CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

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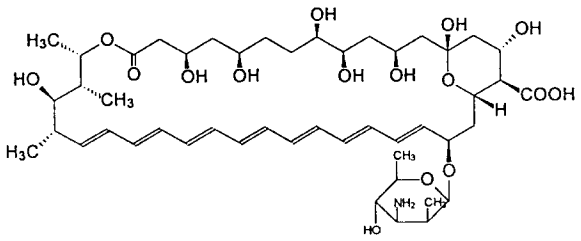
Sponsor: Fujisawa USA, Inc.
Three Parkway North
Deerfield, Illinois 60015

Drug: AmBisome



Structure: Amphotericin

Proposed use: Antifungal

Introduction:

Amphotericin B is a polyene fungicidal antibiotic used to treat systemic mycoses in man. It has strong affinity for ergosterol in fungal cell membranes, and, when amphotericin B binds to ergosterol, the fungal cell begins to leak. Despite its efficacy, the use of Amphotericin B is severely restricted because of a number of side effects, including nephrotoxicity. Conventional amphotericin B (cAmB, Fungizone ®) is solubilized for injection with the bile salt sodium deoxycholate, which dissociates rapidly in the plasma. The released amphotericin subsequently binds to cholesterol sulphate-containing membranes and plasma lipoproteins and this may account for its toxicity in mammals.

In an attempt to reduce the toxicity of amphotericin B while maintaining efficacy, the drug has been intercalated into the membrane bilayer of liposomes. Liposomes are submicroscopic spheres typically composed of phospholipids and sterols having an aqueous core surrounded by a molecular bilayer.

Preliminary toxicology studies with AmBisome® in female mice and rats have shown a reduced toxicity when compared to equivalent doses of conventional amphotericin B. In addition, *in vitro* and *in vivo* study results indicate that AmBisome® has retained the antifungal activity of amphotericin B.

This submission contains final reports of the studies submitted to support the NDA.

Toxicology Studies Summary

1. Routine safety testing of AmBisome in mice and single dose lethality studies .
2. AmBisome and Fungizone single dose pilot study in rats.
3. Pilot single dose pharmacokinetics characterization of AmBisome in female rats.
4. Pilot studies to characterize the acute toxicity of single dose AmBisome in Charles River CD rats.
5. 14-day subacute toxicity study of AmBisome in mice
6. Subacute toxicity study of AmBisome in rats (30 day).
7. A 30 day toxicity and toxicokinetic study of intravenously administered AmBisome in rats.
8. A 30 day toxicity and toxicokinetic study of intravenously administered AmBisome in beagle dogs.
9. 91-Day Toxicity, Toxicokinetic and Post-Dosing Recovery Study of Intravenously Administered AmBisome® (Liposomal Amphotericin B) in Rats.
10. A Pilot segment II reproductive toxicity study of intravenous AmBisome in rats.
11. A Pilot Segment II reproductive toxicity study of intravenous AmBisome in rabbits
12. A Segment I reproduction toxicity study of intravenous AmBisome in rats.
13. A Segment II reproduction toxicity study of intravenous AmBisome in rats.
14. A Segment II reproductive toxicity study of intravenous AmBisome in rabbits.
15. Dye extravasation study.
16. Paravenous and subcutaneous administration of AmBisome and conventional amphotericin B.
17. Evaluation of local tissue reactions to AmBisome: Paravenous and intraarterial administration in rats.

Toxicology Studies Review

1. Routine safety testing of AmBisome in mice and single dose lethality studies. Study report No. 23295105. Study dates February 1994 to October 1995. Non-GLP studies. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive San Dimas, CA 91773.

As required by the USP safety test protocol, each of 31 batches of manufactured AmBisome was routinely tested for safety in a group of five female C57 BL/6 mice (Charles River Laboratories, Wilmington MA) prior to release for patient use. In addition to the safety test, AmBisome was tested for lethality at doses between a 110 and 160 mg/kg in groups of 10

mice each.

The safety test is performed on a pooled sample from four vials which were reconstituted at room temperature with water for injection according to the package insert instructions. Tested batches were manufactured between February 1994 and October 1995. AmBisome was administered intravenously via the tail vein at 100 mg/kg to groups of five healthy female mice. Mice were observed for one hour after injection for signs of acute toxicity and then daily for seven days. In the safety test, if one mouse dies then the batch is retested with 15 mice. If two mice die the batches are re-tested by the same procedure. If three or more mice die the batch failed. There must be at least 90 percent survival for the batch to pass. The LD_{10} and LD_{50} values for each lot were calculated using a computer program by probit/log analysis. Results are shown in the table below

Results

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Animal deaths were recorded between one and four days post injection. Deaths never occurred within one hour of injection. In 13 lots of drug, the number of deaths was so low that LD_{10}'s or LD_{50}'s could not be calculated. For 17 lots the LD_{10} was an average of 115 mg/kg and the LD_{50} was an average of 133 mg/kg. Overall the median LD_{50} value was 150 mg/kg. One lot had a calculated LD_{10} of 100 mg/kg and this lot failed the test. Other lots were released for patient use.

Conclusion

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Test results show that AmBisome batches have a uniformly low acute toxicity in mice (median LD_{50} was 150 mg/kg) compared to conventional amphotericin B (where mice given doses greater than 2.6 mg/kg, die shortly after intravenous injections).

2. AmBisome and Fungizone single dose pilot study in rats. Study # 232-93103. February 1991. NeXstar Pharmaceuticals Inc. , 650 Cliffside Drive, San Dimas, California 91773. Non-GLP study. Fungizone lot number OF28901. AmBisome lot number 0420013ER.

This study was designed to determine the LD_{50} values for AmBisome and Fungizone in female Sprague Dawley rats. Animals were treated by slow injection (over two minutes) into the lateral caudal vein. Animals were observed immediately after injection and one hour later for signs of toxicity. Animals were thereafter checked daily for survival and weighed on days 1, 3, 7, 10 and 14 after injection.

In the Fungizone section of the study, groups of two to four rats were treated with 1.25, 1.50 and 1.75 mg/kg. Both rats treated with 1.75 mg/kg Fungizone and one of the three rats treated with 1.5 mg/kg died within one hour of injection. There were no deaths in the 1.25 mg/kg dose group. Up to 10 % body weight loss was seen in surviving animals.

In the AmBisome section of the study, groups of five animals each were treated with 45, 65 or 80 mg/kg AmBisome. All the rats in the 80 mg/kg group died and four of five of these deaths occurred within the first 24 hours. At 65 mg/kg, three of the five animals died by day 2 post injection. One animal died two days after injection with 45 mg/kg AmBisome.

Conclusion

LD_{50} values for female Sprague Dawley rats were calculated at 1.5 mg/kg for Fungizone and 58 mg/kg for AmBisome.

3. Pilot single dose pharmacokinetics characterization of AmBisome in female rats. Study report No. 232-930106. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive San Dimas, CA 91773. October 1990.

The purpose of this study was to calculate the pharmacokinetic parameters of AmBisome following intravenous administration of AmBisome to rats.

Six female rats received a single intravenous injection of AmBisome at 5 mg/kg via the tail vein. Three animals were bled at each of the following time points after injection: five minutes, 30 minutes, one hour, four hours, eight hours and 24 hours. Plasma amphotericin B concentrations were determined for each plasma sample using an HPLC method. Individual animals were pre-assigned to each time point for bleeding such that no animal was bled more than three times.

The pharmacokinetic parameters of AmBisome determined after a single IV injection in female rats are summarized below.

Table 1. Pharmacokinetic parameters of AmBisome in female rats.

C max	123 μg/mL
Clearance	0.25 mL/min*kg
$T_{½}$ α	0.86 h
$T_{½}$ β	8.7 h
$AUC_{0-\infty}$	330 μg·h/mL
A	108 μg/mL
α	0.013 min^{-1}
B	15.7 μg/mL
β	0.0013 min^{-1}
V_C	0.041 L/kg
V_{SS}	0.12 L/kg
$V_β$	0.19 L/kg
MRT	8.0 h

Summary

In this pilot pharmacokinetics study of AmBisome, the peak plasma drug concentration was 123 μg/ml. The plasma elimination of AmBisome was biexponential with half lives of elimination being 0.86 ($T_{½}α$) and 8.7 hours ($T_{½}β$). Initial volume of distribution was approximately equal to plasma volume in the rat, while steady state volume of distribution was three-fold higher, suggesting uptake into tissues.

4. Pilot studies to characterize the acute toxicity of single dose AmBisome in Charles River CD rats. Study # 232-91100. April 1991. NeXstar Pharmaceuticals Inc., 650 Cliffside Drive, San Dimas, California 91773. Non-GLP study. AmBisome lot numbers 0420013ER, 0420027E and 0421007E.

This report describes three experiments designed to characterize the toxicity of intravenous AmBisome.

In the first experiment, animals were divided into four same sex groups of two animals each. A single 30 mg/kg dose of AmBisome was administered to one group of each sex. Records

were kept of deaths (daily), body weights (days 1, 3, and 7 post treatment) until 7 days post intravenous injection. No male rats died and both animals in both groups of female rats died.

In the second experiment, four groups of female Charles River rats (two groups of two rats and two groups of three rats) were treated with AmBisome either as an undiluted preparation (4.0 mg/ml) or as a diluted preparation (1.0 mg/ml). The two groups of two were each treated with a single intravenous injection of 30 mg/kg AmBisome, with one group receiving an undiluted sample and the other group receiving the diluted sample. The two groups of three were treated with a single injection of 15 mg/kg of either diluted or undiluted AmBisome. Records were kept of clinical signs, deaths, body weights and the study was terminated on day 14. All rats died at 30 mg/kg and one rat which received 15 mg/kg (as 1 mg/ml solution) also died. Thus dose, rather than concentration of drug in the preparation, is the important factor in determining the toxic effects of this drug.

In the third experiment, 15 male and 30 female rats were assigned to single sex groups of five each. Female rats received intravenous AmBisome 14, 17, 21, 25, 30 and 36 mg/kg. The male groups received 70, 84, and 101 mg/kg AmBisome. Record were kept of mortality, body weights and the study was terminated on day 14. All female rats died except one at 14 mg/kg and one at 21 mg/kg. No clinical manifestations were observed in any male rats.

Conclusion: Female Charles River CD rats are more sensitive to the toxic effects of AmBisome than were males.

5. 14-Day Subacute Toxicity of AmBisome in mice. Study Number 232-90100. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive, San Dimas, California 91773. Non-GLP study. AmBisome lot number 0420001R1. May 27, 1990, (R90-0015-AMP-P2-E).

The purpose of this study was to determine the multiple intravenous dose regimen of AmBisome that could be used in mice for a subacute study.

Groups of female mice (strain: C57BL/6 10 animals/group) were administered daily intravenous injections of the following treatments in a maximum injection volume of 0.5 ml/mouse: (1) AmBisome diluted (1:4 with 5% dextrose), 25 mg/kg, (2) AmBisome, 50 mg/kg, (3) AmBisome, 75 mg/kg, (4) non-drug liposomes, 0.4 ml and (5) control buffer (9% sucrose with 10 mM sodium succinate, pH 5.5) for 14 consecutive days.

Initially during the treatment phase, all AmBisome treatment groups showed a slight decrease in physical activity with some piloerection evident. These effects gradually diminished and were not apparent in any of the groups. All surviving animals appeared to be normal after treatment was discontinued.

There were 2 deaths (75 mg/kg). These animals lost 18.4 and 25.7% of their initial body

weights, respectively. The other animals in this treatment group lost body weights during the initial four days of the treatment. After 4 days of treatment, all surviving animals began to gain weight (during the next ten days), and the survivor's average weight returned to its initial value. Maximum weight loss (8% of the average body weight) for the 50 mg/kg treatment group occurred after first injections. At the end of the study, control groups gained slightly more weight (10% vs 5% increase) compared to the 25 mg/kg treatment group. Since kidney function was not evaluated in this study, a NOAEL was not determined.

6. 30-Day Subacute Toxicity of AmBisome in Rats, AmBisome Lot # 0420013E, Vestar, Inc. 650 Cliffside Drive, San Dimas, CA, August 22, 1990, (R90-0016-AMP-P2-E)

The purpose of this study was to establish the dose levels for AmBisome which produced definite subacute toxicity in rats. Groups of female Sprague-Dawley rats (10 animals/group) were administered iv injections (via tail vein) of AmBisome at 25, 50 or 75 mg/kg/day for 30 days. Control groups (2) received either buffered sucrose solution or non-drug containing liposomes.

Mortality:

A summary of the subacute lethality of drug is presented in Table 2. The survivors exhibited a slight to moderate decrease in physical activity throughout the remainder of the injection period.

Table 2 Mortality associated with subacute treatment with AmBisome in Rats

IV Dose (mg/kg/day),	Duration (days)	No. of Death/No.in Group	Day of Death
75	30	9/10	2,2,2,2,3,3,3,3,6
50	30	5/10	2,2,3,3,11
25	30	1/10	5
Non-drug liposomes	30	0/10	-
Buffer	30	0/10	-

Toxicity

Weight loss in the mid and high dose groups averaged , and occurred between day 8 and 10 after the initiation of the study. Beyond that point, the surviving animals in each of the group began to gradually gain weight and were near their initial weight when the study was terminated. White blood cell count, red blood cell count, hematocrit and differential count for surviving animals in all test and control groups were normal. BUN levels were elevated in all treated groups. Creatinine levels were moderately elevated toward the end of the experiment. SGOT and SGPT were extremely high in mid and high dose groups on day 2; SGPT values returned to normal in mid dose group. Histopathology results are summarized in Table 3.

Table 3. Summary of Histopathology Results

Organs	Results
Lung	All groups: mild multifocal peribronchiolitis; a mild multifocal foreign body granulomatous pneumonia in all animals receiving liposomes.
Liver	All treated groups: moderate infiltration of the sinusoids with clusters of macrophages with foamy light brown cytoplasm. Some displacement of the hepatic parenchyma. One low dose animal showed abnormal hepatocytes.
Spleen	All treated groups and drug-free liposome group (3/10): patchy and/or diffuse infiltration of the red pulp with large foamy macrophages.
Kidney	All treated groups: glomerular infiltration by macrophages and interstitial infiltration with lymphocytes, macrophages and neutrophils. Renal tubules mildly to moderately dilated with foamy cytoplasm.
Heart	High dose (1/10) the animal died on day 2: cardiomyopathy.
Brain	normal

Comments: In this 30-day pilot study, deaths were observed at all dose levels. A dose level of AmBisome which produced subacute or cumulative toxic effects in rats was not identified.

7 . 30-Day Toxicity and Toxicokinetic Study of Intravenously Administered AmBisome in Rats, Lot # 0421025E October 28, 1992, (R92-0004-AMP-P2-E)

The objectives of this study were to define the subchronic systemic toxicity and the toxicokinetic profile of AmBisome. Ten groups of male and female Sprague-Dawley rats were administered daily iv doses of amphotericin B as AmBisome (1, 3, 9 or 20 mg/kg/day) for 30 days, for an evaluation of toxicity and 30-dose toxicokinetics (Tox/Tk rats). Injection was given

at 5 ml/kg over a one min period via the tail vein. Rats used were Crl:CD (SD)BR strain, 6 weeks old, for males and for females. Groups 1-4 had 15 animals/sex/group ; group 5, 18 animals/sex/group 5; Group 6, 25 animals/sex/group and groups 7-10, 18 animals/sex/group. Two control groups of rats received either 5% dextrose or non-drug liposomes. These animals (3/sex/group/time-point) were sacrificed following 30 doses to provide plasma for evaluation of the terminal concentration-time profiles, tissues for possible terminal concentration-time profile, and tissue samples for histopathological assessment of toxicity. Groups of 7 to 10, toxicokinetics animals (Tk rats), were administered single iv doses of amphotericin B as AmBisome (1, 3, 9 or 20 mg/kg) and were bled and sacrificed at 0.5, 1, 3, 5, 8 or 24 hr (3/sex/time-point) after single doses. AmBisome concentrations were analyzed by a validated Statistical significance assessments were made at 0.05 or 0.001 levels.

Deaths occurred in 12 of 15 females at 20 mg/kg/day and were attributed to drug. On day 1, two were found dead and four were sacrificed *in extremis*, while on day 2, three animals were found dead and three were sacrificed *in extremis*. Microscopically, the most noteworthy findings in these animals were in the liver, kidney, bone marrow and thymus. The prominent change in the liver was hepatocellular necrosis which ranged from moderate to severe and was centrilobular in distribution. In severe cases, most of the lobular surface was affected. Marked elevations in bilirubin, AST, ALT and alkaline phosphatase were observed where serum was available. In the kidney, mild degenerative processes were accompanied by mild to moderate elevations in BUN and/or creatinine values. The sponsor considered the deaths of 3 males (one each at 1, 3 and 20 mg/kg/day) during the study unrelated to AmBisome treatment. Beside the fact that these three animals died after blood collection, the sponsor did not elaborate further on the cause(s) of deaths.

One animal (20 mg/kg/day female) had clonic convulsions following the second dose. Other clinical signs in this group of females included brown abdominal/urogenital staining, ataxia, lying on side, partly closed eyes and dehydration. Both males and females (20 mg/kg/day) had significantly lower weight gains when compared to the control groups. Following the initial dose, a body weight loss was recorded for the majority of animals. The weight gains for the overall study were significantly lower than those of the control groups. This was also reflected in group mean body weight values which were significantly lower throughout the study. Animals in the 9 mg/kg/day group had slightly lower weight gains (statistically not significant) compared to the control groups. A drug-related reduction in food consumption was noted for males in the 20 mg/kg/day group throughout the study (statistically significant for day 13-20 and 20-27); for females, reductions occurred in the first week; however, for the remainder of the study values were comparable to the controls. No reductions in food consumption were noted for animals at 1, 3 or 9 mg/kg/day and liposome control animals did not differ significantly from dextrose control animals.

There were increased incidences of ureter dilatation and /or thickening, urinary bladder dilatation, enlarged lymph nodes and spleen, and liver abnormalities (discoloration and presence of pale areas or foci) in animals treated with 9 or 20 mg/kg/day AmBisome. Group mean relative liver weights in both male and female animals increased with dose. Relative group mean spleen

weight (in both male and females) were generally significantly larger than controls for doses of 3 or more mg/kg/day. Group mean kidney weights (relative to both body and brain weights) were significantly larger when compared to controls for both males and females (9 or 20 mg/kg/day).

Dose-related and statistically significant decreases in group mean platelet counts were observed for males and females (9 or 20 mg/kg/day) following 27 doses, and females (20 mg/kg/day) following 14 doses, when compared to controls. Drug-related and statistically significant neutrophilia for males and females (9 or 20 mg/kg/day) were noted. On occasion, lymphocyte counts were elevated in groups administered AmBisome, relative to dextrose control. Total WBC for males and females (9 or 20 mg/kg/day) following 14 and 27 doses were increased slightly when compared to controls. Increases in serum BUN concentrations were noted for both males and females following 7, 14 and 27 doses of AmBisome. Dose-related and statistically significant increases in serum AST, ALT and ALP were noted for females (9 or 20 mg/kg/day) following 7, 14 and 27 doses, when compared to controls. A statistically significant and dose-related hypercholesterolemia was observed for males and females (9 or 20 mg/kg/day) following 7, 14 and 27 doses when compared to dextrose control. Dose-related and generally statistically significant decreases in CK and LDH were noted. On occasions, group mean values for several parameters (total protein, Ca, K, Cl and albumin) were noted to be significantly different (either lower or higher, but within normal ranges) when compared to controls (dextrose and/or drug-free liposome).

Microscopically, a change described as foamy cell accumulation was observed in many organs (kidneys, liver, lymph nodes, spleen, injection site and adrenals) in dosed animals and also in the kidneys of liposome control animals. Histologically, large cells (probably macrophages) were observed with an expanding, slightly yellowish and finely vacuolated cytoplasm, which caused the foamy appearance. The severity of the change was dose-related. It was noted predominantly in rats (9 or 20 mg/kg/day) in the kidney, lymph nodes, spleen, adrenals and at injection sites. A transitional cell hyperplasia was observed in the transitional epithelium of the urinary tract (kidneys, ureter and urinary bladder) of males and females at all dose levels. Histologically, it was characterized by a thickened, often basophilic, epithelium with occasional mitotic figures. The hyperplasia was accompanied by a mixed cell infiltration (mainly neutrophils) in the kidneys and ureters of some animals. In the liver, focal to multifocal necrosis was observed in dosed rats with an increased incidence in females, particularly those treated at 9 mg/kg/day; this lesion was also observed in 1 male (3 mg/kg/day) and 1 female (1 mg/kg/day).

Comments: A NOEL could not be determined in this study since urinary transitional cell hyperplasia was observed even at 1.0/kg/day AmBisome.

AmBisome treatment resulted in death or moribundity of approximately 50% of the high dose females before the third dose. The deaths appeared related to liver necrosis, although bone marrow necrosis was also observed. Marked elevations in serum AST, ALT and ALP activity and total serum bilirubin concentration were noted in animals bled prior to sacrifice. Elevations in BUN concentrations were consistent with renal changes: transitional cell hyperplasia, hyalin

and granular casts and tubular vacuolation, necrosis and dilatation. Thus, male and female rats differed in their susceptibility to the acute toxicity of AmBisome.

The target organs were defined as the urinary tract including kidneys, ureter and urinary bladder. A transitional cell hyperplasia was observed in the urinary tract. A neutrophilia was consistent with the microscopic observation of mixed cell infiltration accompanying the hyperplasia in the kidneys and ureters of some animals. An increase in the incidence of tubular basophilia and basophilic material in the kidneys at 20 mg/kg/day was observed. Dose-related increases in BUN concentrations and increased kidney weights were consistent with the observed renal pathology. Liver (8 of 15 females at 9 mg/kg/day and 3 of 13 females at 20 mg/kg/day) had focal to multifocal necrosis in addition to prominent sinusoidal foamy cell accumulation. Serum indicators of liver toxicity were consistent with these findings with increases in AST, ALT and ALP activities as well as increased liver weights. Bone marrow: dose-related mild thrombocytopenia (9 or 20 mg/kg/day) was observed and bone marrow necrosis was observed in animals that died.

Foamy cell accumulation was observed in the liver, kidneys, spleen, lymph nodes, adrenal and injection sites with a dose-related increase in severity. Liposomes are known to be phagocytozed by macrophages of the reticuloendothelial system. In view of their histologic appearance, the foamy cells observed were suspected to be macrophages and, thus, the condition may have represented an adaptive and/or pathologic response.

Toxicokinetics data.

Plasma concentrations of amphotericin B on day 1 were similar in males and females and increased with dose. The $AUC_{0\text{-}24h}$ increased linearly with dose. The overall elimination half life was approximately 10 hours. The overall volume of distribution was 152 ml/kg and mean total clearance was 11.2 ml/h/g. On day 30, amphotericin B plasma concentrations were similar for males and females but higher than respective day 1 concentrations. The overall half life was approximately 10.5 hours. The area under the curve at lower doses was similar to the respective day 1 values, but at higher doses was greater than the incremental increase in dose. The mean overall V was 81 ml/kg, and Clearance was 5.8 ml/h/kg.

Table 4. Plasma Pharmacokinetics Parameter estimates of amphotericin B in rats after a single and 30 daily doses intravenous injections of AmBisome.

Day 1

Dose (mg/kg)	sex	$T_{½}$ (h)	C_{max}	AUC	V (mL/kg)	Cl (mL/hr/kg)
1	M	12	8	72	242	14
	F	7	7	55	177	18
3	M	9	33	288	135	10
	F	7	28	461	64	7
9	M	10	109	988	137	9
	F	6	189	1285	54	7
20	M	8	143	1176	206	17
	F	19	300	2438	197	8

Day 30

Dose (mg/kg)	sex	$T_{½}$ (h)	C_{max}	AUC	V (mL/kg)	Cl (mL/hr/kg)
1	M	9	9	148	88	7
	F	9	9	90	142	11
3	M	10	43	558	75	5
	F	9	29	374	108	8
9	M	20	148	3116	82	3
	F	7	110	1875	50	5
20	M	11	500	6729	47	3
	F	9	380	4418	56	5

Tissue concentrations of amphotericin B increased with dose and higher levels were found in males than in females. Mean tissue concentrations ranged in

males and between 7 and 100 μg/g in females. On day 30, amphotericin B tissue concentrations were considerably higher than on day one . Tissue concentrations increased with dose and were, in descending order: liver = spleen >> kidney >lung > brain for both days 1 and 30. On day 30, tissue concentrations were relatively constant whereas plasma concentrations decreased.

The data seems to suggest that amphotericin B uptake by the reticuloendothelial system is a saturable process which produces a nonlinear increase in plasma concentrations at the two higher doses.

8. 30-Day Toxicity and Toxicokinetic Study of Intravenously Administered AmBisome in Beagle Dogs, Lot # 0421025E (R92-0074-AMP-P2-E)

Seven groups of male and female beagle dogs (age weight for males and for females; 5 animals/sex/group) were administered a daily iv dose of amphotericin B as AmBisome (0.25, 1, 4, 8 or 16 mg/kg/day, 6 ml/kg over a five min period via right or left cephalic vein) for 30 days. Two control groups received either 5% dextrose or non-drug liposomes. For toxicokinetics, serial blood samples were collected at 0.5, 1, 3, 5, 8 and 24 hr post dose on days 1, 14 and 30. AmBisome concentration was analyzed by a validated method. The objectives of this study were to define the subchronic systemic toxicity and the toxicokinetic profile of AmBisome (liposomal amphotericin B) in dogs. Statistical significance assessments were made at 0.05 level.

Mortality

Deaths (moribund sacrifices) of dogs following daily AmBisome administration were as follows: 10/10 animals in the 16 mg/kg/day group, 7/10 animals of the 8 mg/kg group and 1/10 animals of the 4 m/kg dose group.

Toxicity

Red discharge was present in treated animals. Findings (thin, no feces, few feces, vomitus and hypoactive) were noted at morning observations on several days in one or more animals in the treated groups, especially high dose animals (8 or 16 mg/kg/day). Struggling and vocalization were noted in one or more animals of the treated groups during the dosing interval. Vomitus was present in one female (8 or 16 mg/kg/day).

Body weights of males and females (16 mg/kg/day) were significantly lower than both control groups starting on day 3 and continuing throughout the study. Significant body weight depression was present for males (8 mg/kg/day) from day 6 and for females from day 4 to the end of study. With few exceptions, significant body weight depression was present for males and females (4 mg/kg/day) from day 6 onward. Body weights for males and females (0.25 or 1.0 mg/kg/day) were not significantly different from either control group during the study. With few

exceptions, the significant differences in weight gains between control and treated animals were similar to those observed for body weight. Animals in the 0.25 or 1.0 mg/kg/day dose groups exhibited positive gains; males in the 4.0 mg/kg/day group exhibited no gain or slight gain (females); and dogs in the 8 or 16 mg/kg/day groups exhibited a negative gain. Dogs in the 4, 8 or 16 mg/kg/daydose groups ate progressively less than the animals at 1.0 mg/kg/day. Similar quantities of food were consumed by the females at 0.25 and 1.0 mg/kg/day, and both control groups. Males and females (liposome control group, 8 or 16 mg/kg/day) had significantly higher cholesterol levels than the dextrose control group. Animals (1 mg/kg/day or higher) had higher urea nitrogen and creatinine and lower potassium. The urea nitrogen, potassium and creatinine changes were dose-dependent and, in general, became progressively worse with time. Platelet counts were lower for females given 0.25 mg/kg/day or more and for males given 1 mg/kg/day or more; the effect was most apparent at day 6. Fibrinogen was higher from day 2 through day 29 for groups given 4 mg/kg/day or more. For animals given 8 or 16 mg/kg/day, mean corpuscular volume was higher, and mean corpuscular hemoglobin concentration was lower at day 6 and 13. Serum glucose and albumin concentrations were slightly lower, and globulin and triglyceride concentrations were slightly higher; these effects tended to be most notable after the first week. Serum AST activity was higher from day 2 - 29. Serum calcium, inorganic phosphorus and chloride concentrations were slightly to moderately higher.

There were significant increases in relative spleen and kidney weights in males and females beginning at 4 mg/kg. Relative thymus weight was also reduced in females at 4 mg/kg and relative brain weight was increased in males at 4 and 8 mg/kg.

Several animals (8 or 16 mg/kg/day) were noted to have pale kidneys. The incidence of gastric erosions, red foci, or reddened areas in the stomach was increased in animals (8 or 16 mg/kg/day). The incidence of reddened areas in other segments of gastrointestinal tract was also increased in these animals. At the injection sites, the incidence of diffuse reddening or areas of yellow discoloration was generally increased in animals (4 mg/kg/day or higher). Renal tubular nephrosis and hepatic Kupffer cell vacuolation/inclusions were observed in the animals given 1, 4, 8 or 16 mg/kg/day. The renal nephrosis was characterized by tubular regeneration, tubular dilatation, lymphohistiocytic infiltration, nephrocalcinosis, tubular vacuolation/degeneration, and in two animals tubular necrosis (16 mg/kg/day). The renal lesions were mild and generally consisted only of tubular regeneration and nephrocalcinosis (1 mg/kg/day). At the injection sites, there was a slightly increased incidence of hemorrhage, interstitial yellow pigment, and brown pigmented macrophages, especially in males (8 or 16 mg/kg/day). The testes, prostate glands and epididymides of males (8 or 16 mg/kg/day) were essentially underdeveloped from the juvenile state. Other microscopic findings were as follows: Spleen, (lymphoid depletion, pigmented macrophages vacuolated macrophages), Pancreas (acinar atrophy), Thyroid (follicular atrophy), Thymus (involution), immature uterus, vagina, ovaries, Liver (hepatocytic lipid), Esophagus (vacuolation of epithelium, glandular atrophy, subacute inflammation or erosion), Stomach (interstitial mineralization, subacute inflammation, vascular inflammation, parietal cell degeneration, congestion/hemorrhage or mucosal fibrosis), Intestines (congestion/hemorrhage, subacute inflammation, vascular fibrinoid necrosis, mineralization, vascular inflammation),

Muscles (atrophy of myofibers), Mesenteric lymph node (lymphoid depletion), Sternum (vacuolated macrophages or decreased cellularity), Femur (decreased cellularity), Urinary bladder (vascular fibrinoid necrosis), Mediastinal lymph node (vacuolated macrophages)

Unscheduled Sacrifice Animals

Generally, BUN, creatinine and inorganic phosphorous levels were markedly elevated and a few animals were noted with markedly low platelet counts. Increases in BUN and creatinine for moribund animals were as much control levels. Other changes included higher mean corpuscular volume, fibrinogen, and absolute monocyte count, and higher globulin, cholesterol, triglycerides, calcium, chloride concentrations, and AST activity, and lower mean corpuscular hemoglobin concentration, platelet count, absolute lymphocyte count, and serum glucose, albumin, and potassium concentrations. Two animals had notably low serum chloride concentrations.

Comments: Among the three species (mice, rats and dogs) tested, apparently the dog is the most sensitive species. Overt signs of toxicity included weight loss, poor weight gain, and reduced food consumption (4, 8 or 16 mg/kg/day). Clinical pathology showed changes in kidneys (1, 4, 8 or 16 mg/kg/day) histopathologic changes were also observed in kidneys and livers at these doses. Based on these results, a NOEL of iv AmBisome administered to dogs for 30 days was 0.25 mg/kg/day.

Target organs

Direct drug-related (1, 4, 8 or 16 mg/kg/day) renal nephrosis (characterized by tubular regeneration, tubular dilatation, lymphohistiocytic infiltration, nephrocalcinosis, tubular vacuolation/degeneration) was observed. Several animals (8 or 16 mg/kg/day) were noted to have pale kidneys. The effect on urine volume, urine specific gravity, urea nitrogen, creatinine, and inorganic phosphorus were indicative of renal toxicity, characterized by reduced glomerular filtration rate and impaired concentrating ability. Hepatic Kupffer cells vacuolation/inclusions and hepatic lipid infiltration (1, 4, 8 or 16 mg/kg/day) were observed. However, hepatocellular degeneration or necrosis was not found. Elevated serum cholesterol concentration was associated with both AmBisome and control liposome administration (AmBisome contains 52 mg of cholesterol per vial). Muscle atrophy was seen which may have been associated with the declining nutritional status. Reddened areas were observed in the gastrointestinal tract in several animals. Hemorrhage, congestion, inflammation, mineralization, and vascular changes in the gastrointestinal tract may have been secondary to the uremic state. The degree of failure of normal development of the reproductive organs may be due to the declining nutritional status of the animals or it may be the test article effect. Other target organs are: bone marrow (lower platelet counts); lymphatic system (higher absolute monocyte counts and globulins, although neutrophils were unaffected); hematopoietic system (higher mean corpuscular volume, absolute and lower mean corpuscular hemoglobin concentration, but anemia was not present); injection sites (irritation and yellow discoloration).

Table 5. Summary of Pharmacokinetics parameter estimates for amphotericin B in dogs during a 30 day intravenous toxicity study.

Dose (mg/kg)	Study day	$T_{½}$ (h)	$AUC_{0-\infty}$	V_{ss} (mL/kg)	Cl (mL/h/kg)
0.25	1 14 30	7 4 3	2.6 3.2 5.8	1663 385 196	112 77 47
1	1 14 30	9 6 6	11.4 55 119	959 140 67	79 24 9
4	1 14 30	8 9 16	164 923 3040	286 75 37	26 5 2
8	1 14 30	11 19 12	986 4980 5320	138 56 35	10 2 1
16	1 14 30	12 20	2595 13003	73 39	6 1

Table 6. Mean tissue concentrations of amphotericin B in dogs 24 hours after the last dose of the 30 day intravenous study of AmBisome

Dose (mg/kg/day)	Tissue concentration (µg/g)				
	Liver	Kidney	Spleen	Lung	Brain
0.25	94	1.4	116	0.1	0
1	314	15	295	3	0
4		74	765	31	0.3
8		101	1065	47	1.5(n=1)

The plasma concentrations of amphotericin B increased with dose but not in a dose proportional manner. Concentrations also increased with repeated dosing within each dose group, (at each dose group, the respective plasma concentrations were higher at repeated administrations

relative to their concentration on the respective previous sampling day). The pattern of decline in plasma concentrations with time was similar across groups and number of doses, although the curves appear to be biexponential at lower dose levels.

The estimated elimination half life generally increased as dose was increased, from values of approximately at the lowest dose levels to approximately at the highest doses. In general, day 1 AUC increased at each incremental increase of dose, and was higher at each respective dose at each subsequent sampling time [days 14 and 30].

Liver and spleen contain much higher concentrations of amphotericin B compared to the other tissues sampled. Liver and spleen concentrations were comparable
1 mg/kg/day group, respectively). Spleen concentrations continued to increase as dose was increased. Kidney contained the next highest concentrations, generally an order of magnitude lower than spleen values. Lung and brain contained the lowest concentrations of amphotericin B. Concentrations in all tissues increased with dose, but not in a dose proportional manner.

Comparison of tissue concentration data demonstrated tissue/plasma concentration ratios that were very high for liver and spleen. For kidney, the ratio decreased with increasing dose, from approximately 20 (at 0.25 mg/kg/day) to nearly 1 (8 mg/kg/day). Lung/plasma concentration ratios were marginally greater than 1 at 8 mg/kg/day. Brain/plasma ratios were approximately 1 (4 mg/kg/day), but there were insufficient data at higher dose levels to evaluate any further.

9. A 91-Day Toxicity, Toxicokinetic and Post-Dosing Recovery Study of Intravenously Administered AmBisome® (Liposomal Amphotericin B) in Rats, 470R-FUJ-001-95 November 1995. GLP study. AmBisome lot number 0425008E.

This study was designed to provide a toxicological profile of AmBisome® in rats when administered for 91 days, the recovery of histopathological changes following this duration of administration, and the plasma and tissue toxicokinetics of amphotericin B during and after AmBisome® administration.

The two control articles (5% dextrose or liposome control) and the test article (AmBisome ®) were administered intravenously into the lateral tail vein at 5 mL/kg over one minute as detailed in Table 7.

Table 7. Summary of Dose Administration and Animal Assignments

Group	Dose Group	Animals	No. of Doses	Sacrifice Day
1	Dextrose (5%) control	15/sex	91	≥91
2	Liposome Control	15/sex	91	≥91
3	1 mg amphotericin B/kg	30/sex	91	≥91
4	4 mg amphotericin B/kg	30/sex	91	≥91
5	12 mg amphotericin B/kg	30/sex	91	≥91
6	1 mg amphotericin B/kg	18/sex	1	1
7	4 mg amphotericin B/kg	18/sex	1	1
8	12 mg amphotericin B/kg	18/sex	1	1

Animals in groups 1-5 were used to provide toxicology data, immediate post-dosing toxicokinetic data, recovery data, or 4-week post-dosing toxicokinetic data. Animals in groups 6-8 were dosed, bled, and sacrificed (3/sex/group/timepoint) after a single dose to provide initial plasma concentration-time profiles (toxicokinetics) of AmBisome® (amphotericin B).

The appropriate number of vials of AmBisome® were removed from the refrigerator where they were stored. Twelve mL of sterile water for injection was then added to each vial to reconstitute the contents. Each vial was vigorously shaken to ensure mixing of the contents. The contents of each vial were removed with a 20 cc syringe and filtered through a sterile 5 micron filter into a sterile serum bottle. This filtered solution served as a stock 4 mg/mL solution. Each dose level was prepared by adding the correct amount of stock solution to the correct amount of vehicle (5% dextrose) with the exception of male Day 0 when the vehicle was added to the stock solution. The dosing solutions were prepared in sterile serum bottles which were wrapped in aluminum foil. All dosing solutions were utilized within six hours of preparation.

Mortality

Three animals (2 females and 1 male) died during the conduct of the study: one of these deaths was attributed to AmBisome®. A high dose female (animal no. 1348, 12 mg/kg/day AmBisome®) was found dead prior to dose administration on Day 3. Histopathological examination revealed the cause of death to have been due to severe multifocal hepatocellular degeneration and moderate necrosis of the liver. This death was attributed to AmBisome® toxicity. One female in the vehicle control group (Liposome) was sacrificed moribund on Day 17. At necropsy, a very large, purple urinary bladder was noted which was filled with a red fluid (presumed to be blood). The urinary bladder also had a white calculus, 0.3 cm in

diameter. The left kidney of this animal was tan and the adrenal glands were purple and enlarged to 1.5 times the normal size. The calculus in the urinary bladder was considered to have caused the moderate pyelonephritis and moderate suppurative cystitis observed histopathologically. This death was considered incidental to treatment with liposome. A male in the mid dose group (4 mg/kg/day AmBisome®) was found dead on Day 63. Necropsy and histopathological examination of this animal revealed no cause of death. However, given the isolated nature of this death, it was considered to have been unrelated to treatment. There were no clinical signs observed during the study which could be attributed to test article toxicity.

Mean body weight gains were lower for all high dose group animals (12 mg/kg/day AmBisome®) during the first four weeks. In males, this was seen in the first five weeks of the study and at the tenth week and for the female high dose group during the first four weeks of the study.

Text Table 8. Weekly Body Weight Gains During First Five Weeks of Study (g) (S.D.)

		Males					Females				
		Day 1-8	Day 8-15	Day 15-22	Day 22-29	Day 29-36	Day 1-8	Day 8-15	Day 15-22	Day 22-29	Day 29-36
Group 1 Dextrose Control	Mean S.D.	45 (6.4)	43 (6.5)	33 (6.4)	15 (17.8)	21 (5.0)	21 (5.6)	21 (4.0)	19 (6.2)	15 (3.9)	1 (11.8)
Group 2 Liposome Control	Mean S.D.	43 (9.9)	40 (7.4)	32 (8.9)	10 (16.9)	22 (4.2)	24 (3.4)	19 (5.1)	20 (8.0)	16 (5.4)	4 (12.7)
Group 5 12 mg/kg/day	Mean S.D.	38 * (9.3)	33** + (9.3)	24 ** ++ (5.9)	6 (16.9)	15 ** ++ (5.0)	18 ++ (5.3)	20 (6.7)	13 * ++ (5.4)	11 ++ (5.3)	-1 (12.8)

S.D. = Standard Deviation
Significantly different from control group 1: * $p < 0.05$ ** $p < 0.01$ Dunnett's Test
Significantly different from control group 2: + $p < 0.05$ ++ $p < 0.01$ Dunnett's Test

During the recovery phase of the study, group mean body weight gains for males and females in test article treated groups were comparable to the concurrent control groups.

Food Consumption

A statistically significant decrease in group mean food consumption ($p<0.05$) was observed for the female high dose group (12 mg/kg/day AmBisome®) on Days 8 and 29 when compared with the dextrose control group. For the remainder of the treatment phase of the study, food consumption for the female high dose group was lower than the dextrose control group but this difference did not attain statistical significance.

Hematology

Slightly lower platelet counts were observed for male and female high dose groups (12 mg/kg/day AmBisome®) at each of the hematology investigations during the study (Days 36, 57 and 89). These lower counts attained statistical significance ($p<0.05$) for the female high dose animals on Days 36 and 57.

A slight neutrophilia, which was statistically significant, was observed for male and female high dose groups (12 mg/kg/day AmBisome®) on Days 36, 57 and 89 when compared with dextrose and liposome control groups. This neutrophilia led to statistical differences in relative counts between control and high dose groups. The male mid dose group (4 mg/kg/day AmBisome®) also had a slight neutrophilia on Day 57 and the total white blood cell (WBC) count of high dose males was statistically higher than the dextrose controls on Day 89.

During the recovery phase of the study, platelet counts and neutrophil values for the high dose group were comparable to those of the dextrose and liposome control groups. There were no abnormal findings in the other red blood cell parameters or in the red blood cell morphology during the treatment period or during the recovery phase of the study which were attributable to treatment with AmBisome® or liposome control article.

Blood Chemistry

The administration of AmBisome® was associated with elevated blood urea nitrogen (BUN) for mid and high dose males and females (4 or 12 mg/kg/day AmBisome®). These elevated values demonstrated a dose response and were statistically significant ($p<0.05$ or $p<0.01$) for mid and high dose males and high dose females at most of the clinical pathology investigation timepoints (Days 36, 57 and 89) when compared with either the dextrose or liposome control groups. Mid dose females had a statistically significant elevation in BUN values compared with the liposome control only on Day 57. The magnitude of these elevations did not progress during the study. During the recovery period, BUN was still slightly elevated for male and female high dose animals on Day 99 and for high dose females on Day 106. Values had returned to the control range by Day 121.

Table 9. Group Mean BUN Values at Each Clinical Pathology Investigation During Treatment

Group		Day 36		Day 57		Day 89	
		male	female	male	female	male	female
dextrose control	Mean S.D. N	14 1.6 5	15 3.1 5	14 2.9 5	17 3.0 5	13 1.4 5	17 2.9 4
liposome control	Mean S.D. N	14 1.4 4	14 1.9 5	15 1.8 5	14 1.1 5	12 0.7 5	15 2.9 5
AmBisome® 1 mg/kg/day	Mean S.D. N	17 1.7 10	17 1.5 10	16 1.3 10	17 3.3 10	15 2.1 10	18 2.3 10
AmBisome® 4 mg/kg/day	Mean S.D. N	23 ** ++ 4.4 10	24 6.7 10	30 ** ++ 4.1 10	23 + 5.3 10	28 ++ 7.9 10	21 2.6 10
AmBisome® 12 mg/kg/day	Mean S.D. N	48 ** ++ 5.4 10	46 ** ++ 12.7 10	50 ** ++ 4.3 10	46 * ++ 8.3 9	51 ** ++ 10.3 10	42 ++ 8.7 10

S.D. = Standard Deviation. Significantly different from dextrose control group 1: * $p < 0.05$ ** $p < 0.01$ Dunnett's Test. Significantly different from liposome control group 2: + $p < 0.05$ ++ $p < 0.01$ Dunnett's Test

Test-article related increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed for high dose females (12 mg/kg/day AmBisome®) on Days 36, 57 and 89. On the majority of occasions, the difference between the female high dose values and dextrose and/or liposome controls attained statistical significance. There were no differences in ALT or AST values between control and treated male groups which were considered to be of toxicological significance. Test article related increases in alkaline phosphatase values were also observed for the male and female high dose group on Days 36, 57 and 89. On the majority of occasions, the differences between the male and female high dose values and the dextrose and/or liposome controls attained statistical significance.

Text Table 10. Group Mean ALP Values at Each Clinical Pathology Investigation During Treatment

Group		Day 36		Day 57		Day 89	
		male	female	male	female	male	female
dextrose control	Mean S.D. N	133 29.6 5	74 18.9 5	123 28.6 5	69 20.4 5	91 11.4 5	40 8.9 4
liposome control	Mean S.D. N	148 58.2 4	73 21.5 5	91 13.2 5	53 13.2 5	87 22.0 5	40 10.5 5
AmBisome® 12 mg/kg/day	Mean S.D. N	181 31.3 10	124 ** ++ 34.8 10	162** ++ 35.5 10	150 * ++ 29.6 9	122 * + 20.4 10	109** ++ 28.1 10

S.D. = Standard Deviation
N = Number of animals
Significantly different from dextrose control group 1: * $p < 0.05$ ** $p < 0.01$ Dunnett's Test
Significantly different from liposome control group 2: + $p < 0.05$ ++ $p < 0.01$ Dunnett's Test

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Text Table 11. Group Mean ALT Values at Each Clinical Pathology Investigation During Treatment

Group		Day 36		Day 57		Day 89	
		male	female	male	female	male	female
dextrose control	Mean S.D. N	29 2.3 5	27 3.9 5	31 4.2 5	29 7.3 5	33 6.5 5	33 13.5 4
liposome control	Mean S.D. N	32 6.7 4	40 15.5 5	29 7.5 5	29 10.7 5	35 8.3 5	28 7.4 5
AmBisome® 12 mg/kg/day	Mean S.D. N	26 9.4 10	224 * + 240.9 10	26 5.0 10	574** ++ 495.3 9	26 6.0 10	544 1021.6 10

S.D. = Standard Deviation
N = Number of animals
Significantly different from dextrose control group 1: * $p < 0.05$ ** $p < 0.01$ Dunnett's Test
Significantly different from liposome control group 2: + $p < 0.05$ ++ $p < 0.01$ Dunnett's Test

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Text Table 12. Group Mean AST Values at Each Clinical Pathology Investigation During Treatment

Group		Day 36		Day 57		Day 89	
		male	female	male	female	male	female
dextrose control	Mean S.D. N	108 2.7 5	84 10.1 5	113 31.4 5	108 11.9 5	80 20.1 5	83 27.4 4
liposome control	Mean S.D. N	103 10.5 4	79 8.0 5	82 * 11.2 5	81 12.2 5	76 16.8 5	63 8.2 5
AmBisome® 12 mg/kg/day	Mean S.D. N	91 21.4 10	437** ++ 305.0 10	86 * 7.7 10	1115** ++ 798.6 9	89 14.3 10	758 + 883.6 10

Significantly different from dextrose control group 1: * $p < 0.05$ ** $p < 0.01$ Dunnett's Test
Significantly different from liposome control group 2: + $p < 0.05$ ++ $p < 0.01$ Dunnett's Test

A dose-related hypercholesterolemia was observed for mid and high dose males and females (4 or 12 mg/kg/day AmBisome®) throughout the treatment period compared with the dextrose control group. However, the cholesterol values of these groups were similar to those of the liposome control group and, therefore, considered attributable to the cholesterol content of the liposome and of no toxicological significance. Cholesterol values remained statistically elevated for high dose males compared with dextrose control values at the recovery period investigation on Day 121. The liposome control and treated female group values had returned to those of the dextrose controls by Day 121.

Apparent treatment related decreases in lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) were observed for high dose males and females (12 mg/kg/day AmBisome®) and occasionally mid dose groups (4 mg/kg/day AmBisome®) compared with dextrose control values. Liposome control values demonstrated similar decreases. These decreases frequently attained statistical significance compared with the dextrose group mean values. Although there is no clear explanation for this finding, these decreases in LDH and CPK are considered to be of no toxicological significance. Control and treated group values were similar at the Day 121 recovery period investigation.

Necropsy Observations

Macroscopic pathology findings which were considered to be related to the administration of AmBisome® were observed for the liver, spleen and injection sites. The liver findings consisted of tan/white foci and/or pale linear streaks on the lobes of the liver or pale lobes. These findings were noted for 6 of 17 females in the high dose (12 mg/kg/day AmBisome®) and 1 of 17 males in the mid dose group (4 mg/kg/day AmBisome®). The findings were not observed in any animals in the low dose group (1 mg/kg/day AmBisome®) or

in the dextrose control or liposome control groups.

Lesions attributed to the repeated dose administration of AmBisome® were observed at the injection sites (tail) of low, mid and high dose male rats (1, 4 or 12 mg/kg/day AmBisome®, respectively). These lesions consisted of dark/green discoloration, red foci, and a raised lesion. The incidence of these findings was 5 of 17, 1 of 17 and 4 of 18 for the low, mid, and high dose groups, respectively. Similar findings were not observed for female treated groups.

Other findings noted at the terminal necropsy were low in incidence, commonly observed in rodent studies, and considered unrelated to treatment with either liposome or AmBisome®.

Enlargement of the prostate gland was noted sporadically in all groups (including controls) at both terminal and recovery period necropsies. This finding was considered to be related to the bleeding technique from the vena cava which placed undue pressure upon the prostate.

After one, two, and four weeks of recovery, there were no macroscopic findings observed at necropsy which were attributable to treatment. In particular, the findings for the liver were not present macroscopically after one week of recovery.

Organ Weights

The administration of AmBisome® was associated with absolute and/or relative changes in kidney, liver and spleen weights of treated animals at the terminal sacrifice.

Relative kidney weights of mid and high dose males (4 or 12 mg/kg/day AmBisome®) and of low, mid and high dose females (1, 4 or 12 mg/kg/day AmBisome®) were statistically higher than the dextrose control group mean weights. These differences were also apparent when compared with the liposome control group with the exception of mid dose male weights which were not statistically significant. Relative kidney to brain weight ratios also revealed several statistical differences with the dextrose control groups.

The absolute and/or relative mean liver weights were statistically higher than dextrose controls for mid and high dose males (4 or 12 mg/kg/day AmBisome®) and high dose females (12 mg/kg/day AmBisome®). Relative to body weight liver weights were also statistically higher than the liposome controls for high dose males and females.

Group mean spleen weights of mid and high dose males and females (4 or 12 mg/kg/day AmBisome®) were higher than dextrose control group mean weights. These differences attained statistical significance with the exception of mid dose female spleen weights and relative spleen to brain weight ratios. For high dose animals, the spleen weights

were also statistically higher than those of the liposome controls.

After one month of recovery, there were still some differences between group mean control and treated kidney and spleen weights which may have been attributable to AmBisome® treatment. The absolute group mean kidney weight of mid dose females (4 mg/kg/day AmBisome®) was statistically higher than dextrose controls and when adjusted for body weight, left and/or right kidney weight were statistically higher for low, mid and high dose females. Relative spleen to body weight ratios of high dose males and females were statistically higher than ratios observed in dextrose controls.

Histopathology

Histopathological examination of the tissues taken at terminal necropsy revealed dose related accumulations of finely vacuolated macrophages (presumed to contain liposomes/amphotericin B) in the liver, spleen, kidney and mesenteric lymph nodes of low, mid and high dose groups (1, 4 or 12 mg/kg/day AmBisome®). In general, there was little or no tissue reaction to the accumulations of vacuolated macrophages in these tissues. In both males and females, finely vacuolated macrophages were singly distributed in liver lobules. In the females, there were also larger accumulations of similar vacuolated macrophages in the interlobular and subcapsular tissues of the liver. In the spleen, these macrophages were diffusely distributed in the red pulp portion. In the mesenteric lymph nodes, and occasionally in other lymph nodes (submandibular and mediastinal), these macrophages were aggregated in the medullary portion.

The kidneys of most of the high dose male and female rats (12 mg/kg/day AmBisome®) and the kidneys of three low dose males (1 mg/kg/day AmBisome®) had vacuolated macrophages present in the glomeruli of the nephrons. In individual high dose rats (12 mg/kg/day AmBisome®) the kidneys contained areas of tubular separation, tubular dilatation, basophilic tubules (tubular regeneration), and areas of subcapsular fibrosis. These changes suggest earlier or prior tubular damage. Transitional cell hyperplasia was present in the renal pelvis of the kidney and/or in the urinary bladder of almost all of the high dose male and female rats (12 mg/kg/day AmBisome®). Transitional cell hyperplasia was present in kidneys of six male and two female mid dose animals (4 mg/kg/day AmBisome®) and in the kidneys of four low dose females (1 mg/kg/day AmBisome®). In the females with more severe transitional cell hyperplasia there was concurrent subacute inflammation of the renal pelvis (pyelitis).

Multifocal areas of necrosis were present in livers of a few mid and high dose male and female rats (4 or 12 mg/kg/day AmBisome®) and in one low dose male (1 mg/kg/day AmBisome®).

At Days 99 and 106, although reduced in severity, many of the changes present at Day 91/92 were still present. At Day 121, these changes were more reduced but they were not

completely resolved.

Adjacent to the tail vein of at least one or more rats in each of the five groups, there were mild changes secondary to the injection procedures. These changes were one or more of the following: perivascular hemorrhage, perivascular cellulitis and/or focal areas of vascular degeneration. In the lungs of many of the rats, scattered across all five groups, there were focal granulomatous lesions surrounding foreign bodies. These foreign body granulomas contained cross sections of hair and or cutaneous epithelial cells. These were iatrogenic lesions secondary to the multiple intravenous injections into the tail veins of the rats in this study. In two rats, these foreign bodies were surrounded by a purulent material (foreign body abscesses).

Incidental changes were found to be present in varying degrees in the tissues of the control and test animals. These lesions occurred spontaneously and were of similar distribution and severity in the Dextrose control group, Liposome control group and the rats having received 1, 4 or 12 mg/kg/day AmBisome® and were in such small numbers as to have no apparent relationship to the administration of the test material.

The results of this study indicate that the target organs for toxicity of AmBisome® (liposomal amphotericin B) were liver (necrosis) and kidneys (transitional cell hyperplasia) at doses of 1, 4 or 12 mg/kg/for 91 days. In addition, kidneys of some animals given 12 mg/kg/day AmBisome® contained areas of tubular separation, tubular dilation, and basophilic tubules (tubular regeneration). The no-observable-effect level (NOEL) of AmBisome® when administered intravenously for 91 consecutive days was below 1 mg/kg/day AmBisome®

10. A Pilot segment II reproductive toxicity study of intravenous AmBisome in rats. Study # TX944001. Fujisawa Pharmaceutical Co. Ltd. Toxicology Research Laboratory. 1-6 Kashima 2-Chome, Kashima, Yodogawa-ku. Osaka 532, Japan. January 6, 1994.

The purpose of this study was to obtain preliminary information on the teratogenic and/or fetotoxic potential of AmBisome.

Mature, nulliparous, and nonpregnant female rats were bred with untreated male rats and then administered daily intravenous doses (see Table 4) on days 6 through 15 of gestation. Each group consisted of 6 confirmed-mated females. Records were kept of mortality, clinical signs, and body weights. On day 20 of gestation the animals were euthanized and gross examination of the contents of the thoracic, abdominal, and pelvic cavities was done. The uterus of each animal was examined for viable and nonviable fetuses and implantations and resorption rates. A corpora lutea count was made on each ovary. Fetuses were weighed and examined externally for malformations.

Table 13. Treatment protocol for study # TX944001

Group	Dose Group (mg Amphotericin B/kg/day)	Number of females
1	Control (5% glucose)	6
2	5	6
3	10	6
4	15	6
5	20	6

Mortality

Three of six rats administered 20 mg/kg died before the third dose. One animal died in the 15 mg/kg and the 10 mg/kg groups.

Adverse events associated with AmBisome administration included reduced weight gain (seen in all groups but most pronounced in the 20mg/kg dose group) and occasional white foci on the liver surface (in two animals treated with 10 mg/kg/day or more). Drug therapy was also associated with a significant depression in food consumption at 15 and 20 mg/kg/day between days 6 and 9. Food consumption returned to normal after this early period. No abnormal changes ascribable to the treatment were detected in fetuses.

AmBisome is does not show the potential for teratogenicity or fetotoxicity.

11 A Pilot Segment II reproductive toxicity study of intravenous AmBisome in rabbits. Fujisawa Pharmaceutical Co. Ltd. Toxicology Research Laboratory. 1-6 Kashima 2-Chome, Kashima, Yodogawa-ku. Osaka 532, Japan. January 1994.

This study is part of a series of experiments designed to determine the potential of AmBisome to produce teratogenic and fetotoxic effects. The current study was a pilot study designed to aid in the selection of doses for the definitive reproductive toxicology study.

Groups of female NZW rabbits (four or five pregnant rabbits per group) were administered daily intravenous doses of AmBisome on days 6 through 18 of gestation. Dosing details are shown in Table 14. Records were kept of mortality, body weights and clinical signs. On day 29 of gestation, animals were euthanized and examined for gross pathology. Fetuses were weighed and examined externally for malformations.

Table 14. Treatment protocol for study # TX944002

Group	Dose Group (mg Amphotericin B/kg/day)	Number of female rabbits
1	Control (5% glucose)	8
2	5	9
3	10	6
4	15	6
5	20	5

Results

There were no mortalities in any group. Adverse events seen in all groups included decreases in body weight, body weight gain and food consumption, but these changes were only statistically significant at 10 mg/kg and above. At higher doses, abortions ascribable to treatment were observed. Total resorption was observed in one animal given 20 mg/kg/day and resorption rates and total fetal weights were lower for this group (although these decreases were not statistically significantly). The only fetal abnormality associated with AmBisome therapy was brachyury in one animal from the 5 mg/kg/day group.

Adverse effects in the dams included a large nodule in the gall bladder and retention of liquid in the caecum in one animal in the 20 mg/kg group. In the 15 mg/kg group, a small gallbladder and a few foci were observed in the gastric mucosa while large gallbladder and a few foci of the gastric mucosa were observed in an animal of the 10 mg/kg group.

Comment: The 20 mg/kg/day dose exceeds the maximum tolerable dose for reproductive toxicity in rats. Studies using doses lower than 20 mg/kg/day will allow us to distinguish effects of the drug that are due to maternotoxicity from direct embryo-fetal effects.

12. A Segment I reproduction toxicity study of intravenous AmBisome in rats. Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6 Kashima 2-Chome, Kashima, Yodogawa-ku. Osaka 532, Japan. April 1995. AmBisome lot number 042008E

This study was performed to assess the reproductive toxicology (effects on estrous cycle, copulation, fertility, implantation, implantation and embryogenesis) of amBisome in rats.

One hundred and twenty male and one hundred and twenty female Sprague-Dawley rats (Jcl:SD strain) were allocated to groups of 20 animals per sex and administered daily intravenous doses of AmBisome at 5, 10 or 15 mg/kg/day.

Males were treated from 28 days before the start of mating to the conclusion of the mating period. Females were treated from 14 days before mating, through the mating period, to day 6 of gestation. Solvent and placebo control animals were administered 5 % glucose and drug-free liposomes respectively.

Mortality

Seven animals from the 20 mg/kg group died during the study. Four, two, and one animals were found dead after the first, second, and third doses. Necropsy findings included pale foci of the liver (two animals), marked diffuse necrosis in the liver (seven animals), and moderate protein casts in the kidney (one animal).

Body weights were significantly lower in the 10 and 15 mg/kg groups compared to control groups (see Table 18 below). At the 5 mg/kg dose, mean body weight was only about 5 % less than comparable controls, but body weight gain over the period was 47 % of control levels.

Table 15. Body weight and body weight gain during the premating period (male rat)

Dose (mg/kg)		0	0	5	10	15
Bodyweight (g)	Day 8	449	458	443	**437**	**426**
	Day 29	485	480	**459**	**440**	**441**
Bodyweight gain (g)		47	43	**21**	**2**	**3**

In females total weight gains were significantly lower in the 10 and 15 mg/kg groups (see Table 19).

In males, food consumption was significantly lower in the 15 mg/kg groups than in controls throughout the dosing period. In the 5 and 10 mg/kg dose groups, consumption decreased for one week after the start of dosing and in the later dosing period. In females, food consumption was lower in the 10 and 15 mg/kg dose groups only during the first week of dosing.

Diestrus was prolonged in three animals in the 10 mg/kg group and 6 animals in the 15 mg/kg group.

Table 16. Frequency of Estruses

Dose (mg/kg)	0	0	5	10	15
Frequency of estruses during 15 days	3.8	3.7	3.8	**3.1**	**2.5**

At 15 mg/kg, there were decreases in the number of corpora lutea, implantations and live embryos. Since the implantation and resorption rates did not change, the decreases in the numbers of implantations and live embryos were ascribed to the decrease in corpora lutea.

Table 17. Summary of litter data

Dose (mg/kg)	0	0	5	10	15
No of corpora lutes	18	18	17	17	**15**
No of implantations	17	17	16	16	**13**
No of live embryos	16	16	15	15	**12**

There were no other effects on copulation, fertility, or days to copulation associated with this drug.

Conclusion

The administration of AmBisome produced abnormal estrus, decreased numbers of corpora lutea, implantations, and live embryos at doses which also produced death and liver necrosis in female rats.

13 A Segment II reproduction toxicity study of intravenous AmBisome in rats. Study # TX044008. Toxicology Research Laboratories. Fujisawa Pharmaceutical Co. Ltd. 1-6 Kashima 2-Chome, Kashima, Yodogawa-ku. Osaka 532, Japan. October 1994. AmBisome lot number 042012E

This study was designed to assess the embryotoxicity and teratogenicity of AmBisome in rats.

AmBisome was administered to groups of pregnant Jcl:SD rats (23 to 25 rats per group) as daily intravenous injections at doses of 5, 10 or 15 mg/kg via the tail vein. Animals were treated from days 6 to 15 of gestation and control animals received placebo liposomes or 5 % glucose. Animals were subjected to cesarean section on day 20 of gestation and their fetuses were examined morphologically.

Mortality

One animal, in the 15 mg/kg group, died on day 8 of gestation while another, treated with 5 mg/kg AmBisome, died two days after the end of dosing. While no cause of death was determined for the 15 mg/kg animal, the death in the 5 mg/kg animal was associated with hemorrhage of the uterus.

Body weights of animals treated with AmBisome were slightly less (approximately 10 %) than for control animals at the 15 mg/kg dose level. There were also decreases in food consumption at 10 (up to 21 % less than controls) and 15 mg/kg (up to 46 % less than controls) between days 6 and 12 of gestation.

Gross pathology examination showed white foci or spots on the liver and kidneys. Histopathological examinations of affected animals revealed multifocal necrosis and sinusoidal foamy cell accumulation in the livers and suppurative nephritis and basophilic tubules with inflammatory cell infiltration in the kidneys.

The total number of fetuses with visceral abnormalities was increased in the 15 mg/kg group as a result of an increase in the number of fetuses with thymic remnant in the neck. This finding was 50 % more prevalent in animals treated with AmBisome compared to liposome treated animals. Overall the background incidence of this finding from the company's historical data was 8.5 % and the incidence in this study was 12.7 % overall.

Table18. Number of fetuses with thymic remnant in neck

	Control (glucose)	Control (liposome)	AmBisome 5 mg/kg	AmBisome 10 mg/kg	AmBisome 15 mg/kg
No of fetuses with thymic remnant	8	14	10	11	22

Conclusion

AmBisome did not show teratogenic potential at doses up to 10 mg/kg. At 15 mg/kg, a dose which is clearly maternotoxic, AmBisome administration was associated with an increase in the frequency of thymic remnant in the neck of rat pups.

14. A Segment II reproductive toxicity study of intravenous AmBisome in rabbits. Fujisawa Pharmaceutical Co. Ltd. Toxicology Research Laboratory. 1-6 Kashima 2-Chome, Kashima, Yodogawa-ku. Osaka 532, Japan. September 1994. Drug lot number 0422012E. Japanese GLP.

This study was designed to evaluate the embryotoxicity and teratogenicity of AmBisome. Groups of pregnant New Zealand white rabbits (17 or 18 females per dose group) were treated with daily intravenous doses of AmBisome at 3, 7, or 16 mg/kg via the ear vein from days 6 to 18 of gestation. Control animals were treated with placebo liposomes or 5 % glucose. Surviving females were subjected to cesarean section on day 29 of gestation and the fetuses were examined morphologically. Records were kept of the number of corpora lutea, the

number and location of implantations, the number of live and dead fetuses at early and late stages, as well as implantation and resorption rates. Live fetuses were examined for external abnormalities (including the oral cavity) and internal abnormalities. Mean male and female fetal weights were calculated as well as the sex ratio and incidence of abnormal fetuses.

Mortality

One animal in the glucose control group died on day 24 of gestation while another in the 7 mg/kg group died on day 28. Both animals showed soft feces, decreases in body weight and food consumption, lung congestion, and dark red foci in the lung. Two other animals, one in the glucose control group and the other in the liposome control group, suffered hind limb fractures and were sacrificed on day seven.

Toxicity

Differences between AmBisome-treated animals and controls were seen at the two higher doses (7 and 16 mg/kg) in food consumption and bodyweight and these were likely to have contributed to the increased incidence of abortions seen at those doses. Other parameters examined were comparable to controls. Rabbits treated with AmBisome at 16 mg/kg experienced reduced body weight gain compared to control. Body weight in these animals was up to 14 percent less than the body weights of control animals.

Thirteen animals aborted. Two dams in the 7 mg per kg group aborted on days 26 or 29 of gestation, while 11 dams in the 16 mg/kg group aborted between days 22 and 26. In all cases food consumption had been decreased markedly 2 to 16 days before abortion.

Table 19. Food consumption in pregnant rabbits treated with AmBisome

Dose	0(glucose)	0(liposomes)	3	7	16
Days 6-9	151	160	156	135	100*
Days 15-19	134	122	111	53*	32*
Days 24-27	82	97	106	108	93

*Statistically different from controls (glucose and liposome controls) at $p < 0.01$

Conclusion

AmBisome demonstrated no evidence of teratogenic potential. Injection of AmBisome in the rabbit resulted in a dose dependent increase in abortions, which is likely the result of decreased food consumption and body weight.

15. Dye extravasation study. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive San Dimas, CA 91773. AmBisome lot number 0421023. April 1992 Non-GLP study.

This study was designed to determine the potential toxic effects of AmBisome when injected extravascularly.

Test specimens were (1) AmBisome, diluted according to rehydration instructions with 12 mL water for injection (2) AmBisome rehydrated as in (1) but then further diluted 1:7 with D5W (3) Non-drug vehicle (600 mg lipid) was rehydrated with 12 mL of water for injection and further diluted 1:7 with D5W (4) succinate buffered 9% sucrose.

Two healthy New Zealand white rabbits were shaved and injected intradermally with 0.1 mL of test or control solution at two sites on the backs for each solution. Controls consisted of sodium chloride for injection USP (negative control), WFI, USP and 20 percent ethanol in the WFI, USP (positive control). Fifteen minutes after the last intradermal injection dye was injected intravenously via the marginal ear vein. The rabbits were observed for the presence of dye extravasation at intradermal sites at 0, 10, 20, 30, 60, 120 and 180 minutes after dye injection. An estimation of the intensity of staining was achieved according to the following scheme:

No color	0
Light blue colorations	2
Distinct blue throughout	4
Deep blue throughout	8
Ischemic central area surrounded by deep blue halo	16

Results

No dye extravasation was observed in animals tested with AmBisome [consistently scoring 0]. One of four test sites tested with liposomes showed some sign of dye accumulation [scores of two and eight]. Positive controls, water [consistently scoring 8] and ethanol, [scoring a 8 and 16] produced dye accumulation as expected.

Conclusion

AmBisome does not cause irritation when injected intradermally under the conditions of the test

16. Paravenous and subcutaneous administration of AmBisome and conventional amphotericin B. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive San Dimas, CA 91773. AmBisome lot number 0421023. April 1992.

Three groups of Sprague Dawley rats (group I consisting of three females and groups II and III consisting of three males each), were injected with 1 mL of test substance,

subcutaneously on the left and right dorsal flank. Tail injections were given in the artery and lateral vein in doses of 0.2 mL. Groups I and II received AmBisome in the tail and right dorsal flank, while receiving empty liposomes in the left dorsal flank. Group 3 rats received Fungizone in the tail and right dorsal flank and D5W in the left dorsal flank. Twenty four hours after injection, all animals were euthanized by carbon dioxide, and injection sites of the tail, right and left dorsal flank were excised and fixed individually in 10 percent formalin. All samples were sent for pathological evaluation.

Animals in group I showed definite evidence of inflammation at the injection sites, although some injection sites were difficult to locate. Neutrophils and macrophages seemed to be to be the majority of cells throughout the injection sites. Minimal residual injected material could be identified in the macrophages but there was no evidence of necrosis. In group III, reactions were varied. Even though it was difficult to identify some injection sites, other sites showed a rather severe suppurative response.

Conclusion: AmBisome did not produce serious inflammatory or necrosis when injected outside the vein under these conditions.

17. Evaluation of local tissue reactions to AmBisome: Paravenous and intraarterial administration in rats. NeXstar Pharmaceuticals Inc. 650 Cliffside Drive San Dimas, CA 91773. AmBisome lot number 0421011. April 1992.

This study was designed to determine the potential for toxicity if AmBisome was administered adjacent to a vein or through an artery.

Fourteen groups of Sprague Dawley rats, (four rats/group) were used in the study, and injected either paravenously or intraarterially and observed for either one or seven days (see Tables 20 and 21)

Table 20. Experimental protocol for Evaluation of local tissue reactions to AmBisome: Paravenous injections.

Group number	Treatment	Sacrificed
P1C7	Buffer vehicle	7 days post injection
P2L7	Liposomes without drug	7 days post injection
P3F7	Fungizone	7 days post injection
P4A7	AmBisome	7 days post injection
P5C1	Buffer vehicle	1 day post injection
P6L1	Liposomes without drug	1 day post injection
P7F1	Fungizone	1 day post injection
P8A1	AmBisome	1 day post injection

For the intraarterial study animals were treated as follows:

Table 21. Experimental protocol for Evaluation of local tissue reactions to AmBisome: intraarterial injections.

Group number	Treatment	Sacrificed
A1C7	Buffer vehicle	7 days post injection
A2L7	Liposomes without drug	7 days post injection
A3F7	AmBisome	7 days post injection
A4A7	Buffer vehicle	1 day post injection
A5C1	Liposomes without drug	1 day post injection
A6L1	AmBisome	1 day post injection

Results

Paravenous treatment

One day after injection of the control buffer vehicle, symmetrical continuous zones of marked congestion and edema with prominent cellular infiltrates consisting primarily of

polymorphonuclear leukocytes were observed. By day seven, inflammation and cellular responses were partially healed. At liposome-treated sites there was moderate inflammation and a mild cellular response on day one. On day seven these animals showed increased inflammation and cellularity compared to day 1. Fungizone-treated sites showed severe inflammation with marked cellular infiltrates of polymorphonuclear leucocytes, lymphocytes, and macrophages. AmBisome-treated injection sites showed marked local inflammation with moderate cellular response. For AmBisome and Fungizone treated animals, the degree of inflammation and cellular response was nearly identical on day seven compared to day one.

Intraarterial Treatment

One day after intraarterial treatment with control buffer vehicle one of four animals showed evidence of moderate inflammation and cellularity. In the group treated with liposomes without drug moderate inflammation was also occasionally present. AmBisome treated animals showed moderate to marked inflammation with cellular infiltrates at the injection sites in all of animals.

On day 7 mild inflammation was present in the non-drug liposome treatment group and occasionally in the AmBisome treatment group.

Conclusion

Paravenous injections of AmBisome caused local inflammatory responses. Intraarterial injection of AmBisome produces an inflammatory reaction. Seven days post injection, both paravenous and intraarterial inflammatory effects to show evidence of recovery.

Preclinical Toxicology Summary

AmBisome is clearly less toxic to animals than conventional amphotericin B. Although the toxic effects are largely the same, the toxic effects are seen at much lower doses with Fungizone when compared to AmBisome. The target organs for toxicity of AmBisome were the urinary tract (including kidneys, ureter and urinary bladder), liver (including increased liver weights, increased liver enzymes and necrosis) and the bone marrow (thrombocytopenia and bone marrow necrosis). AmBisome has an elimination half life in rats of about 10 hours.

Conclusion

Sufficient information has been provided to support the approval of the NDA and to support the information included in the label.

/S/

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Concurrences:

HFD-590/RAlbrecht /S/ 12/10/97
HFD-590/KHastings /S/ 10/15/97
HFD-590/OMcMaster

Disk:
HFD-530/KHastings
cc:
HFD-590 Division File
HFD-590/Micro/LGosey
HFD-590/MO/JKorvick
HFD-590/SPharm/KHastings
HFD-590/Chem/NSchmuff
HFD-340
HFD-590/CSO/EFrank
HFD-590/Pharm/OMcMaster

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APPLICATION NUMBER: 050740

STATISTICAL REVIEW(S)