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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-632
NDA 21-948

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY TEAM LEADER REVIEW

NDA 21-632 Letter date: April 25, 2003
NDA 21-948 Letter date: August 18, 2005

Applicant:
Vicuron Pharmaceuticals Inc., a subsidiary of Pfizer
235 East 42nd Street
New York, NY 10017

Vicuron Pharmaceuticals, Inc. (King of Prussia, Pennsylvania) was previously known as Versicor, Inc. On September 5, 2005, Pfizer Inc. acquired and began representing Vicuron Pharmaceuticals, Inc. (letter to FDA dated November 1, 2005). Vicuron became a wholly owned Pfizer subsidiary in December 2005.

Drug product: Eraxis™ (anidulafungin) for Injection, 50 mg
The name of anidulafungin during early development was LY303366.

Route of administration: Intravenous

Indications:
NDA 21-632: treatment of esophageal candidiasis
NDA 21-948: treatment of Candidemia and other forms of Invasive Candidiasis

Dosing:
NDA 21-632: 100 mg loading dose on Day 1, followed by 50 mg daily thereafter
NDA 21-948: 200 mg loading dose on Day 1, followed by 100 mg daily thereafter

Subject:
This Team Leader Review addresses apparent discrepancies between the reviews submitted by the primary Pharmacology/Toxicology Reviewer and the anidulafungin label. The portions of the product label discussed include parts of the sections, "Carcinogenesis, Mutagenesis, Impairment of Fertility," and "Pregnancy." The types of nonclinical studies discussed include mutagenicity studies and reproductive studies.

Background:
The Pharmacology/Toxicology Reviewer, Dr. Owen McMaster, submitted a nonclinical pharmacology/toxicology review (dated February 25, 2004) of NDA 21-632 to the FDA/CDER Division File System (DFS) on May 13, 2004. On November 14, 2005, Dr. McMaster submitted an amendment to his original review to readdress the results of the bacterial reverse mutation (Ames) studies submitted by the applicant and to address an Animal Pharmacology and Toxicology section in the product label. (In Dr. McMaster's first review of NDA 21-632, he refers to an earlier proposed trade name of the drug product, [redacted] which Vicuron/Pfizer has since replaced with "Eraxis™".)

Nonclinical pharmacology/toxicology studies were submitted with NDA 21-632. There were no nonclinical pharmacology/toxicology studies submitted to NDA-948; the applicant cross-referenced NDA 21-632 in the original NDA 21-948 cover letter.

Anidulafungin is a fermentation product and a non-competitive inhibitor of 1,3-β-D-glucan synthase, an enzyme required for the synthesis of β-linked glucan polymers which comprise a major portion of the cell wall carbohydrate in many pathogenic fungi. Nonclinical in vitro and in vivo pharmacodynamic studies demonstrated activity of anidulafungin against *Candida* and *Aspergillus* species and *Pneumocystis carinii*.

Pharmacokinetic studies in mice, rats, rabbits, dogs, and cynomolgus monkeys demonstrated that the oral bioavailability of anidulafungin is varied and limited. Data from these studies [redacted] Consequently, Dr. McMaster did not include reviews in his reports of animal studies examining the effects of anidulafungin by the oral route of administration (with one exception), although all studies submitted in support of an NDA are normally reviewed.

Mutagenicity Studies:
Vicuron submitted the following seven mutagenicity studies to IND 21-632:

- Versicor Toxicology Report 5: The effect of LY303366 on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test. March 23, 1995.
- Versicor Toxicology Report 8: The effect of LY303366 on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test. October 24, 1995.
- Versicor Toxicology Report 30: The effect of LY303366 containing impurities on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test. January 11, 1999.
- Versicor Toxicology Report 4: The effect of LY303366 on the in vitro induction of chromosome aberrations in Chinese hamster ovary cells. February 15, 1995.
- Versicor Toxicology Report 1: The effect of LY303366 on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y mouse lymphoma cells. December 13, 1994.
- Versicor Toxicology Report 2: The effect of LY303366 given orally for 2 consecutive days on the induction of micronuclei in bone marrow of ICR mice. January 17, 1995.
- Versicor Toxicology Report 12: The effect of LY303366 given intravenously for 2 consecutive days on the induction of micronuclei in bone marrow of ICR mice. August 8, 1996.

There are no outstanding issues concerning the last three reports and they will not be discussed further.

Versicor Toxicology Report 5: The effect of LY303366 on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test. March 23, 1995.

Report 5 contains five Ames assays, or "studies" as they are designated in the original report. Each study is identified by the date the study was begun (e.g., Study No. 941116AMS3683 was begun on November 16, 1994). These are usually two-day studies.

The potential of LY303366 (anidulafungin) to induce bacterial mutation was evaluated in *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* strain WP2uvrA, with and without metabolic activation. Appropriate positive and negative controls were utilized and a preliminary toxicology study was conducted to select drug concentrations. For these mutagenicity assays, drug concentrations were 125, 250, 500, 1000, and 2000 µg/plate with metabolic activation and 62.5, 125, 250, 500, and 1000 µg/plate without metabolic activation. All assays were run with triplicate plates.

Studies were as follows:

Study No.	Lot No.	Status	Characterization	Results
941116AMS3683	603CD4	Purified	Yes	Positive
941206AMS3683	603CD4	Purified	Yes	Positive
941220AMS3683	917-AXS-159	Purified	No	Negative
941221AMS3683	DTC-124	Purified	No	Negative
950125AMS3683	DTC-132	Crude	No	Negative
950207AMS3683	312SB4	Crude	No	Negative
950214AMS3683	500CB5	Purified	Yes	Negative

Lot 603CD4 was a purified toxicology lot. In the first study (start date, November 11, 1994), the drug with and without activation caused a concentration-related increased incidence of revertant colonies with all tester strains except TA 100. However, the results met the criteria for a positive response (i.e., a two-fold or greater increase in the number of revertant colonies over those in the control plates) only for the plates with activation. The study was repeated with Lot 603CD4 (December 6, 1994) and the results were the same. The applicant's conclusion at this time was the drug is mutagenic. However, because anidulafungin is a fermentation product and studies in previous mutagenicity assays were negative, the applicant suspected the drug lot was biologically contaminated.

Additional studies were undertaken (December 20, December 21, 1994, and January 25, and February 7, 1995) using other drug lots. Each of these studies resulted in negative responses with and without activation. While these tests were conducted, a clinical trial lot (500CB5) became available, and it was tested (February 14, 1995). The results were again negative. The applicant's conclusion was the drug was not mutagenic.

In his review, Dr. McMaster noted that the studies were conducted under GLP conditions except that the concentration and stability of the test article under the conditions of administration were not determined and the test article characterizations (i.e., the percent

purity of each lot) were only available for the toxicology and clinical lots (Lots 603CD4 and 500CB5). Between the two characterized lots, one test result was positive and one was negative. These studies do not yield a clear determination of whether anidulafungin is genotoxic.

Versicor Toxicology Report 8: The effect of LY303366 on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test.

Report 8 contains 32 Ames assays (or modified Ames assays), which are designated as "studies" in the original report and labeled according to the start date of the assay.

The applicant continued testing additional lots of anidulafungin. Plates were run in triplicate. A new toxicity study was conducted (February 1, 1995). Study 950501AMS3683 used the same range of concentrations as in Report 5; all the other studies in Report 8 were conducted at concentrations ranging from 125 to 2000 µg/plate both with and without metabolic activation.

These studies were GLP with the following critical exceptions: The concentrations and stability of the test article under the conditions of administration were not determined; no in-process inspections were made; and test article characterizations were not available for Lots 311SB4 and BGS290. Also, studies 95023AMS3683 and 950523AMS3683A through P were conducted as non-GLP studies.

Study No.	Lot No.	Status	Characterization	Results
950321AMS3683	603CD4	Purified	Yes	Positive
950405AMS3683	501CB5	Purified	Yes	Positive
950418AMS3683A	501CB5	Purified	Yes	Positive
950418AMS3683B	311SB4	Crude	No	Negative
950425AMS3683A	502CB5	Purified	Yes	Negative
950425AMS3683B	503CB5	Purified	Yes	
950501AMS3683	500CB5	Purified	Yes	Negative
950509AMS3683A	504CB5	Purified	No	Negative
950509AMS3683B	BGS290	Crude	No	Positive
950523AMS3683	HPLC fractions	Purified	No	Negative
950523AMS3683A through 950523AMS3683P	HPLC fractions	Purified	No	Negative
950619AMS3683	501CB5	Purified	Yes	Positive
950619AMS3683A	603CD4	Purified	Yes	Positive
950619AMS3683B	502CB5	Purified	Yes	Negative
950621AMS3683				See narrative.
950718AMS3683	603CD4	Purified	Yes	See narrative.
950719AMS3683	501CB5	Purified	Yes	See narrative.

Results from the studies conducted through May 1995 were lot-specific. Lots 603CD4, 501CB5, and BGS290 resulted in increases in revertant colonies in strains TA1535, TA1537, TA98, and WP2uvrA with activation. Treatment with crude starting material (Lot 311SB4), Lots 502CB5, 500CB5, 504CB5, and HPLC fractions of anidulafungin from several lots did not result in the induction of *S. typhimurium* or *E. coli* revertants.

Further studies were conducted (June 19, 1995) with Lots 501CB5, 603CD4 (previously positive lots) and 502CB5 (previously negative lot) with activation using strain TA1537 to investigate the possibility of microbial contamination of the test article. In addition to the usual reverse mutation assay with TA1537 (triplicate plates, positive and negative controls, five concentrations of anidulafungin), cultures using 1000 and 2000 µg/plate of each of the same three lots were treated with activation but using no tester strain (NOTA) to detect colonies that may be contributed by the test article.

The reverse mutation assays with TA1537 confirmed previous positive results of Lots 501CB5 and 603CD4 and negative results of Lot 502CB5. Also, the number of colonies present on the NOTA plates using Lots 501CB5 and 603CD4 was greater than the number of colonies present on the NOTA plates using Lot 502CB5 or the negative control (DMSO), suggesting the positive results in all assays were due to microbial contamination of the lots studied.

Study	Treatment	Lot	µg/plate	Total colonies
950619AMS3683	Drug	501CB5	1000	21 ± 5
		501CB5	2000	19 ± 5
950619AMS3683A	Drug	603CD4	1000	17 ± 5
		603CD4	2000	25 ± 4
950619AMS3683B	Drug	502CB5	1000	7 ± 3
		502CB5	2000	7 ± 3
950619AMS3683	NOTA	501CB5	1000	9 ± 5
		501CB5	2000	8 ± 1
950619AMS3683A	NOTA	603CD4	1000	17 ±5
		603CD4	2000	29 ± 7
950619AMS3683B	NOTA	502CB5	1000	1 ± 1
		502CB5	2000	1 ± 1
	Pos control		2.5	92 ± 10
	Neg control		0.05 mL	6 ± 2

Plates from the June 19 studies were then examined microscopically. Individual colonies were isolated and wet mounted on a microscope slide and examined for microbial contamination. Microbial contaminants were observed on plates using Lots 501CB5 and 603CD4, but not Lot 502CB5. Details of this procedure and the findings were not presented in Report 8.

Attempts to culture the contaminated lots were unsuccessful. Study 95621AMS3683 was conducted to attempt to determine whether the colonies from 950619AMS3683 and

950619AMS3683A were auxotrophs or true revertants from histidine auxotrophy. However, there were difficulties with transferring the colonies to new plates and no conclusions were drawn.

Finally, Lots 501CB5 and 603CD4 were tested again (five plate concentrations from 125 to 2000 µg/plate, with metabolic activation, July 1995) using filtered (0.2 µ filter) and nonfiltered test article. In addition to the plates with TA1537, the highest test article concentration (2000 µg/plate) of each lot and vehicle control (DMSO) plates were treated with metabolic activation but no tester strain (NOTA).

With the exception of TA100, mean colony counts were increased in the assays with nonfiltered test article and not increased in the assays with filtered test article compared with controls. The NOTA plates with test article contained colonies. The applicant concluded that the positive results in the Ames assays were due to microbial contamination of test article lots.

Versicor Toxicology Report 30: The effect of LY303366 containing impurities on the induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test.

Report 30 contains Studies 981020AMT3683 and 981104AMS3683 (Ames assays) which were conducted to determine whether identified impurities in anidulafungin lots induce mutations in the tester strains (*Salmonella typhimurium* TA1535, TA1537, TA98, TA100, and *Escherichia coli* WP2uvrA). These studies were conducted using standard methodologies. For the first set of assays, impurity was spiked with the anidulafungin lot to bring the impurity concentration to approximately One lot of anidulafungin in which the concentration of the impurity was determined to be approximately was used in the study. Results indicated that the presence of either impurity with the active pharmaceutical ingredient did not induce revertant colonies in the tester strains compared with controls.

Discussion, Conclusions, and Recommendations Concerning Reports 5, 8, and 30

In his first review of Report 5, Dr. McMaster stated that results of the bacterial reverse mutation assays were conflicting. In his second review, Dr. McMaster stated the overall conclusion is that anidulafungin is genotoxic. Upon considering Reports 5 and 8 further, Dr. McMaster communicated to the applicant (teleconference, February 3, 2006) that due to the GLP deficiencies in the studies, the applicant had not fully resolved the conflicting assay results and the applicant should conduct an additional study. Dr. McMaster's critical consideration of the Ames studies as a whole takes a legitimate conservative view.

I agree that the evidence from Reports 5, 8, and 30 supports the applicant's position that positive results in some of the Ames assays were due to microbial contamination of those drug lots. There were serious GLP variances with these studies but the weight of evidence indicates the drug lots that tested positive in the Ames assay were contaminated lots and that the negative results came from uncontaminated lots. Therefore, I recommend that the label reflect that anidulafungin was not genotoxic in the bacterial reverse mutation (Ames) assays.

Versicor Toxicology Report 4: The effect of LY303366 on the in vitro induction of chromosome aberrations in Chinese hamster ovary cells. February 15, 1995.

Report 4 contains two studies which examined whether anidulafungin will induce chromosome aberrations in Chinese hamster ovary (CHO) cells: Study 941207CAB3683 was conducted without metabolic activation, and Study 941109CAB3683 was conducted with metabolic activation.

Preliminary toxicity tests were conducted to select the dose ranges. CHO cells were exposed in triplicate assays for four hours to anidulafungin at concentrations of 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, and 25 µg/mL without activation or at concentrations of 200, 225, 250, 275, 300, and 325 µg/mL with activation. Appropriate positive and negative controls were also employed. Approximately 17 or 18 hours post-exposure, Colcemid® was added to two of the three cultures in each treatment group to arrest dividing cells. The remaining culture in each treatment group was used to conduct concurrent cytotoxicity tests. The results of the toxicity tests were used to select the cells from three different concentrations from each study to evaluate chromosomal aberrations. From the study with metabolic activation, the cultures selected were 250, 275, and 325 µg/mL, and from the study without metabolic activation, the cultures were 5, 7, and 12.5 µg/mL. Metaphase cells were selected, washed and fixed, and at least two slides were prepared from each culture. Fifty metaphase figures from each treatment and vehicle control culture and 25 metaphase figures from positive controls were read.

Results were subject to the following criteria: Positive and negative controls should yield chromosome aberrations consistent with historic data; and the test article should be tested to toxicity (i.e., decrease in the mitotic cell count by approximately 50%) or to the limits of solubility.

The applicant's test for positive clastogenicity is defined as a dose-related increase in chromosome aberrations is observed in which the number of aberrations is statistically ($p \leq 0.01$) greater than that of the concurrent control value, as determined by a trend test for binomial distribution.

The combined studies' results are presented in the following table:

Chromosome aberrations in Chinese Hamster Ovary (CHO) cells treated with anidulafungin in the presence of metabolic activation (combined results from two cultures)

	code	Neg. Control	Pos. Control	250 µg/mL	275 µg/mL	325 µg/mL
Cells scored		100	25	100	100	100
No. cells with chromatid gaps	TG	1	4	0	3	1
No. cells with chromosome gaps	SG	1	11	1	2	7
No. cells with chromatid breaks	TB	1	12	0	3	1
No. cells with triradial	TR	0	3	0	0	0
No. cells with quadriradial	QR	0	4	0	0	0
No. cells with interstitial deletion	ID	0	1	0	0	1
No. cells with chromosome breaks	SB	1	10	3	2	0
No. cells with dientric	D	0	1	0	0	0
No. aberrations per cell		0.02	1.24	0.03	0.05	0.02
% cells with aberrations excluding gaps	TA	2	64	3	4	2
% cells with aberrations including gaps	TAG	4	76	4	9	10
% cells with >1 aberrations		0	36	0	1	0
% cells with diplochromosomes		0.5	2	1	0.5	0.5

Discussion, Conclusions and Recommendations Concerning Report 4

The Chinese Hamster Ovary studies (assays) appear to have been generally well conducted. The results of controls were similar to historical data and the applicant selected culture concentrations for evaluation that went up to toxicity. However, the number of cells examined is half the number of the standard acceptable protocol (200 metaphases per concentration).

Dr. McMaster noted in his original review that there is a concentration-related increase in the number of cells with chromosome gaps and a concentration-related increase in the percent of cells with aberrations including gaps. Therefore, he concluded that anidulafungin demonstrated the potential to produce clastogenic effects. However, Dr. McMaster recommended the label state that anidulafungin did not show evidence of genotoxic potential in the CHO assay; he did not provide a reason for the apparent change in interpretation. In his second review, Dr. McMaster stated that anidulafungin increased the number of gaps in Chinese Ovary cells in the presence of metabolic

activation; but in the label, he stated anidulafungin was not clastogenic in the assay. These statements appear quite divergent.

Even though they are scored, chromosome gaps are not counted as aberrations because the DNA is not broken and there is not a clear consensus in the scientific community as to what chromosome gaps mean. Among the findings in these studies, there are no aberrations that approach the number of gaps or demonstrate a dose-related trend. Furthermore, the percent of cells with aberrations excluding gaps is no different in the high dose groups than in the negative controls. Although the number of cells examined is inadequate, there is no indication from these studies that anidulafungin is clastogenic in the Chinese Hamster Ovary cell assay. Therefore, I recommend that the label reflect that anidulafungin was not clastogenic in the Chinese Hamster Ovary cell assay.

Reproductive Studies: Discussion and Conclusions

Vicuron submitted two intravenous animal reproductive studies to NDA 21-632:

- Vicuron Toxicology Report 18: A combined segment I and segment II study of intravenously administered LY303366 on CD rats. July 15, 1999.
- Vicuron Toxicology Report 19: A segment II toxicology study of LY303366 administered intravenously to pregnant New Zealand White rabbits. February 1997.

In Dr. McMaster's initial and second reviews, he recommended that the product label reflect that skeletal changes occurred at maternal doses at or above 2 mg/kg in rats and rabbits. This is a reasonable accounting of the data. Intravenous anidulafungin dosing in rats was at 2, 5, and 20 mg/kg/day and intravenous dosing in rabbits was 5, 10, and 20 mg/kg/day. Skeletal changes were seen in some fetuses at all doses, including controls, of both species. Some of the skeletal changes occurred with greater frequency in the anidulafungin-dosed animals compared with concurrent controls. However, incidences of skeletal changes in both species fall within the range of the applicant's historical controls.

Dr. McMaster also noted there was a reduction in litter weight and an increase in late fetal resorptions in rabbits at doses 2 to 4 times the recommended clinical dose (i.e., 10 or 20 mg/kg/day in rabbits). These statements are correct. However, the study also demonstrated there was maternal toxicity in the high dose group and the number of total resorptions and live fetuses per litter was not different between the controls and treated groups.

These data do not strongly indicate that anidulafungin is causing fetal skeletal changes, litter weight reduction, or increased resorptions, but the findings are serious enough to include them in the product label. A balance is sought to provide cautionary information without overstating the confidence we have that the findings are drug-related. I agree the findings are adequately reflected in the applicant's proposed wording for this section of the label.

Team Leader Review Summary:

The above discussions address apparent discrepancies within Dr. McMaster's reviews of anidulafungin and with his labeling recommendations.

Team Leader Recommendations:

I recommend that the second paragraph under *Carcinogenesis, Mutagenesis, Impairment of Fertility* read as follows:

> Anidulafungin was not genotoxic in the following *in vitro* studies: bacterial reverse mutation assays, a chromosome aberration assay with Chinese hamster ovary cells, and a forward mutation assay with mouse lymphoma cells. Anidulafungin was also not genotoxic when administered to mice in a micronucleus assay.

I recommend that the first paragraph under *Pregnancy Category C* read as follows:

> Embryo-fetal development studies were conducted with doses up to 20 mg/kg/day in rats and rabbits (equivalent to 2 and 4 times, respectively, the proposed therapeutic maintenance dose of 100 mg/day on the basis of relative body surface area). Anidulafungin administration resulted in skeletal changes in rat fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These changes were not dose-related and were within the range of the laboratory's historical control database. Developmental effects observed in rabbits (slightly reduced fetal weights) occurred in the high dose group, a dose that also produced maternal toxicity. Anidulafungin crossed the placental barrier in rats and was detected in fetal plasma.

William H. Taylor, Ph.D., DABT
Pharmacology/Toxicology Team Leader
February 16, 2006

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/s/

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PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-632
SERIAL NUMBER:	000 (RESUBMISSION)
REVIEW # 2	(SEE ORIGINAL REVIEW DATED 2/25/2004)
DATE ORIGINAL NDA RECEIVED	4/25/2003
DATE CURRENT SUBMISSION RECEIVED	5/27/2005
PRODUCT:	ANIDULAFUNGIN
INTENDED CLINICAL POPULATION	Patients with esophageal candidiasis
SPONSOR:	Vicuron Pharmaceuticals. 455 South Gulph Road, Suite 310. King of Prussia PA 19406
New Sponsor (merger)	Pfizer Corporation
DOCUMENTS REVIEWED:	NDA 21632 Pharmacology/Toxicology Sections
REVIEW DIVISION:	Division of Special Pathogen and Transplant Products
PHARM/TOX REVIEWER:	Owen G McMaster, PhD
PHARM/TOX SUPERVISOR:	William Taylor, PhD
DIVISION DIRECTOR:	Renata Albrecht, MD
PROJECT MANAGER:	Donovan Duggan

Date of review submission to Division File System (DFS): November 14, 2005

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

There are no Pharmacology or Toxicology findings that preclude the approval of ANIDULAFUNGIN.

B. Recommendation for nonclinical studies

No additional preclinical studies are being recommended at this time.

C. Recommendations on labeling

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long-term animal carcinogenicity studies of ANIDULAFUNGIN have not been conducted.

PREGNANCY

PREGNANCY CATEGORY C

There are no adequate and well-controlled studies in pregnant women.
ANIDULAFUNGIN should be used during pregnancy only if the potential benefit justifies the risk to the fetus.

NURSING MOTHERS

ANIDULAFUNGIN should be administered to nursing mothers only if the potential benefit justifies the risk. ANIDULAFUNGIN was found in the milk of lactating rats. It is not known whether ANIDULAFUNGIN is excreted in human milk.

OVERDOSAGE

During clinical trials a single 400 mg dose of ANIDULAFUNGIN was inadvertently administered as a loading dose. No clinical adverse events were reported. In a study of 10 healthy subjects administered a loading dose of 260 mg followed by 130 mg daily, ANIDULAFUNGIN was well tolerated

3 of the 10 subjects experienced transient, asymptomatic transaminase elevations (≤3 x ULN).

ANIDULAFUNGIN is not dialyzable.

The maximum non-lethal intravenous dose of ANIDULAFUNGIN in rats was 50 mg/kg, a dose which is equivalent to 10 times the recommended daily dose for esophageal candidiasis

ANIMAL PHARMACOLOGY AND TOXICOLOGY

Liver toxicity, including single cell hepatocellular necrosis, hepatocellular hypertrophy and increased liver weights were observed in monkeys and rats at doses equivalent to times human exposure For both species, hepatocellular hypertrophy was still noted one month after the end of dosing.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Toxicology studies of ANIDULAFUNGIN have mostly been conducted in rats and monkeys and include intravenous studies of up to 13 weeks. These are supported by additional studies in mice and dogs.

In repeat dose studies, the principal clinical finding was a transient infusion reaction consisting of swollen snout, red ears and hypoactivity. These signs were only observed for the first few days of dosing. These effects were not listed among the common adverse events in the clinical trial.

Rats treated with ANIDULAFUNGIN exhibited a regenerative anemia which the sponsor ascribes to excessive blood sampling. No hemolysis was observed in monkeys. *In vitro* exposure of rat or human blood cells to ANIDULAFUNGIN showed that rat erythrocytes were more sensitive than human erythrocytes to the hemolytic effects of ANIDULAFUNGIN (0.7 % hemolysis in humans versus 17 % in rat cells at 8.78 mg/mL ANIDULAFUNGIN). This hemolysis seen in rats seems to be a species specific finding.

In four- and thirteen week studies in rats and monkeys, liver toxicity was observed, including increased liver weights, hepatocellular hypertrophy, increased activity of AST and ALT and liver necrosis. These changes were beginning at doses around 8 fold higher than recommended clinical doses based on AUC comparisons. Animals allowed a one month recovery period after the end of dosing still showed microscopic changes in the liver. Increased liver enzymes have been observed in patients that received high doses of ANIDULAFUNGIN (see Overdose section of the label). Other signs observed in high dose animals included kidney tubular vacuolation, skeletal muscle atrophy and increased spleen, kidney, and lung weight.

ANIDULAFUNGIN injections resulted in skeletal changes in rat fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These bone changes occurred at maternal doses at or above 2 mg/kg/day, a dose equivalent to 0.4 times the recommended clinical maintenance dose (based on body surface area comparisons).

In rabbits at higher doses, (4 to 8 times the recommended dose) there was a reduction in litter weight and increased late resorptions. There was an increase in the number of fetuses and litters with an extra aortic arch at doses two times the recommended dose. Drug was detected in the blood of pups and in the milk of drug-treated rats.

The minimum lethal dose of ANIDULAFUNGIN in Fischer rats was 100 mg/kg. This dose is equivalent to a human dose of 15.9 mg/kg or about 10 times the recommended loading dose. The minimum lethal intravenous dose of ANIDULAFUNGIN in CD-1 mice was greater than 100 mg/kg (8.1 mg/kg human dose or about 5 times the recommended loading dose).

ANIDULAFUNGIN produced inconsistent results (positive and negative results) in the Ames Tests using different batches of drug; the overall conclusion was that ANIDULAFUNGIN was positive in the Ames tests. ANIDULAFUNGIN was negative in the mouse micronucleus assay and mouse lymphoma assay but did increase the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation.

B. Pharmacologic activity
ANIDULAFUNGIN inhibits beta-(1,3) glucan synthesis, damaging fungal cell walls.

C. Nonclinical safety issues relevant to clinical use:

Liver toxicity, including single cell hepatocellular necrosis, hepatocellular hypertrophy and increased liver weights were observed in monkeys and rats at doses equivalent to —— times human exposure for 3 months. For both species, hepatocellular hypertrophy was still noted one month after the end of dosing. These findings have been described in the label under "ANIMAL PHARMACOLOGY AND TOXICOLOGY".

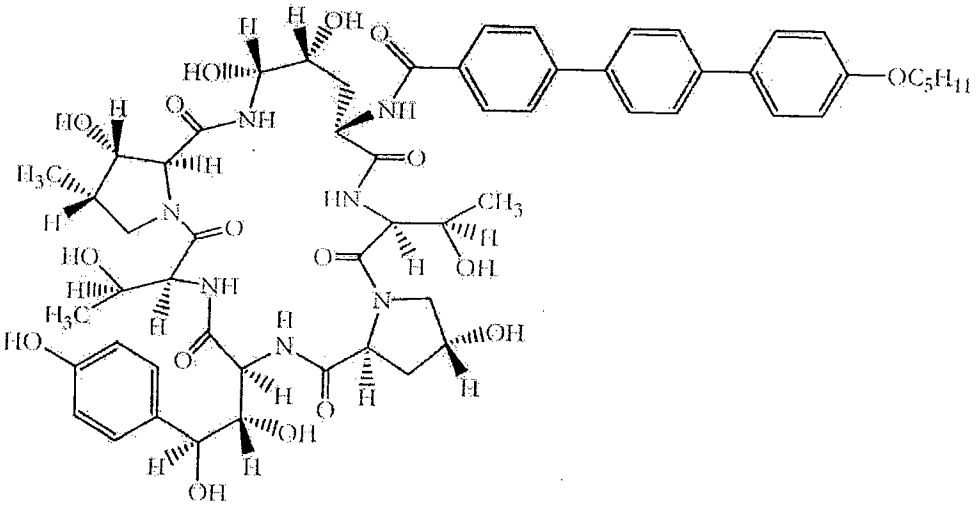
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-632
Date of Original Submission: April 25, 2003
Date of Initial Approvable Action: May 21, 2004
Date of Re-submission: May 27, 2005
Information to sponsor: Yes
Sponsor:
Vicuron Pharmaceuticals
455 South Gulph Road, Suite 310.
King of Prussia PA 19406
New Sponsor (merger): Pfizer Corporation

Manufacturer for drug substance:
[redacted]

[redacted]

Reviewer name: Owen McMaster, PhD.
Division name: Division of Special Pathogen and Immunologic Drug Products
HFD #: 590
Review completion date: November 10, 2005
Drug Trade name: ANIDULAFUNGIN®
Generic name: ANIDULAFUNGIN
Code names: VER002, LY303366, D70013, V-echinocandin
Chemical name: 1-[(4R,5R)-4,5-Dihydroxy-N(2)-[[4"-(pentoxy)[1,1':4",1"-terphenyl]-4-yl]carbonyl]-L-ornithine]echinocandin B
CAS registry number: 166663-25-8
Molecular formula: C_{58} H_{78} N17 O17
Molecular weight: 1140.3
Structure:



Related IND's: IND # 54,597 and IND

Drug class: Echinocandin antifungal

General: ANIDULAFUNGIN is a semi synthetic lipopeptide synthesized from a fermentation product of *Aspergillus nidulans*. Its antifungal activity derives from its ability to inhibit the synthesis of glucan, an essential component of fungal cell walls.

Indication: Treatment of Esophageal Candidiasis

Clinical formulation: ANIDULAFUNGIN is a sterile lyophilized product for intravenous infusion. It requires reconstitution prior to use. A companion diluent, 20% ethanol (w/w) in water for injection, is supplied.

Route of administration: Intravenous

Proposed use: Intravenous administration at a single 100 mg loading dose followed by a 50 mg (0.8 mg/kg) dose thereafter.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

There are no Toxicology findings that would preclude the approval of ANIDULAFUNGIN for the treatment of esophageal candidiasis. The product label outlines the preclinical toxicology findings associated with ANIDULAFUNGIN and appropriate monitoring should allow this product to be used safely as indicated.

Owen G McMaster, PhD.
Pharmacology/Toxicology Reviewer

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Owen McMaster
11/21/2005 02:19:22 PM
PHARMACOLOGIST

William Taylor
11/21/2005 02:23:19 PM
PHARMACOLOGIST

Steven Gitterman
11/22/2005 10:34:28 PM
MEDICAL OFFICER

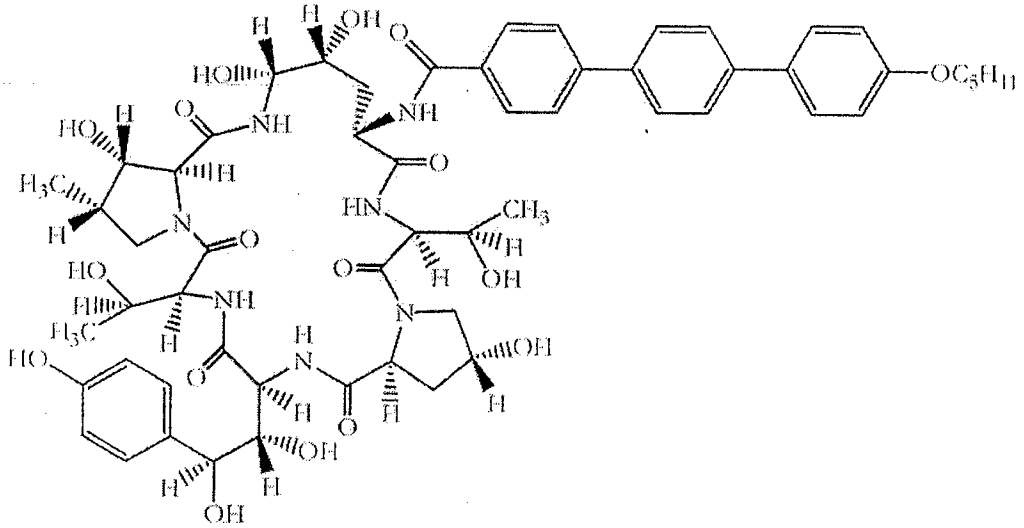
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-632
Date of submission: April 25, 2003
Information to sponsor: Yes
Sponsor: Vicuron Pharmaceuticals.
455 South Gulph Road, Suite 310.
King of Prussia PA 19406

Manufacturer for drug substance :
[
]

Reviewer name: Owen McMaster, PhD.
Division name: Division of Special Pathogen and Immunologic Drug Products
HFD #: 590
Review completion date: February 25, 2004

Drug
Trade name: [ ]
Generic name: Anidulafungin
Code names: VER002, LY303366, D70013, V-echinocandin
Chemical name: 1-[(4R,5R)-4,5-Dihydroxy-N(2)-[[4"-(pentoxy)[1,1':4",1"-terphenyl]-4-yl]carbonyl]-L-ornithine]echinocandin B
CAS registry number: 166663-25-8
Molecular formula: C_{58} H78 N17 O17
Molecular weight: 1140.3
Structure:



Related IND's: IND # 54,597 and IND #

Drug class: Echinocandin

General: Anidulafungin is a semi synthetic lipopeptide synthesized from a fermentation product of *Aspergillus nidulans*. Its antifungal activity derives from its ability to inhibit the synthesis of glucan, an essential component of fungal cell walls.

Indication: Treatment of Esophageal Candidiasis

Clinical formulation: Anidulafungin is a sterile lyophilized product for intravenous infusion. It requires reconstitution prior to use. A companion diluent, 20% ethanol (w/w) in water for injection, is supplied.

Route of administration: Intravenous

Proposed use: Intravenous administration at a single 100 mg loading dose followed by a 50 mg (0.8 mg/kg) dose thereafter.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

Recommendations

Approvability

There are no Pharmacology or Toxicology findings that preclude the approval of [ ].

Nonclinical Studies

The results of Ames Test were positive in some genotoxicity studies and negative in others. The sponsor concluded that the positive results were due to contamination. These studies should have been conducted following Good Laboratory Practice (GLP), but were not performed according to GLP. The sponsor should submit to the agency genotoxicity studies that have been conducted according to GLP guidelines and using multiple batches of the clinical formulation of anidulafungin in order to resolve this issue.

Labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been conducted to evaluate the carcinogenic potential of anidulafungin.

The results of the bacterial reverse mutation assays were conflicting. In some studies anidulafungin was positive and in others the result was negative. The meaning of these conflicting results is unclear. Anidulafungin did not show evidence of genotoxic potential in the chromosome aberration assay with Chinese hamster ovary cells or the forward mutation assay with mouse lymphoma cells. Administration of anidulafungin to mice did not provide evidence of genotoxic potential using the micronucleus assay.

Administration of anidulafungin to rats was not associated with any effects on reproduction, including male and female fertility.

Pregnancy

Pregnancy Category C

[ ] administration resulted in skeletal changes in rat and rabbit fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These bone changes occurred at maternal doses at or above 2 mg/kg, a dose equivalent to 0.4 times the recommended clinical maintenance dose (based on body surface area comparisons). In rabbits at higher doses, (times the recommended dose) there was a reduction in litter weight and increased late resorptions. There was an increase in the number of fetuses and litters with an extra aortic arch at doses two times the

recommended dose. [ ] crossed the placental barrier in rats and was detected in fetal plasma.

[ ] for Injection should be used during pregnancy only if the potential benefit justifies the risk to the fetus. There are no adequate and well-controlled studies in pregnant women.

Nursing Mothers

[ ] for Injection should be administered to nursing mothers only if the potential benefit justifies the risk. [ ] was found in the milk of lactating rats. It is not known whether [ ] is excreted in human milk.

Summary of Nonclinical Findings

Toxicology studies of anidulafungin have mostly been conducted in rats and monkeys and include intravenous studies of up to 13 weeks. These are supported by additional studies in mice and dogs. The sponsor recommends that the drug be given by intravenous administration at a single 100 mg loading dose followed by a 50 mg (0.8 mg/kg) dose thereafter. The longest human exposure has been reported to be 42 days but the typical treatment is expected to be on the order of 10 days. The AUC recorded in patients given the recommended dose is 53 µg*h/mL.

In repeat dose studies, the principal clinical finding was a transient infusion reaction consisting of swollen snout, red ears and hypoactivity. These signs were only observed for the first few days of dosing. These effects were not listed among the common adverse events in the clinical trial.

Rats treated with anidulafungin exhibited a regenerative anemia which the sponsor ascribes to excessive blood sampling. No hemolysis was observed in monkeys. *In vitro* exposure of rat or human blood cells to anidulafungin showed that rat erythrocytes were more sensitive than human erythrocytes to the hemolytic effects of anidulafungin (0.7 % hemolysis in humans versus 17 % in rat cells at 8.78 mg/ml anidulafungin). This hemolysis seen in rats seems to be a species specific finding.

In four- and thirteen week studies in rats and monkeys, liver toxicity was observed including increased liver weights, hepatocellular hypertrophy, increased activity of AST and ALT and liver necrosis. These changes were beginning at doses around 8 fold higher than recommended doses based on AUC comparisons. Animals allowed a one month recovery period after the end of dosing still showed microscopic changes in the liver. Increased liver enzymes have been observed in patients that received high doses of anidulafungin (see Overdose section of the label). Other signs observed in high dose animals included kidney tubular vacuolation, skeletal muscle atrophy and increased spleen, kidney, and lung weight.

LY303366 injections resulted in skeletal changes in rat fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These

bone changes occurred at maternal doses at or above 2 mg/kg, a dose equivalent to 0.4 times the recommended clinical maintenance dose (based on body surface area comparisons).

In rabbits at higher doses, (4 to 8 times the recommended dose) there was a reduction in litter weight and increased late resorptions. There was an increase in the number of fetuses and litters with an extra aortic arch at doses two times the recommended dose. Drug was detected in the blood of pups and in the milk of drug-treated rats.

The minimum lethal dose of LY303366 in Fischer rats was 100 mg/kg. This dose is equivalent to a human dose of 15.9 mg/kg or about 10 times the recommended loading dose. The minimum lethal intravenous dose of LY303366 in CD-1 mice was greater than 100 mg/kg (8.1 mg/kg human dose or about 5 times the recommended loading dose).

LY303366 produced inconsistent results (positive and negative results) in the Ames Test using different batches of drug. The sponsor concluded that some batches of the the drug contained a genotoxic contaminant. LY303366 was negative in the mouse micronucleus assay and mouse lymphoma assay but did increase the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation.

I. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

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TOXICOLOGY STUDIES REVIEW:

1. Study title: The acute toxicity of LY303366 administered intravenously to Fischer rats

Key study findings: The minimum lethal intravenous dose of LY303366 in Fischer rats was 100 mg/kg. Based on body surface area comparisons, this is equivalent to a human dose of 15.9 mg/kg or 954 mg for a 60 kg patient. This is about 10 times the typical loading dose (100 mg).

Study # R13396.
Report # 13
Conducting laboratory: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: June 13, 1996
GLP compliance: Yes
QA report: Yes
Drug lot 135RM6
Purity: [illegible]
Formulation: Drug was dissolved in a vehicle consisting of 2.5% polysorbate 80, 5% mannitol parenteral and 0.3% glacial acetic acid in purified water.

Methods

Groups of Fischer 344 rats, 5/sex/group, were treated intravenously with LY303366 at 0 (vehicle), 20, 50 or 100 mg/kg via the caudal vein. Rats were 8 weeks old and females weighed 122 to 138g while males weighed 154 to 167 g. The rate of infusion was 66 ml/h and the dose volume was 10 mL/kg.

Animals were observed for two weeks after injection and records were kept of clinical observations (daily) and body weights (weekly). At the end of the observation period, animals were euthanized by carbon dioxide and subjected to gross pathology examinations.

Results

Mortality:

There were no deaths among the animals treated with 20 or 50 mg/kg LY303366. All animals treated with 100 mg/kg died within 1 hour of dosing.

Clinical signs:

Clinical signs observed in the 20 or 50 mg/kg groups included swollen muzzles, sternal recumbency and excessive drinking. Other signs seen at 50 mg/kg included red urine, red perineal soiling and decreased feces. Five animals died immediately after dosing and all but one was dead by 30 minutes post dosing. This animal was dead within one hour of dosing.

Gross pathology findings included red mottled kidneys, moist and pink pancreas, slight red jejunum and red (congested) small intestine and cecum. The anterior portion of the eye from three of the 100 mg/kg rats was opaque.

Summary of individual study findings: The minimum lethal intravenous dose of LY303366 in Fischer rats was 100 mg/kg. Based on body surface area comparisons, this is equivalent to a human dose of 15.9 mg/kg or 954 mg for a 60 kg patient. This is about 10 times the typical loading dose (100 mg).

2.The Acute Toxicity of LY303366 administered intravenously to CD-1 mice.

Key study findings: The minimum lethal intravenous dose of LY303366 in CD-1 mice was greater than 100 mg/kg. Based on relative body surface area comparisons, this dose is equivalent to a human dose greater than 8.1 mg/kg (or greater than 4.8 times the typical loading dose)

Study # M03496
Report # 14
Conducting laboratory: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: June 17, 1996
GLP compliance: Yes
QA report: yes
Drug lot 135RM6,
Purity: ( )
Formulation/vehicle: Drug was dissolved in a vehicle consisting of 2.5% polysorbate 80, 5% mannitol parenteral and 0.3% glacial acetic acid in purified water.

Methods

Groups of CD-1 mice, 5/sex/group, were treated intravenously with LY303366 at 0 (vehicle) or 100 mg/kg via the caudal vein. Mice were 6 weeks old and females weighed 21 to 28g while males weighed 27 to 34g. The rate of infusion was 66 ml/h and the dose volume was 10 mL/kg. Animals were observed for two weeks after injection and records were kept of clinical observations (daily) and body weights (weekly). At the end of the observation period, animals were euthanized by carbon dioxide and subjected to gross pathology examinations.

Results:

Mortality: No animals died during the study.

Clinical signs:

The only clinical sign observed was decreased activity in three treated females between 2 to 4 hours after dosing.

Body weights: There was no difference between the mean body weights of the treated or control animals.

Pathology

A unilateral white eye lesion was observed in one control and one treated mouse.

Summary of individual study findings: The minimum lethal intravenous dose of LY303366 in CD-1 mice was greater than 100 mg/kg. Based on relative body surface area comparisons, this dose is equivalent to a human dose greater than 8.1 mg/kg (or greater than 4.8 times the typical loading dose)

3. A Subchronic Toxicity Study in Fischer 344 Rats given LY303366 daily by 20 minute intravenous infusions for one month and companion blood level studies.

Key study findings: Histamine-related anaphylactic response (ataxia, excessive drinking, lethargy, red ears, restlessness, and sternal recumbence) Species-specific hemolysis/regenerative anemia. Hepatotoxicity. Liver and spleen weights increased. Skeletal muscle showed swollen, vacuolated fibers. Vacuolation of the cytoplasm of the proximal tubules of the kidney. Mid dose animals, the lowest dose where we saw liver changes, had an exposure of 135 µg*hr/mL. Patients treated with the 100/50 mg regimen of anidulafungin have an AUC of about 53 µg*hr/mL on day 10. This data suggests that patients receiving the prescribed dose of anidulafungin should not experience these adverse effects.

Study numbers: R10996, R11096, R14196
Report 15
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: June 3, 1996.
GLP compliance: Yes
QA report: Yes
Drug, lot # 135RM6
Purity: [illegible]
Formulation/vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water.

Methods

Groups of Fischer 344 rats, 10/sex/group, were treated intravenously with LY303366 once daily for one month. Females received 0 (vehicle), 2.6, 6.6 or 40 mg/kg via the caudal vein, while males received 0, 3.1, 7.6 and 46 mg/kg LY303366. Rats were 15 to 18 weeks old and females weighed 146 to 177g while males weighed 248 to 301 g. The infusion was conducted over 20 minutes and the dose volume was 5 mL/kg. At the end of the observation period, animals were euthanized by carbon dioxide and subjected to gross pathology examinations.

Records were kept of survival (daily) and clinical observations (daily) body weights (weekly), food consumption (weekly) hematology (week 2 and week 4) clinical chemistry (week 2 and week 4), urinalysis (study termination), toxicokinetics, hepatic enzyme induction, organ weights and morphologic pathology.

Results:

Mortality:

One high dose female died during dosing and one high dose male died after receiving a slightly higher dose than intended (51 mg/kg vs the intended 40 mg/kg)

Clinical signs:

Clinical signs were observed on days 0 to 2 and only in high dose animals. Signs were consistent with a histamine related anaphylactic response and included ataxia, excessive drinking, lethargy, red ears, restlessness, and sternal recumbence. Dose related increases in plasma histamine have been recorded after a 2-hour infusion with LY303366 (Study R21295). Body weights were 12 % lower in high dose males at the end of dosing than in control animals. Food consumption was 12 % (males) and 10 % (females) lower than controls at the end of dosing.

In high dose animals, hematology changes were suggestive of a regenerative anemia and consisted of reductions in erythrocyte counts (10 to 13 %), hemoglobin concentrations (6 %) and packed cell volume (6 %) and increases in mean corpuscular volume and mean corpuscular hemoglobin. Spherocytosis and poikilocytosis were also suggestive of hemolysis. Slight changes in hematology parameters at the low and mid doses were also consistent with this regenerative erythrocyte response. Other hematology changes associated with the high dose included increased leukocyte count (+18 to 39 %), lymphocyte count (+15 to 44 %), platelet counts (+10 to 17 %) as well as decreases in APTT and PT (4 to 10 %). The sponsor also suggests that a slight increase in eosinophils observed when vehicle treated animals are compared to saline controls is related to vehicle administration.

Clinical chemistry changes consistent with moderate hepatotoxicity were detected in the high dose groups. After two weeks of dosing, ALT and AST values were increased by almost 3-fold in high dose animals. At the end of dosing ALT and AST values were increased by 421 and 478 % respectively. Increases were also observed in total bilirubin (120 %), ALP (+ 20 %) and GGT (+126 %). ALP was also increased (11 %) in mid dose males at the end of the study. Other changes included increased cholesterol (+37 to 78 %), increased total protein (up to +11 %), globulin (up 9 to 29 %), calcium (up to +10 %), BUN (up 25 % in high dose males), potassium (up by 8 to 45 %). Urinalysis changes were restricted to high dose males and consisted of slight increase in urine specific gravity, urine protein and occult blood.

Absolute and relative liver weights were increased in high dose animals by up to 45 %. Spleen weights were increased in males in all dose groups (11 to 88 %) and in high dose females (up to 60 %) . These findings were correlated with enlarged spleens, and discoloration of the liver surface. Microscopic examination revealed splenic congestion and increased extramedullary hematopoesis, which correlated with splenomegaly. This finding, in conjunction with the hypercellularity of the bone marrow is taken by the sponsor to indicate a regenerative response to hemolytic anemia. High dose animals also showed multinucleated hepatocytes, (some with enlarged nuclei), granulomatous inflammation, single cell necrosis and hepatocellular

necrosis. Liver discoloration was correlated with hepatocellular necrosis (3 rats), fibrosis (2 rats) or focal lipidosis (1 rat).

LY303366 also produced changes in the skeletal muscle and kidneys of high dose rats. Skeletal muscle myopathy was found in all high dose males and 3/10 females and consisted of muscle fibers which were swollen, vacuolated, eosinophilic hyalinized, fragmented and/or basophilic with central nuclei and sarcolemmal nuclear proliferation (suggesting muscles in various stages of degeneration and subsequent regeneration). In the 19/20 high dose animals, kidneys showed medium to large size vacuoles in the cytoplasm of the proximal tubules which contained brown pigment.

Toxicokinetics

LY303366 produced a 36 % decrease in the mean microsomal benzphetamine N-methylase activity in high dose male (but not female) rats suggesting an inhibition of cytochromes P450 2B or 2C in male rats.

Table 1. Pharmacokinetics of LY303366 in Fischer 344 male rats given daily intravenous infusions for twenty eight days.

		Low (2.6 mg/kg)	Mid (6.6 mg/kg)	High (26 mg/kg)
Day 1	AUC	25.2	89.9	967
	Cmax	2.51	6.68	62.7
Day 7	AUC	53.7	143	1,053
	Cmax	3.20	8.65	58.2
Day 27	AUC	68.7	140	1,202
	Cmax	4.51	9.57	61.3

AUC_{0-} (μg*hr/mL)
Cmax (μg/mL)

Table 2. Pharmacokinetics of LY303366 in Fischer 344 female rats given daily intravenous infusions for twenty eight days.

	Low	Mid	High

Day 1	AUC	42.2	74.3	714
	Cmax	3.84	8.34	38.9
Day 7	AUC	58.5	127	1,122
	Cmax	3.03	8.03	73.7
Day 27	AUC	72.2	166	1,242
	Cmax	3.54	9.50	61.7

AUC_{0-} (μg*hr/mL)
Cmax (μg/mL)

Summary of individual study findings:

LY303366 produces signs of histamine related anaphylactic response when infused (4 to 6 mg/ml, dose volume 5 ml/kg for 20 minutes) in rats. These signs were observed on days 0 to day 2, but were not observed thereafter. Histamine is known to be increased in the plasma after LY303366 infusion. Signs of hemolysis/regenerative anemia are also observed and include reductions in erythrocyte counts, hemoglobin concentration and packed cell volume. In vitro studies have shown that LY303366 produces hemolysis and the sponsor refers to unpublished reports that rat erythrocytes are more sensitive to hemolysis than are human erythrocytes. Hepatotoxicity, including increased liver enzyme activities, and single cell necrosis is also associated with LY303366. Liver and spleen weights are increased. Skeletal muscle in all high dose males and some high dose females showed swollen, vacuolated fibers and 19/20 high dose animals showed vacuolation of the cytoplasm of the proximal tubules of the kidney. Pharmacokinetics for LY303366 was approximately dose proportional. The NOAEL for LY303366 was the low dose in female (3.1 mg/kg) but because of increased spleen weights seen even at the low dose (2.6 mg/kg) in males, no NOEAL could be established for male rats. In terms of exposure, the NOAEL was 72.2 μg*hr/mL for female rats and less than 68.7 μg*hr/mL for male rats. Mid dose animals, the lowest dose where we saw liver changes, had an exposure of 135 μg*hr/mL. Patients treated with the 100/50 mg regimen of anidulafungin have an AUC of about 53 μg*hr/mL on day 10. This data suggests that patients receiving the prescribed dose of anidulafungin probably will not experience any adverse effects.

4. A 4-week continuous intravenous infusion toxicity study with LY303366 in cynomolgus monkeys

Key study findings: hepatotoxicity

Study no: 6080-129

Report # 16
Conducting laboratory and location: [ ]
Date of study initiation: April 26, 1996
GLP compliance: Yes
QA report: yes
Drug lot # 135RM6
Purity: [ ]
Formulation/vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water.
Methods

Groups of cynomolgus monkeys, 3/sex/group, were treated intravenously with LY303366 once daily for four weeks. The five dose groups received either dextrose, vehicle, or 2, 5 or 30 mg/kg LY303366. Monkeys were described as young to adult and weighed 2.5 to 3.1 kg at the beginning of the study. The infusion was conducted over 20 minutes and the dose rate was 15 mL/kg/hr. When not being dosed with drug, animals received lactated Ringers solution at a rate of about 3 ml/kg/hour. At the end of the observation period, animals were fasted overnight, anesthetized with pentobarbital, weighed, exsanguinated and necropsied..

Records were kept of survival and clinical observations (twice daily) body weights (weekly), food consumption (daily) physical Examination (pretreatment, week 1 and week 3) hematology (week 2 and week 4) clinical chemistry (week 2 and week 4), urinalysis (weeks 2 and 4), toxicokinetics (day 1, day 15 and day 29) and ophthalmology (weeks 1 and 3), organ weights and morphologic pathology.

Results:

All animals survived to the scheduled sacrifice and there were no clinical signs that could be ascribed to drug treatment. There were no drug related effects on body weights, food consumption or ophthalmoscopy. Drug-related increases were noted in high dose animals cholesterol levels, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase on days 14 and 28 (see Table 3 below).

Table 3. Clinical Pathology changes recorded after intravenous LY303366 (30 mg/kg) in monkeys (% increase over control values)

		Day 14	Day 28
Cholesterol	Male	72	36

	Female	94	76
Alkaline phosphatase	Male	92	87
	Female	19	22
Alanine amino transferase	Male	94	38
	Female	57	60
Aspartate amino transferase	Male	56	28
	Female	0	12

LY303366 was not associated with any drug related changes in gross pathology, histopathology, absolute or relative organ weights or and did not induce cytochrome P450.

Toxicokinetics:

Table 4. Pharmacokinetics of LY303366 in cynomolgus monkeys after 27 daily intravenous infusions.

	2 mg/kg	5 mg/kg	30 mg/kg
Males AUC	22.6	40.4	495
Cmax	4.48	13.7	110
Females			
AUC	10.2	24.7	402
Cmax	3.97	10.4	106

AUC0-t (μg*hr/mL)
Cmax (μg/mL)

While Cmax and AUC values were approximately dose proportional at the low and mid doses, the increase in AUC and Cmax between the mid and high doses was larger than expected by the increase in dose.

Table 5 Mean Cmax values (μg/mL) at day 15 or day 29 (both sexes combines)

	Day 15	Day 29
2	1.7	4.2
5	4.0	12.1

30	72.8	109

Table 6 AUC values (μg*hr/mL) at day 15 or day 29

	Day 15	Day 29
2	13.6	164
5	20.9	326
30	607	449

Half life values ranged from 1.6 to 7.5 hours on Day 1, to between 3.6 to 12.5 hours on days 15 and 29.

Summary of individual study findings:

Monkeys dosed intravenously with LY303366 at 2, 5 or 30 mg/kg showed few toxic effects. The high dose monkeys showed signs indicative of liver toxicity such as increased liver enzyme activities, but there was no histopathological evidence of liver toxicity. Drug half life ranged between 2 to 12 hours. Cmax and AUC increased with dose, and with repeated dosing. The only exception was that the AUC at the high dose was higher on day 15 than on day 29.

Toxicology Conclusions:

The NOAEL for intravenous LY303366 in cynomolgus monkeys was 5 mg/kg for 4 weeks. High dose monkeys, which showed slight liver enzyme elvations, showed an AUC values around 606 μg*hr/mL. These results predict that liver damage could be expected from exposures to anidulafungin, but that this would occur at exposures higher than the exposures AUC seen in humans after therapy (around 53 μg*hr/mL)

Study 5. 13-week intravenous infusion toxicity study with LY303366 in rats with a 4-week recovery.

Key study findings: Toxic effects in high dose animals. Bone marrow, lung, liver, spleen and skeletal muscle. The exposures observed at this high dose were around 608 μg*hr/mL which is over 11 times the exposure observed at the recommended dose (53 μg*hr/mL).

Study no. [ ]6180-130
Report # 24
Conducting laboratory and location: [ ]

Date of study initiation: September 24, 1996
GLP compliance: Yes
QA report: Yes
Drug, lot # 302SB6
Purity: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water.

Methods

Groups of CDF ® (F-344) BR VAF/Plus ® rats, 10/sex/group, were treated intravenously with LY303366 at 0 (dextrose), 0 (vehicle) or 5, 10 or 30 mg/kg, once daily for thirteen weeks.Two additional groups of animals (5/sex/dose group) were treated for 13 weeks with either vehicle or 30 mg/kg LY303366 and then observed for an additional 4 weeks to assess the reversibility of any toxicology findings which may have been observed at 13 weeks. Rats were approximately 62 days old at initiation of treatment and males weighed between 123 to 188 g and females weighed 118 to 151 g. The infusion was conducted over 20 minutes and the dose rate was 15 mL/kg/hr. When not being dosed with drug, animals received sterile saline solution at a rate of about 1 ml/kg/hour. At the end of the observation period, animals were fasted overnight,
anesthetized with pentobarbital, weighed, exsanguinated and necropsied.

Records were kept of survival and clinical observations (twice daily) body weights (weekly), food consumption (daily) physical examination (pretreatment, weekly) hematology, clinical chemistry (weeks 5, 14 and 18), urinalysis (weeks 2 and 4), toxicokinetics (days 30, 92, 94, 99 and day 106) and ophthalmology (weeks 13 and 18), organ weights and morphologic pathology.

Results

Mortality:

There were no drug related deaths. Mortality and unscheduled sacrifices were ascribed to infusion procedures. Mortality in drug treated groups (1/15 to 3/15) was similar to that in control groups (1/10 to 2/10).
Clinical signs:

Clinical signs were observed at doses of 10 or 30 mg/kg between 10 minutes and 1 hour postdose on days 0 to 4. Signs were consistent with a histamine-related anaphylactic response and included labored, irregular respiration, swollen nose, red ocular discharge, ataxia, lethargy and red ears. At 10 mg/kg, signs were generally seen in fewer animals and lasted for shorter periods.

Body weights:

The dominant effect on bodyweight was that high dose and vehicle-treated males showed lower (up to around 10 % lower) bodyweights than dextrose treated animals. In females, the trend was also seen with the lowest bodyweights being seen in the vehicle and high dose animals. In females, the variation in bodyweights across all groups was not as large.

Food consumption:
Food consumption was generally lower for vehicle treated (males) and high dose animals compared to dextrose control or low and mid-dose animals.

Ophthalmoscopy:

There were no unusual findings from the ophthalmoscopic examination.
Hematology:

In high dose animals, hematology changes included signs of a mild regenerative anemia and consisted of reductions in erythrocyte counts (up to 10 %), and slight (less than 10 %) reductions in hemoglobin and hematocrit concentrations, higher platelet counts, lower prothrombin time and activated partial thromboplastin time.

Clinical chemistry changes consistent with moderate hepatotoxicity were detected in the high dose animals. Changes included increased ALT and AST (3 to 4-fold increases by week 5 and rising to 5 to 6-fold increases by week 14). ALP was also increased (30 to 50 %). Other changes included increased cholesterol (10 % increased in the mid dose group , but 30 to 50 % increased in the high dose). GGT was increased in high dose males and females by week 14 .

Organs affected by LY303366 included the liver, spleen, kidneys and lungs which showed increases in absolute and relative liver weights. At the high dose, relative kidney weights were increased by 14 to 23 %, liver weight was increased by 39 %, spleen weight was up by 59 % and lung weight was increased by 28 %. At the end of the recovery period, liver (+15 %), spleen (+49 %), kidney (+12 %) and lung (+28 %) still showed increased weight.

Macroscopic examination of the animals did not reveal many remarkeable findings. At the high dose, microscopic examination revealed reticulo endothelial hyperplasia in the femoral bone marrow, alveolar macrophage infiltrates in the lung, polykaryocytosis, karyomegaly, sinusoidal lining cell hypertrophy, hepatocellular vacuolation, and individual cell necrosis in the liver. Skeletal muscle showed degeneration, necrosis, or atrophy. Tubular cells in the kidneys showed vacuolation. The mesenteric lymph nodes showed macrophage infiltrates. In the spleen, these animals also showed congestion, macrophage infiltrates and extramedullary erythropoiesis. Other findings included thymic necrosis and vacuolation of the epithelium of the epididymis.

The findings in the bone marrow, lung, liver (hepatocellular hypertrophy in one male (of 5) and one female (of 5) high dose animal) , muscle, kidney, lymph node, spleen and

epididymis were still present at the end of the recovery period but reduced in severity. Liver enzyme levels had returned to normal.

Histopathology: Adequate Battery: yes

Toxicokinetics:

Table 7. Plasma concentrations of LY303366 in rats following intravenous administration of LY303366 for three months.

	AUC ss	Cmax	T ½
	µg*hr/mL	µg/mL	h
5 mg/kg	117	10.6	27.2h
10 mg/kg	220	19.2	27.8h
30 mg/kg	608	69.7	46.5h

Summary

Toxic effects were largely associated with the high dose animals. These included changes in the bone marrow, lung, liver, spleen and skeletal muscle. The exposures observed at this high dose were around 608 µg*hr/mL which is much higher than the exposure observed in the clinic 53 µg*hr/mL.

Study 6. 13-week intravenous infusion toxicity study with LY303366 in cynomolgus monkeys with a 4-week recovery.

Key study findings: Alkaline phosphatase levels were increased (by 20 to 50%) at all doses. Pathology findings in the liver and salivary glands. LY303366, at an intravenous dose of 35 mg/kg produces hepatocellular hypertrophy in monkeys which does not reverse after a four-week "recovery" period. Liver inflammation is observable at 10 mg/kg. This dose produces an

AUCss of 55 μg*hr/mL, an exposure that approximates exposure at the recommended clinical dose.

Study no. [ ] 6180-130
Report # 23
Conducting laboratory and location: [ ]

Date of study initiation: September 19, 1996
GLP compliance: Yes
QA report: yes
Drug, lot # 302SB6
Purity:
Vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water.

Methods

Groups of cynomolgus monkeys, 4/sex/group, were treated intravenously with LY303366 once daily for thirteen weeks. The five dose groups received either dextrose, vehicle, or 5, 10 or 35 mg/kg LY303366. An additional two monkeys/sex, designated as recovery animals, were treated for thirteen weeks, after which treatment was discontinued and animals observed for four additional weeks to assess the reversibility of any changes observed at the end of the thirteen week period. Monkeys were described as young to adult and weighed 2.0 to 3.2 kg at the beginning of the study. The infusion was conducted over 20 minutes and the dose rate was 15 mL/kg/hr. When not being dosed with drug, animals received lactated Ringers solution at a rate of about 3 ml/kg/hour. At the end of the 4 week observation period, animals were fasted overnight, anesthetized with pentobarbital, weighed, exsanguinated and necropsied.

Records were kept of survival and clinical observations (twice daily) body weights (weekly), food consumption (daily) physical examination (pretreatment, week 1 and week 14 and 19) hematology, clinical chemistry and urinalysis (weeks -1, 1, 5, 14, and 18), toxicokinetics (days 30 and 89) and ophthalmology (pretreatment, week 13 and week 18), organ weights and morphologic pathology.

Results.

The only significant clinical finding in monkeys administered LY303366 was a slightly higher incidence of liquid or non-formed feces. This finding was transient and is occasionally seen in monkeys and so was not clearly drug related.

Cholesterol values were occasionally elevated in all three dose levels, but the significance of these findings were confounded by the findings of increased cholesterol in several dextrose-treated animals at various time points. Alkaline phosphatase levels were significantly higher than controls in high dose animals at weeks 1, 5 and 14. It was higher (by 20 to 50 %) in all dose groups in both sexes compared to one or both controls in weeks 5 and 14. The strength of the signal was not always dose related, but the the high dose tended to produce the more robust increases and produced the effect earlier.

The most consistent findings were observed in the liver. These included increased absolute and relative liver weights (increased by 28 % at week 14 in high dose animals). Microscopically, changes included chronic inflammation (two high dose males and one mid dose female), and hypertrophy with some sinusoidal and perisinusoidal cells containing blue staining material (three high dose males). At the end of the recovery period, one male and one female high dose animal still showed hypertrophy with some sinusoidal and perisinusoidal cells containing blue staining material.

The only other gross pathology change was an increase in the relative mandibular salivary gland weight which was increased in mid dose females at week 14 but was also increased in high dose males and females at the end of the recovery period.

Pharmacokinetics

Table 8. Plasma concentrations of LY303366 in monkeys following intravenous administration of LY303366 for three months.

	AUC ss µg*hr/mL	Cmax µg/mL	T ½ (h)
5 mg/kg	23.8	8.9	3.6
10 mg/kg	55.3	20.6	4.1
30 mg/kg	441	103.7	4.7

Summary

LY303366 was administered intravenously at 5, 10 or 35 mg/kg to cynomolgus monkeys for thirteen weeks. An additional group of animals was allowed to recover for four weeks after the end of dosing to assess the reversibilty of any toxic effects. Cholesterol levels were increased at all doses. Increases ranged between 10 and 92 % and were generally dose related. However, on a number of occasions, the cholesterol values were increased in dextrose treated animals. As such, the relationship between the cholesterol level increases and the drug remains unclear. Alkaline phosphatase levels were increased (by 20 to 50%) at all doses. Pathology findings were restricted to the liver and salivary glands. Relative liver weights were increased in high dose animals and microscopically, mid and high dose animals showed inflammation. Hepatocellular hypertrophy, with some sinusoidal and perisinusoidal cells containing blue staining material was observed in (3/8) high dose animals at the end of dosing and in (2/4) animals at the end of the "recovery" period. LY303366, at an intravenous dose of 35 mg/kg produces hepatocellular hypertrophy in monkeys which does not reverse after a four-week "recovery" period. Liver inflammation is observable at 10 mg/kg. This dose produces an AUCss of 55 µg*hr/mL, that approximates the clinical exposure.

Summary of Nonclinical Findings

Toxicology studies of anidulafungin have mostly been conducted in rats and monkeys and include intravenous studies of up to 13 weeks. These are supported by additional studies in mice and dogs. The sponsor recommends that the drug be given by intravenous administration at a single 100 mg loading dose followed by a 50 mg (0.8 mg/kg) dose thereafter. The longest human exposure has been reported to be 42 days but the typical treatment is expected to be on the order of 10 days. The AUC recorded in patients given the recommended dose is 53 μg*h/mL.

In repeat dose studies, the principal clinical finding was a transient infusion reaction consisting of swollen snout, red ears and hypoactivity. These signs were only observed for the first few days of dosing. These effects were not listed among the common adverse events in the clinical trial.

Rats treated with anidulafungin exhibited a regenerative anemia which the sponsor ascribes to excessive blood sampling. No hemolysis was observed in monkeys. *In vitro* exposure of rat or human blood cells to anidulafungin showed that rat erythrocytes were more sensitive than human erythrocytes to the hemolytic effects of anidulafungin (0.7 % hemolysis in humans versus 17 % in rat cells at 8.78 mg/ml anidulafungin). This hemolysis seen in rats seems to be a species specific finding.

In four- and thirteen week studies in rats and monkeys, liver toxicity was observed including increased liver weights, hepatocellular hypertrophy, increased activity of AST and ALT and liver necrosis. These changes were beginning at doses around 8 fold higher than recommended doses based on AUC comparisons. Animals allowed a one month recovery period after the end of dosing still showed microscopic changes in the liver. Increased liver enzymes have been observed in patients that received high doses of anidulafungin (see Overdose section of the label). Other signs observed in high dose animals included kidney tubular vacuolation, skeletal muscle atrophy and increased spleen, kidney and lung weight.

V. GENETIC TOXICOLOGY:

Study 7. The effect of LY303366 on the induction of reverse mutations in *Salmonella typhimurium* and *Escherischia coli* using the Ames Test.

Key findings: LY303366 was positive in one of two lots tested. Additional studies were negative but the purity of the drug product could not be verified in these studies.

Study numbers: 941116AMS3683, 941206AMS3683, 941220AMS3683, 941221AMS3683, 950125AMS3683, 950207AMS3683, 950214AMS3683.

Report # 5
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: November 16, 1994
QA reports: yes
GLP compliance: The studies were non-GLP. The stability of LY303366 under the conditions of administration was not determined. In addition, the drug substance was not characterized in lots 917-AXS-159, DTC-124, DTC-132, 312SB4.

Table 9 Drug lot numbers used in Ames test

Study number	Lot number
941116AMS3683	603CD4
941206AMS3683	603CD4
941220AMS3683	917-AXS-159
941221AMS3683	DTC-124
950125AMS3683	DTC-132
950207AMS3683	312SB4
950214AMS3683	500CB5

Purity: The purity for the LY303366 was determined to be [redacted] for lot 603CD4 (Study numbers 941116AMS3683 and 941206AMS3683) and [redacted] for lot 500CB5 (Study 950214AMS3683).

Methods:

This study was designed to assess the potential of LY303366 to induce bacterial mutation in *Salmonella typhimurium* strains TA1535; TA 1537; TA98 and TA100 and *Escherischia coli* strain WP2uvrA- using the Ames Test. The test was conducted with and without metabolic (S9) activation.

Bacteria were exposed to LY303366 at 125 to 2000 g/plate. In the range-finding study, (50 to 5000 g/plate), no toxicity was recorded but a chemical precipitate was observed at concentrations of 2000, 3000, 4000 and 5000 g/plate. Vehicle control plates were exposed to DMSO. Positive controls were N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2NF), 9-aminoacridine (9AmAc), and 2-aminoanthracene (2AA).

LY303366 was mixed with the appropriate tester strain and agar and incubated for 48 hours at 37 C. Each condition was tested in triplicate. Revertant colonies were counted using an [ ] Automated Colony Counter [ ], except where precipitate prevented the use of this counter. If a precipitate was present, the revertants were hand counted.

LY303366 produced a dose related increase (with at least a doubling in the numbers of revertants) in the TA1535, TA 1537, TA 98 and WP2uvrA- strains with metabolic activation in study 941116AMS3683. The number of revertants counted in the absence of metabolic activation was also increased at least two-fold in the TA1535, TA 1537 strains. The number of revertants also increased with the other strains but the increases produced were less than two-fold. Using a two fold increase as the criterion for a positive result, LY303366 produced a positive response in the Ames test.

In a repeat study (941206AMS3683) using the same lot of drug (603CD4) LY303366 again produced a positive response. The drug produced dose-related increases in the number of revertants in the TA1535, TA 1537, TA 98 and WP2uvrA- strains with metabolic activation and increases in the numbers of revertants in TA1535 without metabolic activation.

Unwilling to accept the positive result, the sponsor repeated the experiment with five additional lots of drug. For four of the lots, there was no characterization available and so the purity could not be determined. In addition, while the lot 603CD4 (used to obtain the two positive results) and lot 500CB5 were stored at [ ] the other lots were stored at temperatures between [ ] Lot 500CB5 was characterized with a potency of [ ] on an anhydrous basis and lot 603CD4 was characterized at [ ] on an anhydrous basis.

LY303366 was negative in all the additional lots tested. Since the storage temperature was not optimal ([ ]) and the lots were not characterized, it is not clear what chemical was tested in experiments 941220AMS3683, 941221AMS3683, 950125AMS3683 and 950207AMS3683. The usefulness of these results in assessing the genotoxic potential of LY303366 remains in doubt. Lot 500CB5 was manufactured for use in a clinical trial, and reported to contain [ ] related substances as impurities. The positive lot (603CD4) was reported to contain [ ] related substances. The sponsor speculated that lot 603CD4 was positive because it contained [ ] impurities which were not present in the other lots. Since no evidence was produced to substantiate this theory, we have to accept the positive result of the lot 603CD4. The negative result from lot 500CB5 raises the question of how the manufacturing process at [ ] is different from the process at [ ] Further testing of randomly selected, well characterized clinical lots could help settle the question.

Summary of study findings

LY303366 was positive in the Ames test in one of two lots tested. Four other lots tested were also negative, but the purity of these lots had not been characterized.

8. The Effect of LY303366 on the induction of forward mutation at the Thymidine kinase locus of L5178Y mouse lymphoma cells.

Key findings: LY303366 did not induce forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells

Study no:941005MLA3683
Report # 1
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: October 5, 1994
GLP compliance: The studies were non-GLP. The stability of LY303366 under the conditions of administration was not determined.

QA reports: yes
Drug, lot # 603CD4
Purity: [ ]
Formulation/vehicle: LY303366 was dissolved in DMSO

This study was designed to determine the mutagenic potential of LY303366 as measured by its ability to induce the heritable loss of thymidine kinase (TK) activity in formerly TK competent L5178Y cells(TK $^{\pm}$). Mutants lack the salvage enzyme and are detected by their resistance to the lethal thymidine analogs, 5-bromo-2'-deoxy-uridine or trifluorothymidine (TFT). The use of rat liver microsomal enzymes (S9) is designed to detect promutagens.

Cultures of L5178Y cells, were treated with LY303366 at doses between 20 and 100 μg/ml (in the presence of S9) or 1 to 40 μg/ml (without S9). Cultures were conducted in triplicates. The doses were selected based on the preliminary toxicity assay in which a concentration dependent cytotoxic response was observed. Mutant colonies were counted using an [ ] Automated Colony Counter [ ], except where precipitate prevented the use of this counter. If a precipitate was present, the revertants were hand counted. Test article was judged to have induced a positive response when a concentration-dependent increase in TK-/- frequency is observed and when the values for mutation index are at least twofold greater than control values.

Drug treated cultures showed mutation frequency values similar to that seen with DMSO. Cultures treated with 3-methylcholanthrene (3MC), which served as the positive control for the activated assay or ethylmethylsulfonate (EMS), which served as the positive control for the nonactivated assay had mutation frequencies 7 to 50 times greater than those seen with LY303366 or DMSO.

Summary of individual study findings: LY303366 showed no evidence of mutagenic potential since it did not induce mutation in L5178Y cells(TK $^{\pm}$).

9. The Effect of LY303366 on the *in vitro* induction of chromosome aberrations in Chinese Hamster Ovary Cells

Key findings: LY303366 increased the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation. LY303366 has demonstrated the potential to produce clastogenic affects

Study no: 941109CAB3683 and 941207CAB3683

Report #: 4
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: November 9, 1994
GLP compliance: Non GLP.
QA reports: yes.
Drug lot # 603CD4
Purity: C 3

This study was designed to determine the potential for LY303366 to induce chromosome aberrations in Chinese Hamster Ovary (CHO) cells. CHO cells were exposed to LY303366 for four hours at concentrations of 5, 7.5, and 12.5 μg/ml without metabolic activation or 250, 275 or 325 μg/ml with metabolic activation. Doses were selected based on preliminary toxicity tests such that at least one treatment would have 40 to 60 % relative growth and two to four treatments would have greater than 60 % relative growth. The test was non-GLP in that the stability of LY303366 under test conditions was not determined. The metabolic activation system consisted of one part thawed rat liver homogenate (S9) added to three parts cofactor mix (cofactor mix contained 15 mg/ml isocitric acid and 8 mg/ml nicotinamide adenine dinucleotide phosphate). Negative control cultures were exposed to vehicle (DMSO) while mitomycin C was the positive control for the nonactivated portion of the assay. Cyclophosphamide served as the control for the activated portion. After the four hour incubation, cells were washed and allowed to incubate in normal (McCoy's complete) medium for an additional 18 hours. At the end of this period, Colcemid was added to the incubation medium to arrest dividing cells in metaphase. About two hours after the addition of Colcemid, metaphase cells were collected by centrifugation, and slides prepared. Three cultures were prepared for each condition, but one was used to assess cytotoxicity. Two slides were prepared from each of the two remaining cultures. Fifty metaphase figures from each treatment and vehicle control culture and twenty-five from one of the two positive controls was read. The test article is identified as clastogenic when a dose related increase in chromosomal aberrations is observed, in which the number of aberrations is statistically greater than that of the concurrent control value as determined by a trend test for binomial distribution.

Results

LY303366 increased the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation. There was no such increase in the absence of metabolic activation.

Table 10. LY303366 exposure resulted in an increase in the percentage of cells with aberrations including gaps in the presence of metabolic activation.

Treatment	Cells scored	Chromosome gaps	Aberrations *(%)
Vehicle control	100	1	4
Cyclophosphamide	25	11	76
LY 250 µg/ml	100	1	4
LY 275 µg/ml	100	2	9
LY 325 µg/ml	100	7	10

*% cells with aberrations including gaps. The most common finding was chromosome gap.

Summary

LY303366 increased the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation. LY303366 has demonstrated the potential to produce clastogenic affects.

10. The Effect of LY303366 given intravenously for two consecutive days on the induction of micronuclei in bone marrow of ICR mice.

Key findings: LY303366 was not clastogenic.
Study no: 941012MNT3683
Report #: 12
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: May 22, 1996
GLP compliance: non-GLP. The stability of LY303366 under the conditions of administration was not determined for the low dose.
QA reports: yes.
Drug lot # 135M6
Purity: [ ]

This study was designed to determine the potential of LY303366 to induce micronuclei *in vivo* in the bone marrow of male and female ICR mice. Mice were treated intravenously on two consecutive days and approximately 24 hours after the second injection, bone marrow was collected and the frequency of micronucleated polychromatic erythrocytes was determined microscopically. Groups of 5 animals per sex were treated with either Group 1, dextrose (5 %), Group 2, vehicle (glacial acetic acid, 0.3 %, polysorbate 80, 2.5 %, and mannitol 5.0 % in purified water) Group 3, 24 mg/kg LY303366, Group 4, 48 mg/kg LY303366, or Group 5, 96 mg/kg LY303366. Group 6 served as the positive control and received cyclophosphamide. Animals treated with vehicle controls and positive controls were expected to yield MN frequencies consistent with historical controls. A test for a positive trend in Poisson data was performed on the micronucleated PCE count for each sex and for both sexes combined.

Results

LY303366 produced a slight increase in micronucleated polychromatic erythrocytes (MPCE) in males at 96 mg/kg, but values were within the range of those observed for historical controls.

Table 11 Micronucleated polychromatic erythrocytes after LY303366

Sex	Treatment	Dose mg/kg	PCE/NCE ratio	MPCE/1000 PCE
Males	5 % dextrose	0	1.1	1.0
	vehicle	0	0.8	1.2
	LY303366	24	1.0	0.8
	LY303366	48	1.1	0.8
	LY303366	96	1.1	**1.6**
	CP		0.3	11
Females	5 % dextrose	0	1.0	0.8
	vehicle	0	1.1	1.2
	LY303366	24	1.4	1.6
	LY303366	48	1.2	1.4
	LY303366	96	1.1	0.8
	CP		0.8	8.6

(CP=cyclophosphamide)

Summary of individual study findings:

LY303366 did not show evidence of clastogenicity. Values obtained with drug were similar to those observed with historical controls.

Study validity: The study was non-GLP since the stability of LY303366 under the conditions of administration was not determined for the low dose. However, it is accepted as a valid study since cyclophosphamide showed a significant increase in micronucleated polychromatic erythrocytes.

11. The Effect of LY303366 given orally for two consecutive days on the induction of micronuclei in bone marrow of ICR mice.

Key findings: LY303366 did not produce an increase in micronucleated polychromatic erythrocytes in male or female mice

Study no: 941012MNT3683
Report #: 2 Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: October 12, 1994
GLP compliance: GLP.
QA reports: yes.
Drug lot # 603CD4
Purity: [redacted]

This study was designed to determine the potential of LY303366 to induce micronuclei *in vivo* in the bone marrow of male and female ICR mice. Mice were treated orally with LY303366 on two consecutive days and approximately 24 hours after the second dose, bone marrow was collected and the frequency of micronucleated polychromatic erythrocytes was determined microscopically. Groups of 5 animals per sex were treated with vehicle (10 % w/v aqueous acacia), Group 1, 50 mg/kg LY303366, Group 2, 1000 mg/kg LY303366, Group 3, or 2000 mg/kg LY303366, Group 4. The positive control group received 25 mg/kg cyclophosphamide.

Table 12. Incidence of multinucleated polychromatic erythrocytes in bone marrow of ICR mice given LY303366 by oral gavage. Wright-Giemsa stain.

Sex	Treatment	Dose	PCE/NCE ratio	MPCE/1kPCE
Male	Vehicle	20 ml/kg	1.1	0.8
	LY303366	500 mg/kg	1.4	1.0
	LY303366	1000 mg/kg	1.6	3.4
	LY303366	2000 mg/kg	1.3	2.2
	CP	25 mg/kg	1.1	5.2
Female	Vehicle	20 ml/kg	1.6	1.2
	LY303366	500 mg/kg	1.3	2.0
	LY303366	1000 mg/kg	1.6	2.2
	LY303366	2000 mg/kg	1.5	1.0
	CP	25 mg/kg	1.5	6.4

Table 13. Incidence of multinucleated polychromatic erythrocytes in bone marrow of ICR mice given LY303366 by oral gavage.Acridine Orange stain.

Sex	**Treatment**	**Dose**		**MPCE/1kPCE**

Male	**Vehicle** **LY303366** **LY303366** **LY303366** **CP**	**20 ml/kg** **500 mg/kg** **1000 mg/kg** **2000 mg/kg** **25 mg/kg**		**1.2** **1.6** **1.0** **1.4** **10.4**
Female	**Vehicle** **LY303366** **LY303366** **LY303366** **CP**	**20 ml/kg** **500 mg/kg** **1000 mg/kg** **2000 mg/kg** **25 mg/kg**		**0.6** **1.4** **0.8** **1.8** **15.0**

Results and Discussion

The administration of LY303366 to mice resulted in an increase in multinucleated polychromatic erythrocytes in males as counted by Wright-Giemsa stain. In female mice the increase was not dose related. The sponsor agrees that the effect was clear in males but seeks to verify the finding by repeating the study and using a different (acridine orange) stain to identify the PCE's and micronuclei. The sponsor attempts to justify the second study by referring to a paper by Hayashi et al which includes a discussion of "staining artifacts" associated with Wright-Giemsa stain. (Hayashi et al. An application of acridine orange fluorescent staining to the micronucleus test. *Mutat. Res. 120,* 241-247).

Using the acridine orange stain, there is no consistent increase in multinucleated polychromatic erythrocytes in males or females. The values in the difference in values between the drug treated females and controls may be due to the abnormally low vehicle control.

Summary

LY303366 did not produce an increase in micronucleated polychromatic erythrocytes in male or female mice.

Genetic toxicology summary:

LY303366 produced inconsistent results (positive and negative results) in the Ames Test using different batches of drug. The sponsor concluded that some batches of the the drug contained a genotoxic contaminant. LY303366 was negative in the mouse micronucleus assay and mouse lymphoma assay but did increase the number of chromosome gaps in Chinese Hamster Ovary cells in the presence of metabolic activation.

CARCINOGENICITY:

Long-term studies in animals have not been conducted to evaluate the carcinogenic potential of anidulafungin.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

12. A combined Segment I and Segment II study of intravenously administered LY303366 in CD Rats

Key study findings: LY303366 injections given for two weeks prior to conception and up until day 19 of gestation resulted in skeletal changes in the fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These changes occurred at doses beginning at 2 mg/kg, a dose equivalent to a human dose of 0.33 mg/kg or 20 mg for a 60 kg patient.

Study numbers: R12896 and R12996
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: August 5, 1996
GLP compliance: Yes
QA reports: Yes
Drug, lot # 135RM6
Purity: [ ]
Formulation/vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water diluted with 5 % dextrose.

This study was performed to evaluate the parental, reproductive and developmental toxicities of LY303366. Male and female CD rats, 20/sex/dose group, were treated intravenously with LY303366 at 0, 2, 5 or 20 mg/kg/day. Males were treated for four weeks, prior to cohabitation, through cohabitation and were killed after six weeks of treatment. High dose male rats were treated with 20 mg/kg for one week and then the dose was increased to 30 mg/kg. Red urine was observed in most of these rats after treatment and so the dose was reduced to 20 mg/kg for the remainder of the study. Females were treated for 2 weeks prior to cohabitation until day 19 of gestation. Females were euthanized on Gestation day 20 to assess reproductive parameters. Fetuses were evaluated for viability, morphology, gender and weight. Necropsies were performed and tissues were collected and weighed. Liver, skeletal muscle and male reproductive tissue were preserved for histological examination and epididymal sperm concentrations and sperm motion parameters were evaluated.

No animals died in this study. Treatment of rats with LY303366 at 30 or 20 mg/kg resulted in red colored urine in all males and two females. Females also exhibited postdose hyperactivity on the first day of drug administration. Animals experienced minor changes in body weight gain and food consumption. One high dose female had an adenocarcinoma of the mammary gland.

Anidulafungin did not affect reproductive performance, sperm concentration or sperm motility.

Fetuses from dams treated with LY303366 up to day 19 of gestation showed more skeletal abnormalities than fetuses from control dams. The most common finding was incomplete ossification of various bones but also included wavy, misaligned and misshapen ribs. Incomplete ossification was seen in the forepaw, hindpaw, sternebra, cervical vertebra, thoracic vertebra, pelvic girdle and skull. These findings were seen at all LY30336 doses and the frequency did not depend on dose.

Summary of individual study findings

In conclusion, LY303366 injections given for two weeks prior to conception and up until day 19 of gestation resulted in skeletal changes in the fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These changes occurred at doses beginning at 2 mg/kg, a dose equivalent to a human dose of 0.33 mg/kg or 20 mg for a 60 kg patient.

13. A segment II developmental toxicity study of LY303366 administered intravenously to pregnant New Zealand White rabbits

Key study findings: All doses: Incomplete ossification and extra aortic arch. Mid/high dose animals: lower fetal weights. High dose animals;late resorptions, abortion.
Study no:BO2396
Conducting laboratory and location: Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company. Greenfield IN 46140.
Date of study initiation: October 4, 1996
GLP compliance: Yes
QA reports: Yes
Drug lot: B02SB6
Purity: C J
Formulation/vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water diluted with 5 % dextrose.

Methods:

This study was performed to evaluate the maternal, reproductive and developmental toxicities of LY303366, when administered to New Zealand White rabbits during organogenesis. Groups of pregnant female New Zealand White rabbits, 20/dose group, were treated intravenously with LY303366 at 0, 5, 10 or 20 mg/kg/day. Rabbits were treated on gestation days 7 through 19 and were euthanized on Gestation day 28 to assess maternal reproductive parameters. Fetuses were evaluated for viability, morphology, gender and weight.

One dam from the high dose group aborted and was terminated on gestational day 22. This dam had been anorectic since day 14. Mean body weight gain was depressed in high dose rabbits between days 17 and 20 (a loss of 1.7 g/day versus a gain of 8.7 g/day in controls). There was no parallel decrease in food consumption over this period.

There was also a slight but significant decrease (-8%) in the male and female (combined) litter weight and a decrease (-7%) in the male litter weight in the high dose group. In the mid dose there was a 6 % decrease in male fetal weight. LY303366 was associated with a slightly increased number of fetuses with incomplete ossification as well as fused misshaped or misaligned sternebrae and fused ribs. High dose dams also showed four times more late resorptions per litter than control animals. There was also a two to three-fold increase in the number of fetuses and litters with an extra aortic arch at all doses of LY303366.

Summary of individual study findings:

LY303366 administration to New Zealand White rabbits was associated with abortion, late resorptions and a number of fetal abnormalities. Incomplete ossification and fetuses with an extra aortic arch were increased at all doses. Mid and high dose animals at high doses (about 4 or 8 times the recommended maintenance dose) showed lower fetal weights. High dose animals showed a four-fold increase in late resorptions and one abortion.

14 Study title: Intravenous developmental and perinatal/postnatal reproduction toxicity study of anidulafungin in rats, including a postnatal behavioral/functional evaluation.

Key study findings: Drug was detected in the blood of pups and in the milk of drug-treated dams.

Study no.: 622-001
Conducting laboratory: [illegible]
Date of study initiation: October 2001
GLP compliance: Yes
QA reports: yes
Drug lot: 793753A
Purity: [illegible]
Formulation/vehicle: Drug was dissolved in a vehicle consisting of polysorbate 80 (2.5 %), mannitol parenteral (5 %) and glacial acetic acid (0.3 %) in purified water diluted with 5 % dextrose.

This study was performed to evaluate the maternal, reproductive and developmental toxicities of LY303366, when administered to — CD®(SD)IGS BR VAF/Plus® female rats from implantation through lactation and weaning on gestation, parturition, lactation and maternal behavior in female rats.and on the development of the offspring of the treated female rats. Five groups of pregnant — CD®(SD)IGS BR VAF/Plus® female rats, 25/dose group, were treated intravenously with LY303366 at 0 (vehicle), 0 (dextrose, diluent) 2, 6 or 20 mg/kg/day. Three additional rats per group were added to the LY303366 groups for pharmacokinetics evaluations. Rats were treated intravenously from day 7 of gestation to day 20 of lactation. Records were kept of body weights, food consumption, clinical signs, abortions, premature deliveries and deaths. On gestational day 20, maternal and fetal blood samples were collected from the pharmacokinetics animals. For the F1 generation, records were also kept of sexual maturation, body weights, long and short term memory, activity, learning, auditory startle habituation response, mating, fertility index and pregnancy rates.

Results:

No dams died during the study. Clinical signs consisted of swollen snouts during gestation and swollen tails (injection site) during gestation and lactation. Maternal body weight gain was reduced by 68 % between days 7 and 10 of gestation. During this time feed consumption was reduced by 6 %. After delivery, maternal body weight gain was reduced by 55% between days 10 and 14 while body weights were reduced by 5 to 6% on days 14 to 20 of lactation. There were no drug related deaths in F1 animals. F1 animals from drug-treated dams were not different from control animals in any of the parameters examined. Drug was detected in the blood of pups and in the milk of drug-treated dams (see Table 14 below).

Table 14. Toxicokinetics: Anidulafungin levels in plasma, pup plasma and milk of anidulafungin treated dams.

Dose	[LY303366] plasma Dams (µg/ml)*	[LY303366] plasma pups (µg/ml)	[LY303366] milk (µg/ml)
2	1.6	0.1	1.1
6	6.2	0.6	3.5
20	18.5	1.9	14.6

*(6h postdose)

Reproductive and developmental toxicology summary:

LY303366 injections resulted in skeletal changes in rat fetuses including incomplete ossification of various bones and wavy, misaligned or misshapen ribs. These bone changes occurred at doses at or above 2 mg/kg, a dose equivalent to 0.4 times the recommended clinical maintenance dose (based on body surface area comparisons).

In rabbits at higher doses, (4 to 8 times the recommended dose) there was a reduction in litter weight and increased late resorptions. There was an increase in the number of fetuses and litters with an extra aortic arch at doses two times the recommended dose. Drug was detected in the blood of pups and in the milk of drug-treated rats.

Owen G. McMaster, Ph.D.
Pharmacology/Toxicology Reviewer, DSPIDP

Concurrences:
HFD-590/DeputyDirector/SGitterman
HFD-590/ActingPharm/ToxTL/SHundley

cc:
HFD-590 Original IND
HFD-590/Biopharm/ChilukuriD
HFD-590/BiopharmTL/ColangeloP
HFD-590/PM/MillerK
HFD-590/Chem/SeggelM
HFD-590/ChemTL/SchmuffN
HFD-590 Division File
HFD-590/Micro/ShanmugamB
HFD-590/MO/IbiaE
HFD-590/MOTL/CavailleCollM
HFD-590/Pharm/McMasterO
HFD-590/ActingPharmTL/HundleyS

HFD-590/Stat/DixonC
HFD-590/StatTL/HigginsKar
HFD-340

Appendix 1. Histopathology Inventory for NDA # 29

Study	15	16	23	24
Species	RAT	MNKY	MNKY	RAT
Adrenals	X*	X*	X*	X
Aorta	X	X	X	X
Bone Marrow smear	X	X	X	X
Bone (femur)	X	X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X*	X*	X*
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube	X			
Gall bladder	X	X	X	X
Gross lesions	X	X	X	X
Harderian gland				
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland				
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X*	X*	X*
Lymph nodes, cervical				
Lymph nodes mandibular		X	X	X
Lymph nodes, mesenteric		X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves		X	X	
Ovaries	X*	X*	X*	
Pancreas	X	X	X	X
Parathyroid	X			X
Peripheral nerve				
Pharynx				

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

X, histopathology performed
*, organ weight obtained

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/s/

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5/13/04 03:33:00 PM
PHARMACOLOGIST

Steve Hundley
5/13/04 04:45:15 PM
PHARMACOLOGIST