	68.1 g	-13	-7	-4
Premating Food Consumption (%)	--	--	--	--
Gestation Food Consumption (%)	--	--	--	--
Mean Cycle Length (days)	4.2	4.1	4.1	4.1
Mean Number Days in Estrus	4.1	4.0	3.9	3.8
Mean Number Days Prior to Mating	2.2	2.4	2.2	2.1
Number of Mating Females	25	25	25	25
Number of Pregnant Females	24	25	25	25
Uterine Findings				
Number Evaluated	24	24	25	25
Mean Number Corpora Lutea	18.7	16.3**	18.0	17.9
Mean Number Implantation Sites	16.3	14.1**	15.5	15.0*
Mean % Pre-implantation Loss	12.3	13.5	13.4	15.9
Mean Number Early Resorptions	0.7	0.9	0.7	2.6**
Mean Number Late Resorptions	0.0	0.0	0.0	0.1
Mean Gravid Uterus Weight (g)	83.5	73.0	80.3	62.8
Litters from Treated F_0 Females				
Number of Litters Evaluated	24	24	25	25
Mean Number Live Embryos	15.5	13.2**	14.8	12.2**
Mean Number Total Resorptions	0.8	0.9	0.7	2.8**
Mean Number Dead Embryos	0.7	0.9	0.7	2.6**
Mean % Post-implantation Loss	5.2	7.2	4.2	18.8**

Mean Fetal Weight (g)				
Males	3.57	3.58	3.55	3.04**
Females	3.36	3.46	3.35	2.95**
Fetal Sex Ratio (% Male)	49.5	50.6	47.9	46.3
Fetal Anomalies[b]				
Gross External				
Number Litters Fetuses Examined	24/371	24/317	25/370	25/305
Noteworthy Findings	--	--	--	--

*p ≤ 0.05, ** p ≤ 0.01 (statistical significance was determined by Dunnett's or Wilcoxon tests).
-- No noteworthy finding.

a. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
b. Only compound-related noteworthy findings are listed.

GD = Gestation day; GLP = Good Laboratory Practice; NA = Not applicable; RPT = Report.

Table excerpted from the package.

Conclusions:
Female SD rats were treated p.o., daily, with temsirolimus 2 weeks prior to co-habitation through GD 6 and C-sectioned on GD 20. Temsirolimus doses were 0.1, 0.3, and 1 mg/kg/day.

Treatment with temsirolimus at doses up to 1 mg/kg or 6 mg/m2 did not affect the estrous cycle, mating index, fertility index, or the conception rate. Negative results on these fertility parameters may be due to insufficient exposure, since no maternal toxicity was observed. No TK data is available to evaluate maternal drug exposure.

Effects in female rats were observed at HD (6 mg/m2/day or 42 mg/m2/week). This dose is approximately 3-fold the human recommended intravenous dose of 25 mg/week. Toxicokinetic data were not available for the oral study reviewed; however, based on the summary of absorption studies, the oral bioavailability was low and estimated to be only 5% in rats, in 4-cycle toxicity studies. Effects in this study consisted of uterine and embryo-fetal findings:

Uterine findings:
- ↑pre-implantation loss resulting in ↓number of implantation site per litter
- ↑embryo-fetal resorption resulting in ↑post-implantation loss and ↓number of live embryos per litter

Embryo-fetal finding:
- ↓weight

Since TK parameters are not available, a comparison to human based on exposure levels cannot be made. The above comparison was made based on the weekly dose, adjusted to the body surface area.

Embryofetal development

Study title: CCI-779: oral (gavage) developmental toxicity study in mated female rats

Key study findings: Temsirolimus administered to mated rats, p.o. daily, from GD 6-17, at doses up to 0.45 mg/kg, resulted in uterine and embryo-fetal toxicities at the high-dose.

The HD (0.45 mg/kg or 2.7 mg/m2) had an AUC_{0-24} of 70 ng.hr/mL, which is approximately 0.05-fold the mean AUC achieved in patients after multiple administration at the recommended dose of 25 mg i.v. Uterine and embryo-fetal toxicities consisted of:

- Uterine: ↑resorption, ↑post-implantation loss, ↓litter size.
- Embryo-fetal: ↓fetal weight, and ↓ossification of sternabrae and vertebral centra.

Study no.: [redacted] 98204
Report# RPT-45268
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]
Date of study initiation: Jan 10, 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779, Batch/Lot# RA0622, [redacted] pure

Methods

Doses: 0, 0.05, 0.15, and 0.45 mg/kg/day
0, 0.3, 0.9, and 2.7 mg/m2/day
Given on GD 6-17; sacrificed on GD 20
Vehicle: water, polysorbate 80, phosal 50 PG and ethanol (0.7%)

Group No./ Identification	Dose Level (mg/kg/day)	Concentration[a] (mg/mL)	Animal Numbers Females
1 Vehicle control	0.00	0.00	1501-1525
2 CCI-779	0.05	0.010	2501-2537[b]
3 CCI-779	0.15	0.030	3501-3537[b]
4 CCI-779	0.45	0.090	4501-4537[b]

[a] Dose Volume: 5 mL/kg/day

[b] Twelve per group were designated toxicokinetic animals

Table excerpted from the package.

Formulation analysis: all samples analyzed were within accepted range (±10%), with the exception of dosing formulation prepared for use in Week 1 at 0.01 mg/mL, which was between 88.7% and 89.6% of nominal.

Species/strain: SD rats
♀s: 77-84 days of age; 256-314 g
Number/sex/group: 25/group
Route, formulation, volume: oral, gavage, 5 mL/kg
Satellite groups used for toxicokinetics: additional (12/group) mated rats
Study design: mated ♀s were treated from GD 6 through 17 and sacrificed on GD 20.
Parameters and endpoints evaluated:

- Maternal: mortality, clinical signs, BW, food consumption, uterus weight and content (status of implantation).

- Embryo-fetal: gross appearance of placenta, fetal weight, detailed external examination, defining the sex, detailed internal examination on ~half the fetuses in each litter, skeletal examination for the remaining half, examination of fixed head

$$\text{Preimplantation loss (\%)} = \frac{\text{No. of corpora lutea} - \text{no. of implants}}{\text{No. of corpora lutea}} \times 100$$

$$\text{Post implantation loss (\%)} = \frac{\text{No. of implants} - \text{no. of live fetuses}}{\text{No. of implants}} \times 100$$

Toxicokinetics: blood samples were collected on GD 17 (a separate group consisting of 12 mated rats/group) for evaluation of CCI-779 and sirolimus. Collection time-points: 0.5, 1, 2, 4, 8, and 24 hrs.

Results

Toxicokinetics: samples were sent to Wyeth, PA for analysis of TK parameters. TK was presented in a separate report (# RPT-46563)

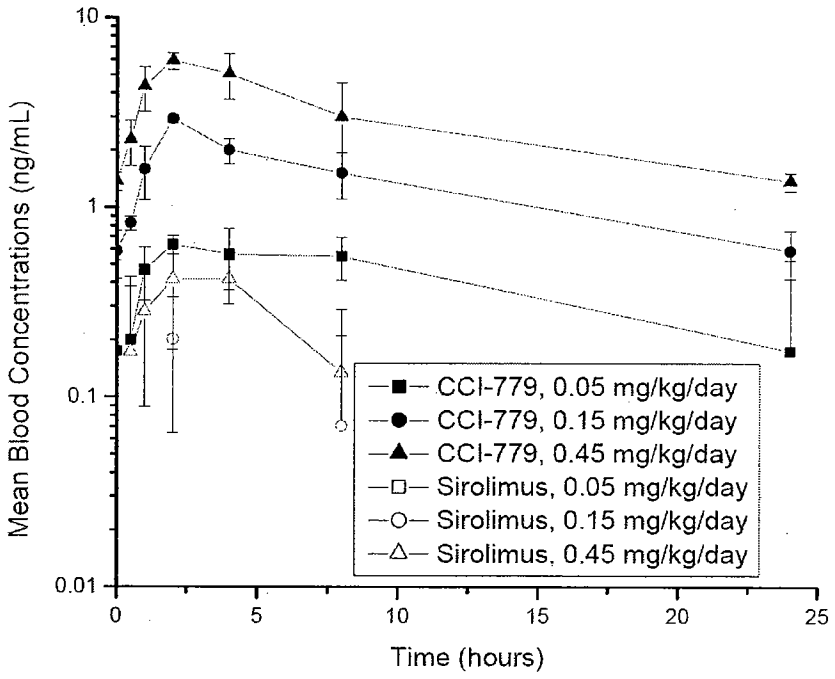
Blood levels of CCI-779 and sirolimus were determined using a validated LC/MS/MS assay

Analyte	CCI-779 Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{0-24}/D	$t_{1/2}$[a] (hours)
CCI-779	0.05	0.637 ±0.035	2.0	10.1 ±1.8	201 ±35	11.1
	0.15	2.93 ±0.07	2.0	32.0 ±2.4	213 ±16	11.4
	0.45	5.94 ±0.31	2.0	69.8 ±8.4	155 ±19	11.4
Sirolimus	0.05	0 ±0	0	0[b] ±0	0[b] ±0	ND
	0.15	0.201 ±0.068	2.0	1.01[b] ±0.71	6.71[b] ±4.75	ND
	0.45	0.418 ±0.054	4.0	3.61 ±0.87	8.01 ±1.93	ND

a: Determined using the mean concentrations in WinNonlin.

b: Data were estimated from the concentration profile: most concentrations were BQL therefore were not included in the statistical evaluation.

ND: Not determined.



Dam

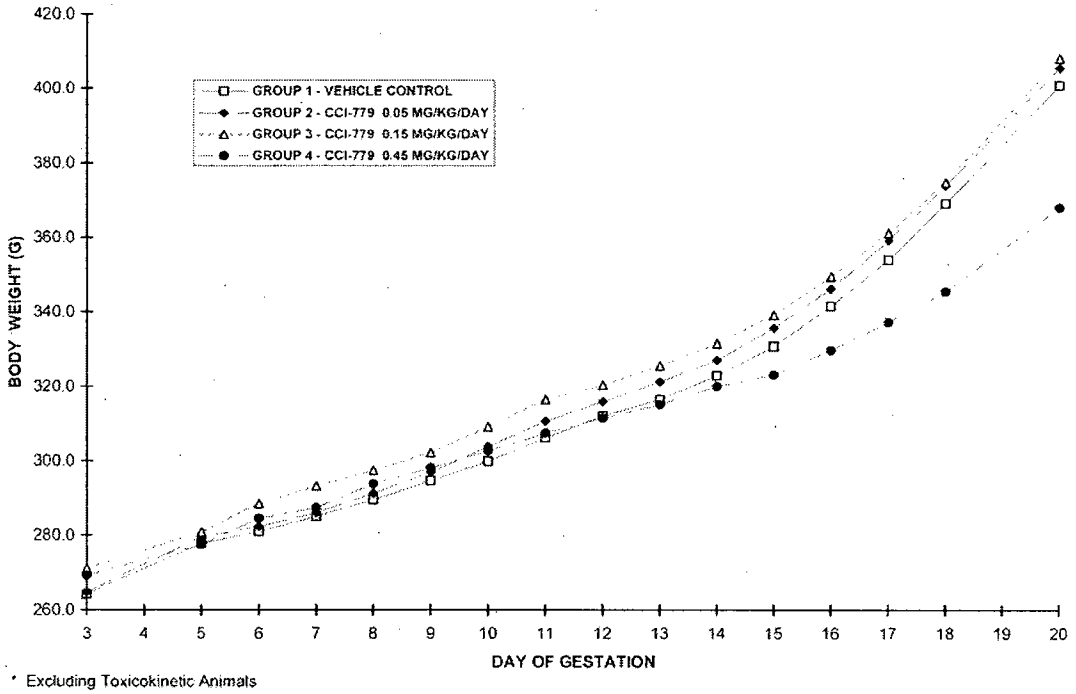
Mortality: no test article-related mortality. The death of 1 animal in the TK group was attributed to complications related to blood collection.

Clinical signs:

- Liquid red/brown vaginal discharge in 1 HD ♀. This ♀ had full litter resorption. Brown liquid/mucoid vaginal discharge was also seen in 2 HD TK ♀s.

Body weight:

- Statistically significant ↓BW gain of up to 30% was seen in HD ♀s. ↓BW gain started from GD 14.
- When corrected for the gravid uterine weight, BW gains were comparable for HD and control animals (overall BW gain: HD was 36 g and Control was 39 g), indicating that the ↓BW gain in HD ♀s were secondary to the ↓litter size and fetal weights.



Graph excerpted from the package.

Food consumption: no effect

Gross pathology: no drug-related finding

Uterine findings:

HD

- Minimally reduced pregnancy rate (24 out 25 mated rats were pregnant)
- ↑early resorption. One dam had total litter resorption
- ↑post-implantation loss
- ↓litter size due to increased resorption and post-implantation loss
- ↓Gravid uterine weight, due to reduced litter size and fetal weight

	Control	LD	MD	HD
# Rats mated	25	25	25	25
# Rats pregnant	25	25	25	24
Pregnancy rate	100%	100%	100%	96%
Total resorption	0	0	0	1
Gravid uterine weight (g)	80	82	78	50
Pre-implantation loss	11.9%	10.7%	11.5%	[A]13.5% [B]13.6%
Post-implantation loss	4.7%	4.7%	8.9%	[A] *42% [B] *40%

[A]: including the animal with total resorption.
[B]: excluding the animal with total resorption.
*: statistically significant.

Dosage (mg/kg)	----0 (Control)----	----------0.05----------	----------0.15----------	----------0.45----------
Uterine Findings				
Number Examined	25	25	25	23 (24)[c]
Mean Number Corpora Lutea	16.6	16.6	16.7	16.2 (15.9)[c]
Mean Number Implantations	14.5	14.8	14.7	13.9 (13.7)[c]
Mean % Preimplantation Loss	11.9	10.7	11.5	13.6 (13.5)[c]
Mean Gravid Uterine Weight (g)	80.3	82.3	77.6	50.4
Litters from Treated F_0 Females				
Number Litters Evaluated	25	25	25	23 (24)[c]
Mean Number Live Fetuses	13.8	14.1	13.4	8.7** (8.4**)[c]
Mean Number Resorptions	0.7	0.7	1.3	5.2** (5.3**)[c]
Mean Number Early Resorptions	0.7	0.7	1.3	5.1** (5.3**)[c]
Mean Number Middle Resorptions	0.0	0.0	0.0	0.0 (0.0)[c]
Mean Number Late Resorptions	0.0	0.0	0.0	0.1 (0.1)[c]
Mean Number of Dead Fetuses	0.0	0.0	0.0	0.0 (0.0)[c]
Mean % Postimplantation Loss	4.7	4.7	8.9	39.7** (42.2**)[c]

*p ≤ 0.05, ** p ≤ 0.01 (statistical significance was determined by Dunnett's or Fisher's Exact tests).

c. Value in parentheses includes dam with total resorption.

Table excerpted from the package.

Embryo-fetal

Fetal weight: the mean fetal weight was statistically significantly reduced at HD (♂s and ♀s)

Sex ratio:

MD: ↑percentage of ♂ fetuses in the litter (effect was non-dose-dependent)

Dosage (mg/kg)	----0 (Control)----	----------0.05----------	----------0.15----------	----------0.45----------
Litters from Treated F_0 Females (cont'd)				
Mean Fetal Weight (g)				
Males	3.84	3.90	3.85	3.70
Females	3.63	3.72	3.63	3.45
Combined males and females	3.73	3.80	3.75	3.53*
Fetal Sex Ratio (% males)	47.9	44.9	57.6*	44.4
Fetal Anomalies[d]				
Gross External				
Number Litter/Fetuses Examined	25/346	25/352	25/335	23/201
Noteworthy Findings	-	-	-	-

*p ≤ 0.05, ** p ≤ 0.01 (statistical significance was determined by Dunnett's or Fisher's Exact tests).
- No noteworthy findings + Noteworthy finding present.

d. Only compound-related noteworthy findings are listed.

Table excerpted from the package.

External and visceral findings: low incidence and/or non-dose-dependent findings were observed in all groups and were comparable to the historical control.

Skeletal findings:

↑incidence of common skeletal findings, i.e. ↓ossification of sternebrae and vertebral centra

	Control # examined		LD # examined		MD # examined		HD # examined	
	Litter	Fetuses	Litter	Fetuses	Litter	Fetuses	Litter	Fetuses
	25	174	25	175	25	165	23	103
Sternabrae: ↓ossification	7 (28%)	10 (5.7%)	9 (36%)	14 (8%)	11 (44%)	13 (7.8%)	12 (52%)	*19 (18%)
Vertebral centra: ↓ossification	20 (80%)	41 (23%)	10 (40%)	50 (28%)	17 (68%)	54 (33%)	22 (96%)	*59 (57%)

* Statistically significant

Conclusions:

Temsirolimus administered to mated rats, p.o. daily, from GD 6-17, at doses up to 0.45 mg/kg, did not cause clear maternal toxicity, as effects seemed to be secondary to the uterine and embryo-fetal toxicities. Statistically significant uterine and embryo-fetal toxicities were reported at the HD. The HD (0.45 mg/kg or 2.7 mg/m2) had an AUC_{0-24} of 70 ng.hr/mL, which is approximately 0.05-fold the mean AUC achieved in patients after multiple administrations at 25 mg i.v (the recommended dose).

- Maternal toxicity was secondary to the uterine and embryo-fetal toxicities: reduced BW at HD was due to the ↓gravid uterine weight and red/brown vaginal discharge in 1 HD ♀ may have been due to the total resorption in this animal.

- Uterine effects were observed at HD (0.45 mg/kg or 2.7 mg/m2) and consisted of: ↑resorption, ↑post-implantation loss, ↓litter size (due to resorption and post-implantation loss).

- Embryo-fetal effects were observed at HD and consisted of: ↓fetal weight, and ↓ossification of sternabrae and vertebral centra.

Table 3.1-1: Pooled Summary of Single- and Multiple-Dose Pharmacokinetic Parameters in Whole Blood of Healthy Subjects and Patients Following 25 mg Intravenous Temsirolimus

	Healthy Subjects	Patients	
Mean (SD)	Single-dose (N=51)	Single-dose (N=13)[a]	Multiple-dose (N=11)[b]
Temsirolimus			
C_{max} (ng/mL)	592.4 ± 101.9	585.4 ± 83.1	443.0 ± 109.2
$t_{1/2}$ (h)	17.7 ± 4.5	17.3 ± 5.9	NC
AUC (ng·h/mL)	2276 ± 340	1627 ± 425	1349 ±231.8
CL (L/h)	11.4 ± 2.4	16.2 ± 3.5	19.0 ± 3.0
V_{dss}	189.6 ± 55.2	172.3 ± 39.4	NC
Sirolimus			
C_{max} (ng/mL)	57.4 ± 14.3	55.4 ± 31.8	34.5 ± 19.3
$t_{1/2}$ (h)	73.3 ± 23.2	54.6 ± 1.5	NC
AUC (ng·h/mL)	5479 ± 1799	4151 ± 1600	3793 ± 1466
CL/f_m (L/h)	4.9 ±1.2	6.9 ± 2.6	7.4 ±2.5
Composite			
AUC_{ratio}[c]	2.44 ± 0.83	2.68 ± 1.22	2.98 ±1.47
AUC_{sum} (ng·h/mL)	7755 ± 1874	5778 ±1722	5141 ± 1345

Note: Temsirolimus was administered as a 30-minute infusion.
Abbreviations: NC = not calculated (no subjects provided data); SD = standard deviation.
a. For C_{max} N=5 and for $t_{1/2}$ and V_{dss} N=2.
b. For temsirolimus C_{max} N=7, sirolimus C_{max} N=3.
c. AUC ratio denotes quotient of sirolimus: temsirolimus AUCs

Table excerpted from the package.

Study title: CCI-779: oral (gavage) development toxicity study in mated female rabbits

Key study findings: Temsirolimus administered to mated rabbits, p.o. daily, GD 6-18, at doses up to 0.9 mg/kg, resulted in uterine toxicities starting from the LD and embryo-fetal toxicities starting from the HMD, both at sub-therapeutic exposures.

- Uterine: ↓mean gravid uterine weight, ↑late and total resorptions, ↑post-implantation loss, ↓litter size
- Embryo-fetal: ↓fetal weight, omphalacele, fused sternebrae, bifurcated sternabrae, ↑incidence of notched ribs/ incomplete ossification of pubic bone/ incomplete ossification of frontal bone

Report no.: RPT-45267
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]
Date of study initiation: Jan 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: CCI-779; Batch/Lot # RA0622; [redacted] pure

Methods
Doses: 0, 0.06, 0.2, 0.6, 0.9 mg/kg/day
0, 0.72, 2.4, 7.2, 10.8 mg/m2/day

C, LD, LMD, HMD, HD
Administered on GD 6-18 (sacrificed on GD 29)
Vehicle (control): water, polysorbate 80 (0.2%), phosal 50 PG (9.1%), ethanol (0.7%)

Group No./ Identification	Dose Level (mg/kg/day)	Concentration[a] (mg/mL)
1 Vehicle control	0.00	0.00
2 CCI-779	0.06	0.03
3 CCI-779	0.20	0.10
4 CCI-779	0.60	0.30
5 CCI-779	0.90	0.45

[a] Dose Volume: 2 mL/kg/day

Table excerpted from the package

Formulation analysis: samples were within ±10% of the nominal concentrations.

Species/strain: New Zealand White (SPF) rabbits
Number/sex/group: 20/group
Route, formulation, volume: oral, gavage, 2 mL/kg/day
Satellite groups used for toxicokinetics: 12/group
Study design: mated rabbits were dosed p.o. daily, from GD 6-18 and sacrificed on GD 29
Parameters and endpoints evaluated:
Maternal:

- Mortality and clinical signs
- Body weight and food consumption
- Weight of gravid uteri
- Corpora lutea count
- Uterine content (status of implantation)

Embryo-fetal:

- Gross appearance of placenta
- Number of live fetuses
- Sex of the fetus
- Fetal weight
- Visceral and skeletal abnormalities

Toxicokinetics: parameters for temsirolimus and sirolimus were evaluated.
Blood samples were collected on GD18 at the following time-points: pre-dose, 0.5, 1, 2, 4, 8, 12, and 24 hrs post-dose

Results

Toxicokinetics: samples were shipped to Wyeth, PA for analysis of TK parameters. TK was presented in a separate report (# RPT-46564)

Blood levels of CCI-779 and sirolimus were determined using a validated LC/MS/MS assay.

Analyte	CCI-779 Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{0-24}/D	$t_{1/2}$[a] (hours)
CCI-779	0.06	2.48 ±0.25	4.0	26.0 ±1.8	433 ±30	10.7
	0.2	8.24 ±0.98	2.0	76.8 ±6.3	384 ±31	12.2
	0.6	18.9 ±2.5	2.0	189 ±17	315[b] ±29	ND
	0.9	28.6 ±6.9	4.0	261 ±24	290[b,c] ±27	7.67
Sirolimus	0.06	1.26 ±0.90	4.0	10.6[d] ±5.5	NA	NC
	0.2	3.04 (n=2)	4.0	37.5 ±14.1	NA	NC
	0.6	7.84 ±0.37	2.0	93.5 ±23.9	NA	NC
	0.9	9.82 ±3.19	0.5	98.5 ±18.4	NA	NC

a: Determined from the mean concentrations using WinNonlin, ver. 2.1.
b: Significantly different from 0.06 mg/kg/day.
c: Significantly different from 0.2 mg/kg/day.
d: Data were estimated from the concentration profile with most sirolimus concentrations were BQL.
NA: Not applicable; CCI-779 was administered to the animals, thus the dosage of sirolimus that animals actually received was not available.
NC: Not calculated; since there were detectable blood CCI-779 concentrations throughout the 24 hour sampling period (Tables 1, 3, 5 and 7), the presence of sirolimus in blood reflected both its conversion from CCI-779 and its elimination from the systemic circulation, the apparent terminal half-life ($t_{1/2}$) of sirolimus was not calculated.
ND: Not determined because the blood concentration-time profiles did not support the definition of the terminal phase by at least 3 data points.

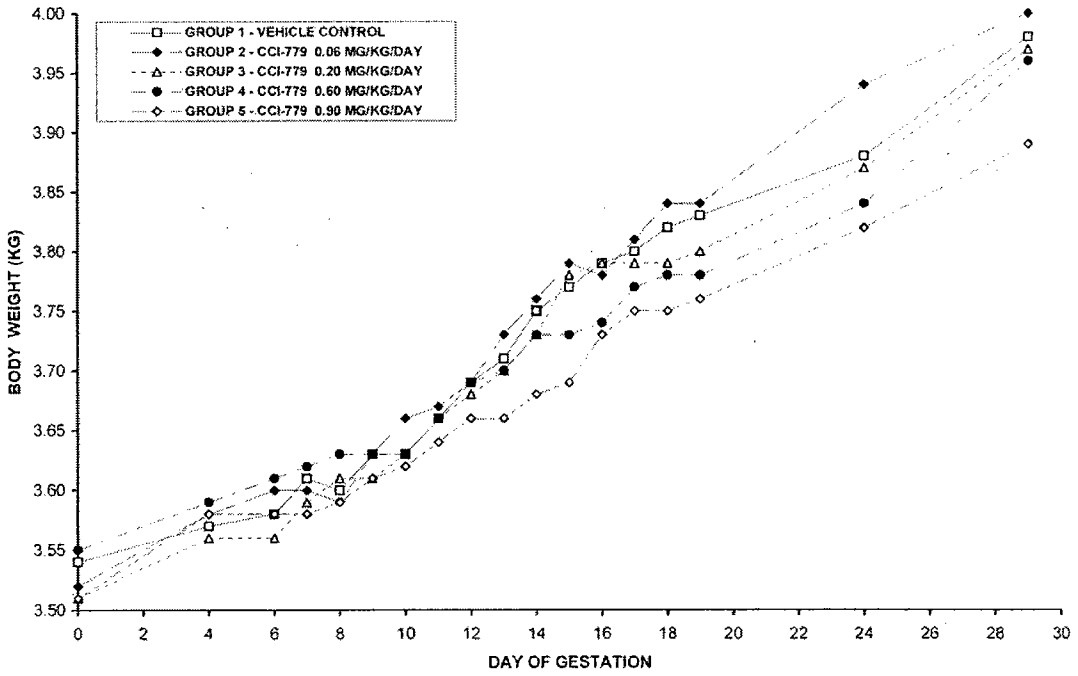
Table excerpted from the package.

DOE

Mortality: no drug-related mortality

Clinical signs: no drug-related effect

Body weight: ↓BW gain of ~30% at HMD and HD.
↓BW gains were secondary to the ↓fetal weight and ↑resorption/post-implantation loss, since BWs were comparable to the control when corrected for the uterine weight.



Graph excerpted from the package.

Food consumption: slightly ↓food consumption at 0.6 (HMD) and 0.9 (HD) mg/kg

Gross pathology: no drug-related effect

Pregnancy rate: 100% in all groups, except for LMD which had a pregnancy rate of 85% (17/20). This effect may not be drug-related as it was not seen in higher dose groups.

Uterine findings:
Effects were most evident at HMD and HD:

- ↓Mean gravid uterine weight, starting from the LD. ↓Weight was 15% at HD.
- Statistically significantly ↑late (↑9-fold at HMD and HD) and total resorptions (↑4-fold at HMD and ~↑6-fold at HD)
- ↑Incidence of post-implantation loss, starting from the LD. The increase was statistically significant at HMD and HD (~↑5% at HMD and ~↑8% at HD)
- ↓litter size, starting from the LD (as a result of increased resorptions and post-implantation loss): ~↓11% at HMD and ~↓18% at HD

EMBRYO-FETAL
Fetal weight: ↓weight at HMD and HD; statistically significantly reduced at HD (↓8%)

External findings:
Omphalacele (protrusion of intestines at the base of the umbilical cord) at LD (1/135 fetuses) and HD (3/146 fetuses= 2%).

Skeletal findings:

- Fused sternebrae at HMD (2.5% of fetuses) and HD (5.5% of fetuses)
- Bifurcated sternabrae at HMD (2.5% of fetuses) and HD (3.4% of fetuses)
- ↑Incidence of notched ribs at HD (2.7% of fetuses at HD vs 0.6% in control)
- ↑Incidence of incomplete ossification of pubic bone at HD (7.5% of fetuses at HD vs 3% in control)
- Statistically significant ↑ in the incidence of incomplete ossification of frontal bone at HMD (~9% vs 0.6% in control) and HD (~8% vs 0.6% in control)

Dosage (mg/kg)	**--0 (Control)--**	**-------0.06------**	**-------0.20-------**	**-------0.60-------**	**-------0.90-------**
Number of Dams	**20**	**20**	**20**	**20**	**20**
Toxicokinetics (RPT-46564)					
Number Evaluated	0	12	12	12	12
AUC_{0-24} (ng•h/mL) (Whole Blood)					
Temsirolimus					
GD 18	NA	26.0 ± 1.8	76.8 ± 6.3	189 ± 17	261 ± 24
Sirolimus					
GD 18	NA	10.6[c] ± 5.5	37.5 ± 14.1	93.5 ± 23.9	98.5 ± 18.4
Treated F_0 Dams/Does					
Number Mated	20	20	20	20	20
Number Pregnant	20	20	17	20	20
Number Died or Sacrificed Moribund	1[b]	1[c]	0	0	0
Number Aborted or with Total Resorptions	0	1	0	0	0
Number Littering Prior to Scheduled Necropsy	2	0	0	0	0
Clinical Observations	–	–	–	–	–
Necropsy Observations	–	–	–	–	–
Body-Weight Gain (%)[d]					
GD 6 to 19	0.25 kg	-4	-4	-28	-28
Food Consumption (%)[d]					
GD 6 to 19	2198 g/animal	2	-2	-6	-7
Uterine Findings					
Number Examined	18	19	17	20	20
Number of Abortions	0	1	0	0	0
Mean Number Corpora Lutea	10.0	8.6	9.6	10.0	10.0
Mean Number Implantations	9.1	7.5	8.4	9.0	8.6
Mean % Preimplantation Loss	7.8	15.3	13.8	9.4	13.6
Mean Gravid Uterine Weight (g)	585.6	493.3	508.0	530.6	492.7
Litters from Treated F_0 Females					
Number Litters Evaluated	18	19	17	20	20
Mean Number Live Fetuses	8.9	7.1	7.6	7.9	7.3
Mean Number Resorptions	0.2	0.4	0.8	1.0**	1.3**
Mean Number Early Resorptions	0.1	0.2	0.5	0.0	0.3
Mean Number Middle Resorptions	0.0	0.0	0.0	0.0	0.0
Mean Number Late Resorptions	0.1	0.2	0.2	1.0**	1.0**
Mean Number of Dead Fetuses	0.0	0.0	0.0	0.0	0.0
Mean % Postimplantation Loss	1.7	4.5	9.2	11.1**	14.8**
Mean Fetal Weight (g)					
Males	46.4	49.2	45.2	44.3	42.8*
Females	45.8	47.1	45.5	41.8	41.5*
Combined males and females	45.9	48.0	45.4	43.1	42.2*
Fetal Sex Ratio (% males)	48.0	56.2	47.5	53.2	47.7

Litters from Treated F_0 Females (cont'd)					
Fetal Anomalies[e]					
Gross External					
Number Litters/Fetuses Examined	18/161	19/135	17/129	20/159	20/146
Noteworthy Findings	–	–	–	–	–
Visceral					
Number Litters/Fetuses Examined	18/161	19/135	17/129	20/159	20/146
Abdomen - omphalocele	0/0	1/1	0/0	0/0	3/3
Skeletal					
Number Litters/Fetuses Examined	18/161	18/130	17/129	20/159	20/146
Frontal bone - incomplete ossification	1/1	1/3	1/1	8*/14	7*/11
Pubic bone - incomplete ossification	4/5	4/7	2/3	4/5	7/11
Ribs - notched	1/1	0/0	0/0	1/1	4/4
Sternebrae - fused	0/0	2/2	1/1	4/4	5/8
Sternebrae - bifurcated	0/0	0/0	0/0	2/4	3/5

*$p \leq 0.05$, ** $p \leq 0.01$ (statistical significance was determined by Wilcoxon's, Dunnett's or Fisher's Exact test).
– No noteworthy finding.

a. Estimated from the concentration profile. Most concentrations were below quantification limits.
b. Euthanized subsequent to delivering litter on Day 28; not compound related.
c. Euthanized GD 25 subsequent to abortion probably resulting from aspiration of dosing formulation; not compound related.
d. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
e. Only compound-related noteworthy findings are listed.

AUC = Area under the concentration-versus-time curve; GD = Gestation day; GLP = Good Laboratory Practice; NA = Not applicable; NZW = New Zealand White; RPT = Report.

Conclusions:
Temsirolimus administered to mated rabbits, p.o. daily, GD 6-18, at doses up to 0.9 mg/kg, did not cause clear signs of maternal toxicity: ↓BW gain in the treated animals were due the reduced litter size and ↓fetal weights.

Uterine findings started from the LD (0.06 mg/kg or 0.72 mg/m2). At LD, the AUC_{0-24} was 26 ng.h/mL for CCI-779; this is approximately 0.02-fold the AUC reported in cancer patients after multiple administration of temsirolimus at the recommended human dose of 25 mg i.v. Embryo-fetal effects were most apparent at the HMD (0.6 mg/kg or 7.2 mg/m2) and HD (0.9 mg/kg or 10.8 mg/m2). At HMD, the AUC_{0-24} was 189 ng.hr/mL for CCI-779; this is approximately 0.14-fold the human AUC at the RD of 25 mg i.v.

Uterine effects consisted of the following: ↓mean gravid uterine weight, ↑late and total resorptions, ↑post-implantation loss, ↓litter size.

Embryo-fetal effects consisted of the following: ↓fetal weight (HMD and HD), omphalacele (LD and HD), fused sternebrae (HMD and HD), bifurcated sternabrae (HMD and HD), ↑incidence of notched ribs (HD)/ incomplete ossification of pubic bone (HD)/ incomplete ossification of frontal bone (HMD and HD).

Prenatal and postnatal development

No study was conducted.

2.6.6.7 Local tolerance

A separate study was not conducted.
Injection site reactions were observed in all groups, including the control groups, in some of the repeat dose toxicology studies. Injection site reactions were not dose limiting. The

following represents the findings in the chronic toxicology study in monkeys (Report# RPT-43566)

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Females --			
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
	No. in group:	4	4	4	4	4	3	4	3
INJECTION SITE/S	Number examined:	4	4	4	4	4	3	4	3
EROSION		0	0	1	0	0	0	0	0
NEUTROPHILIC INFLAMMATION		1	0	1	2	2	0	4	1
MIXED CELL INFLAMMATION		0	1	0	1	0	0	1	0
HEMORRHAGE		3	4	4	4	4	2	4	3
FIBROSIS		1	2	1	2	2	1	4	1

Table excerpted from the package.

2.6.6.8 Special toxicology studies

Qualification of impurities:

To qualify potential changes in drug substance impurity profiles during optimization of the synthetic process, a 2-week i.v. impurity qualification study in rats, a bacterial reverse mutation assay, and a chromosome aberration assay were conducted using Lot 7636-126 of temsirolimus. Lot 7636-126 was manufactured according to a modification of the original synthesis. This yielded material with higher levels of impurities than would be anticipated in drug substance lots manufactured for clinical use. The two in vitro genotoxicity studies conducted with this lot are reviewed (see below). For additional information on impurities, see page 147, "Overall Conclusions and Recommendations".

Study title: CCI-779: Bacterial reverse mutation test with salmonella typhimurium and Escherichia coli

Key study findings: CCI-779, containing [redacted] impurities was non-mutagenic in the Bacterial Reverse Mutation Test with Salmonella typhimurium and Escherichia coli.

Report no.: GTR-37662
Volume #, and page #: Module 4
Conducting laboratory and location: BioReliance, Rockville, MD
Date of study initiation: April 1999
GLP compliance: Yes
QA: Yes
Drug, lot #, and % purity: CCI-779, Lot# 7636-126, [redacted] strength (containing [redacted] impurities)

Methods

Concentrations: initial and confirmatory assays with a minimum of five dose levels of test article (selected from 5000, 1800, 600, 200 and 75 µg/plate). Solutions were adjusted to compensate for the [redacted] strength of the test article.

Vehicle/negative control: DMSO
Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, methyl methanesulfonate and 2-aminoanthracene

Tester Strains:

- Salmonella: TA98, TA100, TA1535 and TA1537

- E.coli: WP2 uvrA

Activation system: aroclor-induced rat liver

Criteria for valid assay:

- All Salmonella tester strain cultures must demonstrate the presence of the deep rough mutation (rfa) and the deletion in the uvrB gene.
- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- All WP2 uvrA cultures must demonstrate the deletion in the uvrA gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 uvrA, 10-60.
- To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x\ 10^9$ cells/mL.
- The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic dose levels are required to evaluate assay data.
- A dose level is considered toxic if one or both of the following criteria are met: (1) A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must he accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn.

Additional information on study design and criteria for positive results:

- The study was done in the presence and absence of rat liver S9 activation.
- All dose levels of test article, vehicle controls and positive controls were plated in triplicate.
- The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate.
- For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test article. Data sets for strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response were equal to or greater than three times the mean vehicle control value. Data sets for strains TA98, TA100 and WP2 uvrA were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than two times the mean vehicle control value.

Results and conclusions:

CCI-779 was negative in the Ames assay at up to 5000 µg/plate in the presence or absence of S9 activation with any of the tester strain used (Salmonella TA98, TA100, TA1535 and TA1537 and E. coli WP2 uvrA).

Precipitate was generally formed at concentrations of temsirolimus ≥ 1800 µg/plate. Occasionally precipitate was observed at 600 µg/plate, one concentration lower than 1800.

Study Title: CCI-779: In vitro mammalian chromosome aberration test

Key study findings: Under the conditions tested, temsirolimus with impurities of [redacted] was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.

Report no.: RPT-38890
Volume #, and page #: Module 4
Conducting laboratory and location: [redacted]
Date of study initiation: August 1999
GLP compliance: Yes
QA: Yes
Drug, lot #, and % purity: CCI-779, Lot# 7636-126, [redacted] strength (containing [redacted] impurities)

Methods
Concentrations: Solutions were adjusted to compensate for the [redacted] strength of the test article.

- Preliminary assay: the maximum concentration tested was 5000 µg/mL. At least a 50% reduction in mitotic index was observed at doses >15 µg/mL in ±S9 4 hr exposure groups and at ≥ 5 µg/mL in the 20-hr exposure group.
- Definitive assay: based on the findings in the preliminary assay, the concentrations chosen ranged from 1.5-150 µg/mL for ±S9 4-hr exposure groups, and from 0.5-75 µg/mL for the non-activated 20-hr exposure group. Dose levels for the non-activated 20-hr exposure group were changed after the initial assay did not yield a dose level with at least 50% mitotic inhibition and sufficient scorable metaphase cells. The levels in the repeat assay ranged from 1 to 75 µg/mL.

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	1.5, 3.1, 6.25, 10, 12.5, 15, 25, 50, 75, 100, 150
	20 hr	0 hr	0.5, 0.75, 1.5, 3.1, 6.25, 12.5, 25, 50, 75
S9-activated	4 hr	16 hr	1.5, 3.1, 6.25, 10, 12.5, 15, 25, 50, 75, 100, 150

Table excerpted from the package.

Vehicle/negative control: DMSO
Positive controls: Mitomycin C was used as the positive control in the –S9 study at final concentrations of 0.25 and 0.5 µg/mL. Cyclophosphamide was used as the positive control in the +S9 study at final concentrations of 25 and 50 µg/mL. For both positive controls one dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Test system: human peripheral lymphocytes (HPBL):
± S9 for 4 hours
–S9 for 20 hrs

Activation system: aroclor-induced rat liver

Criteria for valid assay:
The frequency of cells with structural chromosome aberrations in the untreated and solvent controls must be within the historical range for negative controls. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative. to the solvent control.

Additional information on study design and criteria for positive results:

- Selection of dose levels for the chromosome aberration assay was based on a reduction in the mitotic index relative to the solvent control.
- The test article was considered to induce a positive response when the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). A reproducible significant increase at the high dose only with no dose response or a reproducible significant increase at one dose level other than the high dose with no dose response was considered positive. The test article was concluded to be negative if no statistically significant increase was observed relative to the solvent control.

Results:
Visible precipitate was observed in treatment medium at concentration levels of 1500 and 5000 µg/mL in all exposure groups, and at 500 µg/mL in the non-activated 20 hour continuous exposure group. Below is the tabulated summary of the findings. Results of individual studies at higher concentrations of temsirolimus than what has been presented in the following Table have been reviewed; since there was no relevant finding, they are not presented here.

Treatment	S9 Activation	Treatment[1] Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell[2] (Mean ± SD)	Cells With Aberrations[3] (%) Numerical	Structural
DMSO	-	4	4.1	200	0.010 ± 0.141	0.5	0.5
CCI-779							
10 µg/mL	-	4	2.5	200	0.010 ± 0.100	0.0	1.0
25 µg/mL	-	4	2.3	200	0.005 ± 0.071	0.0	0.5
75 µg/mL	-	4	1.7	200	0.010 ± 0.100	0.0	1.0
MMC, 0.5 µg/mL	-	4	2.5	200	0.170 ± 0.438	0.0	14.5**
DMSO	+	4	3.9	200	0.000 ± 0.000	0.5	0.0
CCI-779							
6.25 µg/mL	+	4	2.8	200	0.005 ± 0.071	0.5	0.5
15 µg/mL	+	4	2.3	200	0.010 ± 0.100	0.5	1.0
50 µg/mL	+	4	1.9	200	0.005 ± 0.071	0.0	0.5
CP, 25 µg/mL	+	4	1.3	200	0.220 ± 0.541	0.0	17.5**
DMSO	-	20	4.4	200	0.005 ± 0.071	0.0	0.5
CCI-779							
5 µg/mL	-	20	3.6	200	0.000 ± 0.000	0.0	0.0
15 µg/mL	-	20	2.4	200	0.010 ± 0.141	0.0	0.5
20 µg/mL	-	20	2.1	200	0.010 ± 0.100	0.0	1.0
MMC, 0.25 µg/mL	-	20	1.8	200	0.170 ± 0.390	0.0	16.5**

[1] Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
[2] Severely damaged cells were counted as 10 aberrations.
[3] *, p≤0.05; **, p≤0.01; Fisher's exact test.

Table excerpted from the package.

Conclusions:
Under the conditions tested, temsirolimus with impurities of [redacted] was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Also see under each study reviewed.

Major Temsirolimus-Related Findings in Repeat-Dose Toxicity Studies with Temsirolimus

	-----Mice-----	-----Rats-----	---Monkeys---
Depletion of Lymphoid Cells and Associated Findings			
Lymphoid atrophy (thymus and lymphoid tissues)	X	X	X
Decreased peripheral blood lymphocytes	X	X	X
Bone marrow hypocellularity (depletion of lymphoid elements)		X	
Hyperglycemia and Related Findings			
Hyperglycemia		X	
Vacuolation of pancreatic islet cells		X	
Cataract		X	
Hepatocellular vacuolation		X	
Reproductive, Male (Testes and Secondary Findings)			
Testes, tubular degeneration/necrosis, decreased weight	X	X	X
Testicular tubular giant cells	X	X	X
Atrophy and decreased weight of accessory sex organs (epididymides, prostate, seminal vesicles)		X	
Oligo/aspermia or immature spermatocytes in the epididymides	X	X	X
Bone fractures (likely associated with decreased testosterone)		X	
Reproductive, Female			
Atrophy of ovaries, uterus, and/or cervix	X	X	
Ovarian follicular or luteal cysts		X	
Inflammation			
Inflammation and/or ulceration of the skin	X	X	
Rash of skin			X
Inflammation of the large intestine			X
Increased neutrophils, fibrinogen, and/or serum proteins	X	X	X
Heart			
Myocardial degeneration		X	
Lipids			
Cholesterol, increased	X	X	X
Lung			
Pulmonary alveolar macrophages		X	

Table excerpted from the package.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The sponsor is pursuing approval of temsirolimus for "treatment of advanced renal cell carcinoma". Temsirolimus will be administered i.v. once a week (25 mg/week).

Because of the proposed route of administration, mainly the i.v. studies were reviewed. Reproductive toxicology studies were conducted orally. Because the metabolic profile of the drug after oral administration to rats and monkeys resembles that after i.v. administration to humans, 2 oral studies (safety pharmacology and repeat-dose toxicology) were also reviewed. The oral studies did not reveal any new information, with the exception of hepatotoxicity which was more pronounced after oral drug administration in the repeat dose toxicology. This may be due to the oral route of administration and the first pass liver effect. For a summary of nonclinical findings, see pages 3-5 of this review.

The sponsor submitted several studies for qualification of impurities. Two of the studies (i.e. in vitro genotoxicity assays of Lot 7636-126 containing [redacted] impurities) were reviewed. Both the Ames test and the clastogenicity assay (human peripheral lymphocytes) were negative. Based on the summary of toxicology findings, the toxicity profile of this lot was comparable to those observed in the toxicity studies reviewed within this NDA. The sponsor provided a comparative summary of the highest nonclinical and clinical exposure to specified impurities/degradants in the drug substance and drug product (see Tables below). For each impurity, the exposure in the toxicity studies was greater than the exposure at the clinical dose when expressed as a % impurity level and when expressed as mg/kg for both the drug substance and the drug product.

Drug substance: impurities/degradants

		Clinical Batch Highest Level			Toxicology Batch Highest Level		
Identifier	Name	RRT	%	mg/kg[a]	RRT	%	mg/kg
[redacted]							

BRL = Below reporting limit; NA = Not applicable; NR = Not reported; RRT = Relative retention time.

a. The clinical mg/kg level was estimated based on the % impurity and a 25 mg dose [redacted] for a 60 kg human.
b. Method L17677-035.
c. Batch 7636-126, impurity qualification batch.
d. Batch OM7612.
e. Batch RA0622.
f. Quantitated by liquid chromatography/mass spectrometry as a group of products and, therefore, does not have a specified RRT in the purity method.

Drug products: degradants

Appears This Way
On Original

Identifier	Name	Clinical Batch Highest Level RRT	%	mg/kg[a]	Toxicology Batch Highest Level RRT	%	mg/kg
Degradants							
[redacted]							

BRL = Below the reporting limits; NA = Not applicable; NR = Not reported; RRT = Relative retention time.

a. The clinical mg/kg level was estimated based on the % impurity and a 25 mg dose [redacted] for a 60 kg human.
b. Method L21063-085.
c. The toxicity limit was based on results of an impurity qualification oral toxicity study using impurity qualification batch L24300-016, analytical method 20196-165. [redacted] seco-temsirolimus, is a component of temsirolimus in vivo in all species examined.
d. Batch 2001B0205, impurity qualification batch, intravenous administration.
e. Quantitated by liquid chromatography/mass spectrometry as a group of products and, therefore, does not have a specified RRT in the purity method
f. Method L17677-035.
g. Batch 7636-126, impurity qualification batch, intravenous administration.
h. Initial analyses of batch 7636-126 used Method L17677-035, qualified for purity but not oxidative/hydrolysis degradants. In Sep 2003, a method qualified for oxidative/hydrolysis degradants (L27385-004) determined the oxidative/hydrolysis degradants to be [redacted]%. Based on the comparability of these degradants in this batch between 1998 and 2003, the estimated level of oxidative/hydrolysis degradants at the time of the toxicity studies was estimated to be [redacted]

Conclusions: There are no Pharmacology/Toxicology issues which preclude approval of temsirolimus for the requested indication.

Unresolved toxicology issues: None

Recommendations: None

Primary Reviewer: _____
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Pharmacologist

Supervisory Concurrence_____ Concurrence Yes _X_
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APPENDIX/ATTACHMENTS
None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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