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RESEARCH**

APPLICATION NUMBER:

021825Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Deferiprone (Ferriprox)

Date: October 10, 2011

To: File for NDA 21825

From: John K. Leighton, PhD, DABT

Director, Division of Hematology Oncology Toxicology

Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Dr Bailey and labeling and supervisory memorandum provided by Dr. Saber. I also had discussions with Dr Gehrke, assigned to this project to conduct a labeling review. I concur with Dr Saber's conclusion that Ferriprox may be approved for the proposed indication and that no additional nonclinical studies are needed.

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/s/

JOHN K LEIGHTON
10/11/2011

MEMORANDUM

Date: October 8, 2011
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology
To: File for NDA # 21825
Drug: Ferriprox[®] (deferiprone) film-coated tablets
Re: Approvability for Pharmacology and Toxicology
Applicant: ApoPharma, Inc.

Background and results of nonclinical studies

Ferriprox (deferiprone) is an iron chelator for oral administration, indicated for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate. Deferiprone forms 1:3 iron:drug complexes of Fe(III) at physiological pH. It also binds to other metals such as copper, aluminum and zinc but with a lower binding affinity (G.J. Kontoghiorghes, *Analyst* 1995; v120: 845-851).

Deferiprone is approved in several countries. It was approved in Europe in 1999; hence there are extensive and well documented clinical data with this drug. The application for Ferriprox was a rolling NDA submission. Nonclinical studies were submitted to the FDA and were reviewed by Dr. David Bailey as part of IND and NDA reviews, with concurrence by Dr. Lanionu. A summary of information from the IND review relevant to the product label was incorporated into the NDA review by Dr. Bailey. The following summary of nonclinical findings is based on the NDA review by Dr. Bailey with supplemental information from the Applicant's submission.

Repeat-dose oral toxicology studies were conducted in rats and monkeys. Adverse findings related to the pharmacologic effects of the drug were evident in animals. Findings included anemia, reduced red cell parameters, and bone marrow hypocellularity. Other drug-related toxicities included: mammary gland hyperplasia and tumors (see table below), and findings in the liver (e.g. increases in liver weight and liver enzymes, formation of fat vacuoles and/or hepatic centrilobular necrosis/degeneration), in rats. The magnitude of effects in the liver varied depending on whether rats were loaded with iron prior to treatment or were naïve (non-iron-loaded). Other effects attributed to the drug were increased weight of pituitary, adrenal, and heart with no clear microscopic correlates.

Group	Mammary tumor findings			Occurrence during the study
Group 1; naïve control (no iron load)	-			-
Group 2, iron loaded control	1/5	female	fibroadenoma	Recovery sacrifice
Group 3, LD drug and iron load	1/20	female	adenocarcinoma	Terminal sacrifice
Group 4, MD drug and iron load	1/20	male	adenocarcinoma	Terminal sacrifice
Group 5, HD drug and iron load	1/20	female	fibroadenoma	Terminal sacrifice
Group 6, MD drug, no iron load	2/5	female	fibroadenoma	Recovery sacrifice
	1/16	female	fibroadenoma	Terminal sacrifice
	2/4	female	fibroadenoma	Unscheduled death

LD: low-dose; MD: mid-dose; HD: high-dose

Deferiprone was positive in several *in vitro* and *in vivo* genotoxicity assays and was teratogenic when administered to rats or rabbits during the period of organogenesis. Results of genotoxicity and teratogenicity studies are reported in sections 13.1 and 8.1 of the label, respectively.

In his review, Dr. Bailey pointed to the deficiencies due to the lack of the following studies:

1. Electrophysiology study in hERG cells.
2. Lifetime carcinogenicity studies.
3. Fertility and early embryonic development study in rats

Subsequently, results of a fertility and early embryonic development study as well as results of a hERG cardiovascular assessment were submitted to the Agency for review. Based on the fertility study conducted in rats, sperm counts, motility and morphology were unaffected by treatment with deferiprone. There were no effects observed on male or female fertility or reproductive function at doses tested. The highest dose was 25% of the maximum recommended human dose based on body surface area.

Results of the hERG assay are used to assess the potential for a drug to cause QT prolongation. Deferiprone did not block the potassium channel at relevant drug concentrations in this assay. The highest concentration of the drug used in the study, 3000 µM, resulted in only 2% inhibition of the hERG mediated potassium current. Therefore, from a nonclinical perspective, this drug has minimal potential for causing QT prolongation. Moreover, extensive clinical experience with deferiprone in thalassemia suggests that this drug removes iron from the heart, an effect expected to improve cardiac function in patients.

The need to conduct carcinogenicity studies was communicated to the Applicant in 2004. Based on the review by Dr. Bailey, the Applicant agreed to conduct the studies. The following is from Dr. Bailey's review regarding this issue:

“The sponsor has proposed conducting a 2-year carcinogenicity study in rats and a 6-month study in p53 knockout mice during Phase 4. The range-finding studies to support dose levels for the carcinogenicity studies are currently underway. Final

reports will be submitted with protocols for the 2 carcinogenicity studies for SPA review by March 2009, with initiation of the 2 carcinogenicity studies in August 2009. Final report for the mouse study is scheduled for December 2010 and the rat report for December 2012.”

It appears that these studies have not been conducted to date. Considering that deferiprone was unequivocally genotoxic and was tumorigenic in rats in the 52-week toxicology study, carcinogenicity studies are not deemed necessary.

Ferriprox label

The following sections of the label were revised to reflect the nonclinical findings and/or to make the wording compliant with 21CFR201.57 on content and format of the label: HIGHLIGHTS, Warnings and Precautions (section 5), Pregnancy (section 8.1), Nursing Mothers (section 8.3) Mechanism of Action (section 12.1), and Carcinogenesis, Mutagenesis, Impairment of Fertility (section 13.1).

The established pharmacologic class for deferiprone is “iron chelator”. This information is under Indications and Usage of the HIGHLIGHTS.

The Applicant proposed (b)(4) for deferiprone pregnancy. Based on the evidence of genotoxicity in *in vitro* and *in vivo* studies and the teratogenicity in animal studies, the Agency proposes Pregnancy Category D. As for other drugs with Pregnancy Category D designation, the summary information on embryofetal toxicity is provided under Warnings and Precautions of the label.

See below the agreed upon language for sections 5.3, 8.1, 8.3, 12.1, and 13.1 of the FULL PRESCRIBING INFORMATION of the label following discussions with the Applicant.

5.3 Embryofetal toxicity

Based on evidence of genotoxicity and developmental toxicity in animal studies, Ferriprox can cause fetal harm when administered to a pregnant woman. In animal studies, administration of deferiprone during the period of organogenesis resulted in embryofetal death and malformations at doses lower than equivalent human clinical doses. If (b)(4) is used during pregnancy or if the patient becomes pregnant while taking (b)(4), the patient should be apprised of the potential hazard to the fetus. Women of reproductive potential should be advised to avoid pregnancy when taking Ferriprox [see *Use in Specific Populations (8.1) and Nonclinical Toxicology (13.1)*].

8.1 Pregnancy

Pregnancy Category D [see *Warnings and Precautions (5.3), Nonclinical Toxicology (13.1)*]

Based on evidence of genotoxicity and developmental toxicity in animal studies, Ferriprox can cause fetal harm when administered to a pregnant woman. In

animal studies, administration of deferiprone during the period of organogenesis resulted in embryofetal death and malformations at doses lower than equivalent human clinical doses. There are no studies in pregnant women, and available human data are limited. If this drug is used during pregnancy or if the patient becomes pregnant while taking [REDACTED]^{(b) (4)}, the patient should be apprised of the potential hazard to the fetus.

Skeletal and soft tissue malformations occurred in offspring of rats and rabbits that received deferiprone orally during organogenesis at the lowest doses tested (25 mg/kg per day in rats; 10 mg/kg per day in rabbits). These doses were equivalent to 3% to 4% of the maximum recommended human dose (MRHD) based on body surface area. No maternal toxicity was evident at these doses.

Embryofetal lethality and maternal toxicity occurred in pregnant rabbits given 100 mg/kg/day deferiprone orally during the period of organogenesis. This dose is equivalent to 32% of the MRHD based on body surface area.

8.3 Nursing Mothers

It is not known whether deferiprone is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for adverse reactions in nursing infants from Ferriprox, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

12.1 Mechanism of Action

Deferiprone is a chelating agent with an affinity for ferric ion (iron III). Deferiprone binds with ferric ions to form neutral 3:1 (deferiprone:iron) complexes that are stable over a wide range of pH values. Deferiprone has a lower binding affinity for other metals such as copper, aluminum and zinc than for iron.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with deferiprone. However, in view of the genotoxicity results, and the findings of mammary gland hyperplasia and mammary gland tumors in rats treated with deferiprone in the 52-week toxicology study, tumor formation in carcinogenicity studies must be regarded as likely.

Deferiprone was positive in a mouse lymphoma cell assay in vitro. Deferiprone was clastogenic in an in vitro chromosomal aberration test in mice and in a chromosomal aberration test in Chinese hamster ovary cells. Deferiprone given orally or intraperitoneally was clastogenic in a bone marrow micronucleus assay in non-iron-loaded mice. A micronucleus test was also positive when mice predosed with iron dextran were treated with deferiprone. Deferiprone was not mutagenic in the Ames bacterial reverse mutation test.

A fertility and early embryonic development study of deferiprone was conducted in rats. Sperm counts, motility and morphology were unaffected by treatment with deferiprone. There were no effects observed on male or female fertility or reproductive function at the highest dose which was 25% of the MRHD based on body surface area.

Recommendation:

Nonclinical studies needed in support of the proposed indication have been conducted and reviewed by the Agency. Deferiprone is considered genotoxic, carcinogenic, and teratogenic. It is recommended that this drug be used in a serious disease, when other therapies are considered inadequate. Women of reproductive potential should be advised to avoid pregnancy when taking Ferriprox. Based on the Indications and Usage of the label, Ferriprox is indicated for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate. There are no nonclinical issues at this time to preclude approval of Ferriprox (deferiprone) for the proposed indication considering the life-threatening nature of the disease and lack of adequate chelation therapy.

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/s/

HALEH SABER
10/08/2011



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-825
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 06/30/09
PRODUCT: FERRIPROX® (deferiprone)
INTENDED CLINICAL POPULATION: Patients with iron-overload from chronic transfusion therapy or iron-induced cardiac disease.
SPONSOR: ApoPharma Inc.
200 Barmack Drive
Toronto, Canada M9L 2Z7
U.S. AGENT: Cato Research
4343 South Alston Avenue
Durham, NC 27713
Telephone: 919-361-2286
DOCUMENTS REVIEWED: Electronic Submission in EDR
REVIEW DIVISION: Medical Imaging and Hematology Drug Products (HFD-160)
PHARM/TOX REVIEWER: David E. Bailey, Ph.D.
PHARM/TOX SUPERVISOR: Adebayo A. Laniyonu, Ph. D.
DIVISION DIRECTOR: R. Dwaine Rieves, MD.
PROJECT MANAGER: Hyon-Zu Lee
Date review submitted to DARRTS: September 22, 2009

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: APPROVAL, with safety warnings in label.

B. Recommendation for nonclinical studies: Carcinogenicity studies to be conducted post marketing.

C. Recommendations on labeling:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy



(b) (4)

8.6 Women of Childbearing Potential



13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility



Pharmacologic activity

Diferiprone is readily absorbed from the gut and as a chelator preferentially binds iron in the body in a 1:3 iron (Fe III):ligand complex and is excreted in the urine and by the fecal route via the bile.

Diferiprone is a bidentate iron chelator that at physiological pH preferentially forms 1:3 iron:ligand complexes of Fe(III). Neither deferiprone nor the 1:3 iron:ligand complex carries a charge at physiological pH, which aids in crossing cell membranes. The deferiprone:iron complexes are not absorbed from the intestine, thus deferiprone does not aid in dietary absorption of iron. Diferiprone mobilizes iron from the iron transport protein, transferrin, and from the resulting lysosomal degradation product hemosiderin and is excreted in the urine and by fecal route via the bile.

Nonclinical safety issues relevant to clinical use

Deferiprone is:

A positive transspecies genotoxin in all assays conducted except Ames test, and thus presumed to be a transspecies carcinogen, which implies a carcinogenic risk to humans.

A potent teratogen in rats and rabbits at all doses tested.

A suspect carcinogen in a rat 12 month chronic toxicity study with numerous malignant tumors in male and female rats in all deferiprone treated groups, with none in male or female control animals.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-825
Review number: 02
Submission: RUP 25 01-JULY-2009

Information to sponsor: No
Sponsor: ApoPharma Inc.
Toronto, Ontario, Canada

Agent: Lynda Sutton
Cato Research
4363 South Alston Avenue
Durham, NC 27713
Telephone: 919-361-2286

Manufacturer for drug substance: Apotex Pharmachem Inc.
Brantford, Ontario, Canada

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Products
HFD #: 160
Review completion date: September 9, 2009

Drug: Registered name: FERRIPROX®
Trade name: deferiprone
Chemical name: 3-hydroxy-1, 2-dimethylpyridin-4-one
Common name: APO-066
CAS registry number:
Molecular weight: 139.15
Structure:

Relevant INDs/NDAs/DMFs: IND# 45,724; DMF # 10,867

Drug class: Iron chelator

Intended clinical population: Patients with iron overload resulting from chronic transfusion therapy or for prevention of cardiac disease in patients with iron overload.

Clinical formulation: 500 mg – film coated tablet
100 mg/mL – aqueous oral solution

Clinical dose: 75 – (b)(4) mg/kg/day (25 - 33 mg/kg, tid)

Route of administration: Oral

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

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA number 21-825 are owned by ApoPharma Inc. or are data for which ApoPharma Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA number 21-825 that ApoPharma Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that ApoPharma Inc. does not own, is for descriptive purposes only and is not relied upon for approval of NDA number 21-825.

Studies reviewed within this submission:

Oral fertility and early embryonic development study of APO-066 in rats.

Studies not reviewed within this submission: None.

TOXICOLOGY**Reproductive and developmental toxicology:**

Fertility and early embryonic development

Study Title: Oral fertility and early embryonic development study of APO-066 in rats.**Key study findings:****Study number:** 82002860**Volume and page #** Located in EDR, Module 4**Conducting laboratory:**

(b) (4)

Sponsor: ApoPharma Inc
200 Barmac Drive
Toronto, Ontario
Canada M9L 2Z7**Agent:** Lynda Sutton
Cato Research
4363 South Alston Avenue
Durham, NC 27713
Telephone: 919-361-2286**Report Date:** June 29, 2009
Date of study initiation: December 12, 2008
Date of in-life study: December 22, 2008 – March 5, 2009**GLP Compliance:** Yes
QA Report: Yes
Drug: APO-066, Lot #JA2957, Batch S-10168
Assumed 100% purity

Design:

This study was designed to evaluate the effects of APO-066 on male and female fertility and reproductive performance, and effects on early embryonic development in Crl:CD(SD) rats. Animals were individually housed except during cohabitation period. When randomized to study, animals were 9 weeks old and males weighed 259-329 g and females weighed 183-266 g. The drug was administered twice daily by oral gavage, yielding total daily doses of 0, 30, 60 or 150 mg/kg/day of APO-066. Control animals received vehicle composed of 0.5% w/v of CMC in sterile saline for injection.

The study was conducted in two phases wherein males were treated for 28 days prior to cohabitation with naïve females in Phase 1, with dosing continuing for at least 10 weeks in males whereupon they were subjected to a gross necropsy. In Phase 2, females were treated for 14 days prior to cohabitation with naïve males, and dosing continued through gestation day 7. On gestation day 13 they were euthanized with CO₂ and subjected to caesarean sectioning and gross necropsy. Group designations are shown in the tables below.

Phase 1: Treated males mated to naïve females.

Group	Number Animals		Dose Level	Dose Level	Dose Concentration
	Male	Female	(mg/kg/b.i.d.)	(mg/kg/day)	(mg/mL/b.i.d.)
1 (Control)	22	22	0	0	0
2 (Low)	22	22	15	30	3
3 (Mid)	22	22	30	60	6
4 (High)	22	22	75	150	15

Phase 2: Treated females mated to naïve males.

Group	Number Animals		Dose Level	Dose Level	Dose Concentration
	Male	Female	(mg/kg/b.i.d.)	(mg/kg/day)	(mg/mL/b.i.d.)
1 (Control)	22	22	0	0	0
2 (Low)	22	22	15	30	3
3 (Mid)	22	22	30	60	6
4 (High)	22	22	75	150	15

Clinical signs were recorded daily, with individual body weight and food consumption recorded twice weekly throughout the study. Stage of estrous cycle was determined daily on females during and 2 weeks prior to cohabitation with males. Hematology parameters were evaluated just prior to cohabitation and prior to terminal sacrifice. Gross necropsy

was conducted on all adult males and females with tissues discarded without histopathology except reproductive organs in Phase 1 males were examined microscopically. Treated males were sacrificed after at least 10 weeks of APO-066 treatment and sperm evaluations conducted. Dams were sacrificed on presumed gestation day 13 with numbers of corpora lutea, implants, resorptions, live and dead fetuses recorded. External and soft tissue examination was conducted on all fetuses.

Results (NNF: No Noteworthy Findings):

Survival: All animals assigned to study survived to scheduled termination.

Clinical Signs: The only consistent finding in males and females in both Phase 1 and 2 was a clear, oral discharge observed during day 1-3 of dosing in the males of the 60 mg/kg/day group and males and females of the 150 mg/kg/day group.

Food Intake: In Phase 1 males, food intake was decreased through Day 21. In Phase 2, female food intake was increased during pre-mating period, but did not continue during the gestation period. By gestation day 13, food intake was comparable to control values for all APO-066 groups.

Body Weight: In Phase 1 males, body weight was decreased through Day 21, correlating with decreased food intake. In Phase 2, female body weight was increased during pre-mating period correlating with food intake, but did not continue during the gestation period. By gestation day 13, food intake and body weight was comparable to control values for all APO-066 groups.

Estrous Cycle: Treatment of females with APO-066 in Phase 2 had a notable effect on the length of the estrous cycle in all treated groups. Females were observed to be in prolonged diestrus for 4 or more days, resulting in an increase in the time from cohabitation to confirmed copulation. This delay in confirmed mating had no effect on fertility or reproductive performance.

Hematology: RBC, HGB, WBC, differential WBC, HCT, PT, APTT, platelet count, reticulocyte count, blood smear, red cell distribution, mean platelet volume, and calculated MCV, MCH and MCHC were recorded.

Blood samples were collected on the day of first cohabitation and just prior to termination. Phase 1 males in 150 mg/kg/day group had increased mean platelet volume (MPV) and decreased prothrombin time (PT). Phase 2 females in 60 and 150 mg/kg/day groups had increased MPV and total platelet count and decreased PT.

Sperm Evaluation: Samples collected from caudal end of right epididymis for sperm analysis at terminal sacrifice of Phase 1 males. NNF for sperm counts, morphology and motility in all groups treated with APO-066.

Organ Weights: Phase 1 males had the following organs weighed: left and right epididymis, prostate, seminal vesicles with coagulating gland, and left and right testes. Absolute organ weights were unaffected by treatment with APO-066, however due to the retarded growth rate and resultant lesser body weight, the organ-to-body weight ratios were significantly increased for both testes and left epididymis of males of the 150 mg/kg/day group. These fluctuations in organ weights did not affect male fertility.

Histopathology of male reproductive organs: NNF

Gross Necropsy: NNF

Fetal External and Soft Tissue Exams: NNF

Caesarean Section Results:

Phase 1 summary of reproductive parameters are shown in the table below.

PARAMETER	TREATMENT GROUP			
	1 Control (0.5% CMC in saline)	2 APO-066 (30mg/kg/ day)	3 APO-066 (60mg/kg/ day)	4 APO-066 (150mg/kg/ day)
M/F Assigned	22/22	22/22	22/22	22/22
M/F Deaths	0/0	0/0	0/0	0/0
Pregnant (#/%)	21/95	21/95	22/100	21/95
Total Live Fetuses	297	322	352	309
Mean Litter Corpora Lutea	16.3	17.1	17.4	16.2
Live Litters (#/%)	21/95	21/95	22/100	21/95
Mean Litter Implants	15.0	16.0	16.4	15.1
Mean Litter Resorptions	0.9	0.6	0.4	0.4
Mean Fetal Death	0	0	0	0
Mean Litter Live Pups	14.1	15.3	16.0	14.7
Pre-implantation Loss (%)	8.7	6.6	5.6	7.1
Post-implantation Loss (%)	5.4	3.9	2.5	2.5
Female Fertility Index	100	100	100	100
Male Fertility Index	100	100	100	100

The number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were unaffected by treatment. Indices for pre-implantation loss, post-implantation loss, male fertility and female fertility were also unaffected by treatment.

Male Sperm Analysis:

Phase 1: A summary of sperm analysis parameters is shown in the table below.

GROUP	1	2	3	4
COMPOUND	Control (0.5% CMC in saline)	APO-066 (30 mg/kg/day)	APO-066 (60 mg/kg/day)	APO-066 (150 mg/kg/day)
No. Males Examined	22	22	22	22
Motility (%)	95.6	95.0	93.4	93.2
Epididymal Count (10⁶ Sperm/Gram)	811.8	995.9	804.9	1,064.5
Sperm Morphology (% Abnormal)	1.1	0.9	1.1	1.0
Male Fertility Index	100	100	100	100

When male rats in Phase 1 were administered 30, 60 or 150 mg/kg/day of APO-066 for at least 10 weeks, there were no drug or dose related effects observed in any group on sperm motility, caudal epididymal sperm count or sperm morphology when compared to control groups.

Caesarean Section Results:

Phase 2 summary of reproductive parameters are shown in the table below.

PARAMETER	TREATMENT GROUP			
	1 Control (0.5%CMC in saline)	2 APO-066 (30 mg/kg/ day)	3 APO-066 (60 mg/kg/ day)	4 APO-066 (150 mg/kg/ day)
M/F Assigned	22/22	22/22	22/22	22/22
M/F Deaths	0/0	0/0	0/0	0/0
Pregnant (#/%)	21/95	19/90	21/95	22/100
Total Live Fetuses	317	297	311	363
Mean Litter Corpora Lutea	17.1	17.0	17.9	18.2
Live Litters (#/%)	21/100	19/100	21/100	22/100
Mean Litter Implants	16.0	16.3	16.0	17.0
Mean Litter Resorptions	1.0	0.6	1.2	0.5
Mean Fetal Death	0	0	0	0
Mean Litter Live Pups	15.1	15.6	14.8	16.5
Pre-implantation Loss (%)	5.5	4.3	8.3	5.7
Post-implantation Loss (%)	5.9	3.9	8.6	3.1
Female Fertility Index	95	90	95	100
Male Fertility Index	95	90	95	100

The number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were unaffected by treatment. Indices for pre-implantation loss, post-implantation loss, male fertility and female fertility were also unaffected by treatment.

Overall conclusions and recommendations:

Conclusions: Fertility, reproductive performance, fetal survival and development were not affected in male or female rats receiving up to and including 150 mg/kg/day (0.25 X MHD on mg/m² basis) of oral APO-066, prior to and during gestation. The information presented in this application is adequate to support the approval of APO-066 with adequate warnings in the label.

Unresolved toxicology issues: Carcinogenicity studies to be conducted post approval.

Recommendations: From the perspective of nonclinical pharmacology and toxicology, the information provided in this NDA is adequate. Therefore, this drug application is recommended for APPROVAL, with adequate warnings in the label.

APPENDIX/ATTACHMENTS: NONE

COMMENTS FOR SPONSOR: NONE

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-21825	ORIG-1	AOPHARMA INC	FERRIPROX (DEFERIPRONE)

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/s/

DAVID E BAILEY
09/22/2009

ADEBAYO A LANIYONU
09/22/2009

MEMO TO FILE**NONCLINICAL PHARMACOLOGY AND TOXICOLOGY**

RE: Response to letter providing timeline for submission of ClinStat RU and brief outline of nonclinical protocol for rat fertility study.

Submission: RUP C 001, 26-NOV-2008.
NDA number: 21-825

Information to sponsor: Yes. See Conclusion below.

Sponsor: ApoPharma Inc.
Toronto, Ontario, Canada

Agent: Lynda Sutton
Cato Research
4363 South Alston Avenue
Durham, NC 27713
Telephone: 919-361-2286

Manufacturer for drug substance: Apotex Pharmachem Inc.
Brantford, Ontario, Canada

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Products
HFD #: 160
Date: December 5, 2008

Drug: Registered name: FERRIPROX®
Trade name: deferiprone
Chemical name: 3-hydroxy-1, 2-dimethylpyridin-4-one

Common name: APO-066
Molecular weight: 139.15

Relevant INDs/NDAs/DMFs: IND# 45,724; DMF # 10,867

Drug class: Iron chelator

Intended clinical population: Patients with iron overload resulting from chronic transfusion therapy or for prevention of cardiac disease in patients with iron overload.

Clinical formulation: 500 mg Fe – film coated tablet
100 mg Fe/mL – aqueous oral solution

Clinical dose: 75 – (b)(4) mg Fe/kg/day (25 - 33 mg/kg, tid)
Route of administration: Oral

Background

The division provided the sponsor with comments and later a face-to-face meeting on November 13, 2008 discussing deficiencies in the submission of the nonclinical Reviewable Unit of the NDA.

In the division Reviewable Unit Letter dated June 27, 2007, 3 deficiencies were listed.

1. Electrophysiology study in hERG cells.
2. Lifetime carcinogenicity studies.
3. Fertility and early embryonic development study in rats.

Current Status

In the current submission the sponsor proposed a timetable for addressing the deficiencies mentioned in the Reviewable Unit Letter.

1. The Electrophysiology study has been submitted and that requirement has been fulfilled.
2. Carcinogenicity studies. The sponsor proposed conducting a 2-year carcinogenicity study in rats and a 6-month study in p53 knockout mice during Phase 4. Sponsor's response is adequate.
3. Fertility study in rats. The sponsor will be initiating this study in a contract research lab in 3-4 weeks, with a target date of June 2009 for final report. The brief outline of the protocol appears adequate for a study and the timetable for submission of the final report appears acceptable. It should be noted that the protocol describes a study of " male and female fertility" and not just " female fertility" as indicated in the cover letter from Lynda Sutton of Cato.

Conclusion:

From the perspective of nonclinical pharmacology and toxicology, the sponsor should be informed that the brief outline of the protocol appears adequate for the male and female fertility study, and the timetable for submission of the final report appears acceptable.

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/s/

David Bailey
12/16/2008 06:07:34 AM
PHARMACOLOGIST

NONCLINICAL PHARMACOLOGY AND TOXICOLOGY REVIEW

RE: Response to RUP Letter and request for information from sponsor.

Submission: RUP 001 BP, 19-March-2008
NDA number: 21-825
Review number: 02

Information to sponsor: Yes. See "Comments to Sponsor" section of this review.

Sponsor: ApoPharma Inc.
Toronto, Ontario, Canada

Agent: Lynda Sutton
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4363 South Alston Avenue
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Manufacturer for drug substance: Apotex Pharmachem Inc.
Brantford, Ontario, Canada

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Products
HFD #: 160
Review completion date: August 4, 2008

Drug: Registered name: FERRIPROX®
Trade name: deferiprone
Chemical name: 3-hydroxy-1, 2-dimethylpyridin-4-one

Common name: APO-066
Molecular weight: 139.15

Relevant INDs/NDAs/DMFs: IND# 45,724; DMF # 10,867

Drug class: Iron chelator

Intended clinical population: Patients with iron overload resulting from chronic transfusion therapy or for prevention of cardiac disease in patients with iron overload.

Clinical formulation: 500 mg Fe – film coated tablet
100 mg Fe/mL – aqueous oral solution
Clinical dose: 75 – (b)(4) mg Fe/kg/day (25 - 33 mg/kg, tid)
Route of administration: Oral

Background

The division has provided the sponsor with two letters describing deficiencies in the submission of the nonclinical Reviewable Unit of the NDA, and requesting additional information from the 52-week rat study.

In the division Reviewable Unit Letter dated June 27, 2007, three deficiencies were listed:

1. Electrophysiology study in hERG cells.
2. Lifetime carcinogenicity studies.
3. Fertility and early embryonic development study in rats.

In the division letter of September 18, 2007, further information was requested to address tumor incidence in the 52-week repeat dose rat study.

Current Submission

In the current submission the sponsor provided information to address the issues described in the two letters. The sponsor proposed a timetable for addressing the deficiencies mentioned in the Reviewable Unit Letter.

1. The Electrophysiology study is currently underway and the report will be submitted upon finalization. The final study report must be submitted and reviewed prior to approval.
2. Carcinogenicity studies. The sponsor has proposed conducting a 2-year carcinogenicity study in rats and a 6-month study in p53 knockout mice during Phase 4. The range-finding studies to support dose levels for the carcinogenicity studies are currently underway. Final reports will be submitted with protocols for the 2 carcinogenicity studies for SPA review by March 2009, with initiation of the 2 carcinogenicity studies in August 2009. Final report for the mouse study is scheduled for December 2010 and the rat report for December 2012.

Sponsor's response is adequate.

3. Fertility and early embryonic development. The sponsor is proposing this as [REDACTED] (b) (4).

[REDACTED] (b) (4) The final study report must be submitted for review prior to approval.

Tumor incidence issue:

The letter dated September 18, 2007 asked the sponsor to have the study pathologist address the incidence of tumors in the 52-week rat study. Tumor incidence was not presented in tabular form or discussed in the final study report.

The study pathologist presented tabular data and discussed in length the results of the 52-week study, and justified why it does not predict potential carcinogenicity in a 2-year study. Deficiencies in the 52-week study include inadequate numbers of animals with only 10/sex/group, and tumor incidence, although higher than expected, falls in the range of historical tumor incidence data provided by the animal supplier, [REDACTED] (b) (4).

Therefore, with statistical power very weak and tumor incidence just slightly higher than historical incidence, it cannot be statistically concluded that there is a significant increase in tumor incidence. This will be determined in the 2-year rat and 6-month mouse carcinogenicity studies.

The sponsor's response is adequate.

Conclusion:

The sponsor has adequately addressed the agency concerns mentioned in the letters of June 27, 2007 and September 18, 2007 relating to the two carcinogenicity studies and the tumor incidence in the 52-week rat study. However, the electrophysiology study and the fertility and embryonic development study reports must be submitted and reviewed prior to approval.

Comments to Sponsor.

A. In the division Reviewable Unit Letter dated June 27, 2007, three deficiencies were listed.

1. Electrophysiology study in hERG cells.
2. Lifetime carcinogenicity studies.
3. Fertility and early embryonic development study in rats.

The sponsor has adequately addressed the agency concerns relating to the two carcinogenicity studies discussed in the submission, RUP 001 19 March 2008.

It should be noted by the sponsor that:

- a. The two carcinogenicity study protocols for SPA review must be provided separately and not in the same submission.
- b. The sponsor must provide final study reports for the electrophysiology study and the fertility and early embryonic development study for review prior to approval.

B. In the division letter of September 18, 2007, further information was requested to address tumor incidence in the 52-week rat repeat dose study.

The sponsor has adequately addressed this issue in the submission dated March 19, 2008 designated, RUP 001 BP 19-March-2008.

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

/s/

David Bailey
8/4/2008 01:30:08 PM
PHARMACOLOGIST

Adebayo Lanionu
8/4/2008 02:32:57 PM
PHARMACOLOGIST



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-825
SERIAL NUMBER: RUP 000
DATE RECEIVED BY CENTER: 12/22/06
PRODUCT: FERRIPROX®
INTENDED CLINICAL POPULATION: Patients with iron-overload from chronic
transfusion therapy or iron-induced cardiac
disease.
SPONSOR: ApoPharma Inc.
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Toronto, Canada M9L 2Z7
U.S. AGENT Cato Research
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DOCUMENTS REVIEWED: Electronic Submission in EDR
REVIEW DIVISION: Medical Imaging and Hematology Drug Products
(HFD-160)
PHARM/TOX REVIEWER: David E. Bailey, Ph.D.
PHARM/TOX SUPERVISOR: Adebayo A. Lanionu, Ph. D.
DIVISION DIRECTOR: R. Dwaine Rieves, MD.
PROJECT MANAGER: Hyon-Zu Lee

Date of review submission to Division File System (DFS): June 27, 2007

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	7
2.6.1 INTRODUCTION AND DRUG HISTORY.....	7
2.6.2 PHARMACOLOGY.....	10
2.6.2.1 Brief summary	10
2.6.2.2 Primary pharmacodynamics	10
2.6.2.3 Secondary pharmacodynamics	11
2.6.2.4 Safety pharmacology	11
2.6.2.5 Pharmacodynamic drug interactions.....	12
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	12
2.6.4 PHARMACOKINETICS/TOXICOKINETICS	12
2.6.4.1 Brief summary	12
2.6.4.2 Methods of Analysis	12
2.6.4.3 Absorption	12
2.6.4.4 Distribution.....	12
2.6.4.5 Metabolism.....	12
2.6.4.6 Excretion.....	12
2.6.4.7 Pharmacokinetic drug interactions.....	12
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	12
2.6.6 TOXICOLOGY.....	13
2.6.6.1 Overall toxicology summary	13
2.6.6.2 Single-dose toxicity	18
2.6.6.3 Repeat-dose toxicity	19
2.6.6.4 Genetic toxicology.....	34
2.6.6.5 Carcinogenicity.....	42
2.6.6.6 Reproductive and developmental toxicology.....	43
2.6.6.7 Local tolerance	43
2.6.6.8 Special toxicology studies	43
2.6.6.9 Discussion and Conclusions	43
2.6.6.10 Tables and Figures.....	43
2.6.7 TOXICOLOGY TABULATED SUMMARY	43
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	44
APPENDIX/ATTACHMENTS: COMMENTS TO SPONSOR	45

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: It is premature to consider approvability at this time without considering results from other disciplines.

B. Recommendation for nonclinical studies:

Deficiencies:

An electrophysiology study in hERG cells.

Lifetime carcinogenicity studies.

Fertility and early embryonic development study to evaluate male and female fertility and general reproductive performance.

C. Recommendations on labeling – Labeling recommendations are not included in this review due to deficiencies in the data.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Multiple of human dose

The sponsor has made the statement in numerous sections of this submission that APO-066 is relatively nontoxic with single dose maximum tolerated doses in animals in the 500-800 mg/kg range. That statement is correct, and would normally be significant; however, with the daily maximum human dose of ^{(b)(4)} mg/kg as indicated in the proposed labeling in this submission, the safety margin is very low based on body weight (5-8 X MHD), and in most cases the multiple of the MHD based on surface area is less than 1.0.

Toxicokinetics

In toxicokinetic studies, in both rats and monkeys, C_{max} and AUC_{0-7h} generally increased with increasing doses and was noticeably higher in non-iron-loaded animals. T_{max} and $t_{1/2}$ were essentially the same for all groups and were not dose, sex, iron-loading or duration of treatment dependent.

Single dose toxicity

In single dose toxicity studies in naïve and iron-loaded rodents the maximum tolerated dose was in the range of 500-800 mg/kg. This is only 5-8 times the MHD based on body weight and less than one based on area. In mice and rats primary effects were lethargy, abnormal gait, labored breathing, low body temperature, ptosis, prostration and lethality. In guinea pigs eye discharge, weakness, lethargy and lethality were the signs observed. Foci of cardiac and skeletal muscle necrosis were observed histopathologically.

Repeat dose toxicity study: Rat

In a 12-month repeat dose toxicity study, iron-loaded rats were administered oral APO-066 at doses of 75, 150 and 200 mg/kg/day, and naïve rats received 150 mg/kg/day. At all dose levels of APO-066, there was a high incidence of malignant tumors in males and females, independent of whether animals were naïve or iron-loaded. Organs involved were liver, lung, mammary gland, skin and thyroid.

There was evidence of iron removal from tissues at the two high doses of APO-066 of 75 and 100 mg/kg bid to naïve rats. Iron removal resulted in iron deficiency anemia that was severe enough to cause death in some animals with hemoglobin levels as low as 1.4-2.3 g/dL. Iron loading conferred some protection against the APO-066 induced anemia effects. Treatment with APO-066 also induced serum changes indicating hepatic effects in naïve rats and at all doses caused changes in thyroid weight and morphology, and mammary gland lobular hyperplasia, independent of iron-loading. Animals did not completely recover from drug effects during the 4-week post-dose recovery period with iron deposition, bone marrow hypocellularity and thyroid effects remaining.

Repeat dose toxicity study: Cynomolgus Monkeys

In a 12 month repeat dose toxicity study, APO-066 given orally to naïve cynomolgus monkeys at 75 mg/kg twice daily for 52 weeks was without significant affect (1.5 X the MHD based on body weight and ≤ 1 based on surface area). Treatment of iron-loaded monkeys by nasogastric intubation with APO-066 at doses of 37.5, 75 and 100/125 mg/kg bid for 52 weeks was without evidence of treatment related effects, except for the pharmacological responses due to iron loading and the administration of iron chelators. During week 14, the high dose was increased to 125 mg/kg bid. The NOAEL for naïve, non-iron-loaded animals was 75 mg/kg bid (150 mg/kg/day). The NOAEL for iron-loaded animals was 100/125 mg/kg bid (200/250 mg/kg/day). A drug effect dose was not defined. However, the top doses were near the lethal dose in a 3-month monkey study.

Since no drug effects were observed, this study would be considered inadequate without information from a 3-month preliminary study in monkeys. In that study 6 of 13 non-iron-loaded monkeys died from treatment with 250 mg/kg/day for 18 days, reduced to 200 mg/kg/day for 2 days and reduced to 150 mg/kg/day thereafter. The animals died of drug induced iron deficiency anemia and resultant effects of anorexia, body weight loss,

emaciation, bone marrow hypocellularity and hemoglobin levels in the range of 1.8 – 2.7 g/dL. No drug effects were observed at 100 mg/kg/day and below.

A more comprehensive cardiovascular safety pharmacology evaluation than what was done in this monkey study, with evaluation of QTc intervals at first dose, with more tracings and more leads would be desirable. However, with EKGs recorded after steady state was reached in this study, it is considered more valuable to have results of an electrophysiology study with hERG cells than to repeat the in vivo study.

Genetic toxicology:

The Salmonella/E. coli reverse mutation assay was negative.

The following genotoxicity studies were positive:

- Mouse lymphoma cell TK+/- forward gene mutation assay.

- Chromosomal aberration assay in Chinese Hamster Ovary.

- Chromosomal aberration assay in human lymphocytes.

- Micronucleus test in naïve male mice bone marrow erythropoietic cells.

- Micronucleus test in iron-loaded and naïve male and female mice bone marrow erythropoietic cells.

APO-066 is found to be a positive transspecies genotoxin and thus considered a transspecies carcinogen, which implies a carcinogenic risk for humans.

Carcinogenicity:

Classic lifetime carcinogenicity studies have not been conducted; however, the carcinogenic potential of APO-066 can be derived from the 12 month repeat dose study in rats. A high incidence of multiple metastatic tumors were observed in all groups of rats receiving APO-066, independent of whether they were naïve or iron-loaded. Organs involved were liver, lung, mammary gland, skin and thyroid.

Reproductive toxicology:

Teratology studies were conducted in rats and rabbits.

In rats, doses administered were 25, 50 and 75 mg/kg/day to groups of 20 presumed pregnant rats from day 6-15 of pregnancy. The NOAEL for maternal toxicity was 75 mg/kg/day (0.2 X the MHD based on surface area). There were soft tissue and skeletal malformations at all doses given, therefore, the NOAEL for fetal malformations was not defined and was less than 25 mg/kg/day (≤ 0.06 X the MHD based on surface area).

In rabbits, doses administered were 10, 25, 50 and 100 mg/kg/day to groups of 16 presumed pregnant rabbits from day 6-19 of pregnancy. Maternal and embryo lethality were observed at 100 mg/kg/day. Drug related teratogenicity was observed at all dose levels. The NOAEL for maternal toxicity was 50 mg/kg/day (0.2 X the MHD based on

surface area). The NOAEL for soft tissue and skeletal fetal malformations was not defined and was less than 10 mg/kg/day (<0.04 X the MHD based on surface area).

It can be concluded that APO-066 is unequivocally a positive genotoxin in multiple species, a potent teratogen and a potent carcinogen.

B. Pharmacologic activity

Deferiprone is a bidentate iron chelator that at physiological pH preferentially forms 1:3 iron:ligand complexes of Fe(III). It is readily absorbed from the gut and as a chelator it preferentially binds iron in the body in a 1:3 iron Fe(III):ligand complex. Neither deferiprone nor the 1:3 iron:ligand complex carries a charge at physiological pH, which aids in crossing cell membranes. The deferiprone:iron complexes are not absorbed from the intestine, thus deferiprone does not aid in dietary absorption of iron. Deferiprone mobilizes iron from the iron transport protein, transferrin, and from the resulting lysosomal degradation product hemosiderin. Deferiprone is excreted in the urine and by the fecal route via the bile.

C. Nonclinical safety issues relevant to clinical use

APO-066 is:

Unequivocally a positive transspecies genotoxin in multiple assays, and thus presumed to be a transspecies carcinogen, which implies a carcinogenic risk to humans.

A potent soft tissue and skeletal teratogen in rats and rabbits at all doses tested.

A potent carcinogen in rat 12 month chronic toxicity study with numerous malignant tumors at all APO-066 dose levels tested. Tumor types observed included hepatocellular carcinoma, lung metastasis malignant histiocytoma, lung metastasis hepatocellular carcinoma, mammary adenocarcinoma, mammary carcinoma, mammary fibroadenoma, skin keratoacanthoma, skin malignant fibrous histiocytoma, skin fibrocyte fibroma and thyroid follicular cell adenoma.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-825
Review number: 01
Submission: RUP 001 21-DEC-2006

Information to sponsor: Yes. See “Comments to Sponsor” section of this review.

Sponsor: ApoPharma Inc.
Toronto, Ontario, Canada

Agent: Lynda Sutton
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Manufacturer for drug substance: Apotex Pharmachem Inc.
Brantford, Ontario, Canada

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Products
HFD #: 160
Review completion date: June 22, 2007

Drug: Registered name: FERRIPROX®
Trade name: deferiprone
Chemical name: 3-hydroxy-1, 2-dimethylpyridin-4-one
Common name: APO-066
CAS registry number:
Molecular weight: 139.15
Structure:

Relevant INDs/NDAs/DMFs: IND# 45,724; DMF # 10,867

Drug class: Iron chelator

Intended clinical population: Patients with iron overload resulting from chronic transfusion therapy or for prevention of cardiac disease in patients with iron overload.

Clinical formulation: 500 mg – film coated tablet

Clinical dose: 75 – (b) (4) mg/kg/day (25 - 33 mg/kg, tid)

Route of administration: Oral

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

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA number 21-825 are owned by ApoPharma Inc. or are data for which ApoPharma Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA number 21-825 that ApoPharma Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that ApoPharma Inc. does not own, is for descriptive purposes only and is not relied upon for approval of NDA number 21-825.

Studies reviewed within this submission:

APO-066: 52 Week oral (gavage) toxicity study in the iron-loaded and non-iron-loaded Sprague Dawley rat.

APO-066: 52 Week oral (gavage) toxicity study in the iron-loaded and non-iron-loaded Cynomolgus monkey.

Ames/Salmonella-E. coli reverse mutation assay with APO-066.

L5178Y Mouse lymphoma cell TK +/- forward gene mutation assay.

In vitro mammalian chromosome aberration assay in Chinese Hamster Ovary with APO-066.

In vitro chromosome aberration assay in human lymphocytes with deferiprone and deferoxamine.

In vitro micronucleus test in male mouse bone marrow erythropoietic cells with APO-066.

APO-066: Evaluation of micronucleus induction potential of APO-066 in iron-loaded and naïve male and female mice.

Studies not reviewed within this submission:

Inhibition of (Lack of) cytochrome P450 enzymes by APO-066.
Non-GLP preliminary pharmacology screen.

Comparison of in vivo iron mobilization potentials of iron chelators in a rat model.
Non-GLP preliminary pharmacology screen.

Comparison of metabolite profile in human, monkey and rat liver microsomal incubations for Phase I and Phase II metabolism.
Non-GLP preliminary pharmacology screen.

References:

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10. Rai, B.L., et al. Synthesis and physiological properties of 4-pyridinones. *J Med Chem.* 1998;41(18):3347.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Diferiprone is readily absorbed from the gut and as a chelator it preferentially binds iron in the body in a 1:3 iron (Fe III):ligand complex and the complex is excreted in the urine and by the fecal route via the bile.

2.6.2.2 Primary pharmacodynamics

Deferiprone is a bidentate iron chelator that at physiological pH preferentially forms 1:3 iron:ligand complexes. Deferiprone has a high affinity for Fe(III) compared with Fe(II). Neither deferiprone nor the 1:3 iron:ligand complex carries a charge at physiological pH, which aids in crossing cell membranes. The chelate is somewhat more lipophilic than deferiprone. The deferiprone:iron complexes are not absorbed from the intestine, thus deferiprone does not aid in dietary absorption of iron.

Deferiprone mobilizes iron from the iron transport protein, transferrin, and from the resulting lysosomal degradation product hemosiderin. The major pathway for iron removal from diferric transferrin exhibits saturation kinetics with respect to deferiprone concentration. Iron removal from diferric transferrin is subject to allosteric regulation by several endogenous anions, primarily chloride and bicarbonate, which facilitate faster iron removal under physiological conditions. Iron mobilization by deferiprone from ferritin and hemosiderin is a slow process and the protein shell limits the access of deferiprone to the iron core.

Deferiprone has not been subject to structured proof of concept studies by the sponsor like most drugs would have been. Proof of concept has been studied in depth and is available in the literature, much of which dates back 20-30 years. Selected pertinent references are shown on page 9 of this review. Numerous species have been used to assess the effectiveness of deferiprone to remove iron from the intact animal using ^{59}Fe as a tracer, including mouse, rat, gerbil, guinea pig, marmoset, and cebus monkey. Naïve and iron-loaded animals have been used, with iron-loading being accomplished by use of ferrocene, carbonyl iron, and iron dextran. Iron excretion was increased by deferiprone in all animals tested. Iron excretion is dose dependent, and primarily by the fecal route via the bile. However, a greater amount is excreted in the urine in monkeys and humans than in small animals. After a single dose, urinary excretion is complete within 24 hours, whereas, fecal excretion is not complete before 48 hours.

In the liver, deferiprone preferentially removes iron from periportal hepatocytes, Kupffer's cells, and portal macrophages, with no mobilization of mitochondrial or peroxisomal iron. In cardiac and brain iron burden, some nonclinical studies have shown a reduction with the use of deferiprone; however, the results have been inconsistent and not conclusive. Studies have not demonstrated a removal of iron from the spleen. Deferiprone has been shown to remove the iron deposits from the red blood cell membranes of thalassemic mice, which increased red cell survival.

In the intact animal iron chelation and elimination from the body is demonstrated in the 12 month rat study and the 3 month preliminary range finding study in monkeys. In the rat study a significant number of deaths in the APO-066 treated groups were attributed to iron deficiency anemia and resultant effects. Hemoglobin levels ranged from 1.4 - 2.3 g/dL in the animals that died. There were 3/14 male deaths in the high dose iron-loaded group, and 5/14 male deaths and 1/14 female deaths in the mid dose naïve group.

In the monkey study 6/13 non-iron-loaded monkeys died from treatment with APO-066 at decreasing doses of 250/200/150 mg/kg/day. The animals died of iron deficiency anemia and resultant anorexia, body weight loss and emaciation. Hemoglobin values were in the range of 1.8-2.7.

2.6.2.3 Secondary pharmacodynamics

Deferiprone limits free radical mediated reactions of hydroxylation, oxidation, oxidative degradation and peroxidation of lipids. Deferiprone-iron complex protected against cell injury in a cellular model of hypoxia-reoxygenation injury. Deferiprone does not react with hydroxyl radicals and nitrogen-centered radicals. Deferiprone has demonstrated a protective effect against iron-catalyzed, free radical-mediated cell injury in rat neonatal beating myocardial cell cultures.

Deferiprone is selective for Fe(III), but in displacement studies Cu(II) was the most efficient at displacing Fe(III). Al(III) and Pb(II) were somewhat active with Zn(II) with Co(II) showing no activity.

In acute iron toxicity studies, oral deferiprone significantly decreased mortality in rats receiving extremely high single doses of iron by blocking absorption from the GI tract.

2.6.2.4 Safety pharmacology

Safety pharmacology information is very limited and detailed information is lacking.

Only hypersalivation was noted in a behavioral pharmacology screen with deferiprone in rats at an oral dose of 100 mg/kg. In mice, oral doses of deferiprone of 300 mg/kg induced body temperature decreases, hypoactivity and ataxia. In cardiovascular and renal function tests with deferiprone, oral doses of 100 mg/kg, only increased urine volume and increased electrolyte excretion were noted.

A definitive cardiovascular study was not performed; however, limited cardiovascular information was collected and is presented in evaluation of the 12 month toxicity study in cynomolgus monkey in this review. Heart rate, systolic, diastolic and mean arterial blood pressure, and EKG waveforms from Leads I, II, and III were unaffected by treatment.

2.6.2.5 Pharmacodynamic drug interactions: No study conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

In toxicokinetic studies, in both rats and monkeys, C_{max} and AUC_{0-7h} generally increased with increasing doses and was noticeably higher in non-iron-loaded animals. T_{max} and $t_{1/2}$ were essentially the same for all groups and were not dose, sex, iron-loading or duration of treatment dependent.

2.6.4.2 Methods of Analysis

2.6.4.3 Absorption

2.6.4.4 Distribution

2.6.4.5 Metabolism

2.6.4.6 Excretion

2.6.4.7 Pharmacokinetic drug interactions

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Single dose toxicity studies:

In single dose toxicity studies in naïve and iron-loaded rodents, the maximum tolerated dose was in the range of 500-800 mg/kg. This is only 5-8 times the MHD based on body weight and less than one based on surface area. In mice and rats the primary effects were lethargy, abnormal gait, labored breathing, low body temperature, ptosis, prostration and lethality. In guinea pigs eye discharge, weakness, lethargy and lethality were the clinical signs observed. Foci of cardiac and skeletal muscle necrosis were observed histopathologically.

Repeat dose toxicity study: Rat

In a 12-month repeat dose toxicity study, iron-loaded rats were administered oral APO-066 at doses of 75, 150 and 200 mg/kg/day, and naïve rats received 150 mg/kg/day. At all dose levels of APO-066, there was a high incidence of malignant tumors in males and females, independent of whether animals were naïve or iron-loaded. Organs involved were liver, lung, mammary gland, skin and thyroid.

At high doses, there was evidence of iron removal from tissues. High doses of APO-066 of 75 and 100 mg/kg bid to naïve rats induced iron deficiency anemia that was severe enough to cause death in some animals. The anemia was associated with bone marrow hypercellularity. Iron loading conferred some protection against the APO-066 induced anemia effects.

Treatment with APO-066 also induced serum changes indicating hepatic effects in naïve rats and at all doses caused changes in thyroid weight and morphology, and mammary gland lobular hyperplasia, independent of iron-loading. Animals did not completely recover from drug effects during the 4-week post-dose recovery period with iron deposition, bone marrow hypocellularity and thyroid effects remaining.

For a complete list of organs and tissues collected and organs weighed, see the table on the next page.

List of tissues collected and organs weighed in repeat dose studies.

Study	006	006	007	007
Species	Rat	Rat	Cyno	Cyno
Adrenals	X	O	X	O
Bone (femur, sternum)	X/X		X/X	
Brain	X	O	X	O
Cecum	X		X	
Cervx/ Uterus/Vagina	X/X/X		X/X/X	
Colon	X		X	
Duodenum	X		X	
Epididymis	X		X	
Esophagus	X		X	
Eye/Optic nerve	X/X		X/X	
Fallopian tube	X		X	
Gall bladder			X	
Gross lesions	X		X	
Harderian gland	X		X	
Heart	X	O	X	O
Ileum	X		X	
Jejunum	X		X	
Kidneys	X	O	X	O
Liver	X	O	X	O
Lungs	X	O	X	O
Lymph node, cervical	X		X	
Lymph nodes mandibular	X		X	
Lymph nodes, mesenteric	X		X	
Mammary Gland	X		X	
Ovaries	X	O	X	O
Pancreas	X		X	
Pharynx	X		X	
Pituitary	X	O	X	O
Prostate	X	O	X	O
Rectum	X		X	
Salivary gland	X		X	
Sciatic nerve	X		X	
Seminal vesicles	X		X	
Skeletal muscle/nerve	X/X		X/X	
Skin	X		X	
Spinal cord	X		X	
Spleen	X	O	X	O
Stomach	X		X	
Testes	X	O	X	O
Thymus	X	O	X	O
Thyroid/Parathyroid	X/X	O	X/X	O
Tongue	X		X	
Trachea	X		X	
Urinary bladder	X		X	

X = histopathology performed. O = Organ weights

Repeat dose toxicity study: Cynomolgus Monkeys

In a 12 month repeat dose toxicity study, APO-066 given orally to naïve cynomolgus monkeys at 75 mg/kg twice daily for 52 weeks was without significant affect. Treatment of iron-loaded monkeys by nasogastric intubation with APO-066 at doses of 37.5, 75 and 100/125 mg/kg bid for 52 weeks was without evidence of treatment related effects, except for the expected pharmacological responses due to iron loading and the administration of iron chelators. During week 14, the high dose was increased to 125 mg/kg bid. The NOAEL for naïve, non-iron-loaded animals was 75 mg/kg bid (150 mg/kg/day). The NOAEL for iron-loaded animals was 100/125 mg/kg bid (200/250 mg/kg/day). A drug effect was not defined. However, the top doses were near the lethal dose in a 3-month monkey study.

Since no drug effects were observed in this study it would be considered inadequate without information from a 3-month preliminary study in monkeys. In that study 6 out of 13 non-iron-loaded monkeys died from treatment with 250 mg/kg/day for 18 days, reduced to 200 mg/kg/day for 2 days and reduced to 150 mg/kg/day thereafter. The animals died of drug induced iron deficiency anemia and resultant effects of anorexia, body weight loss, emaciation, bone marrow hypocellularity and hemoglobin levels in the range of 1.8 – 2.7 g/dL. No drug effects were observed at 100 mg/kg/day and below.

Genetic toxicology:

The Salmonella/E. coli reverse mutation assay was negative.

The following genotoxicity studies were positive:

- Mouse lymphoma cell TK+/- forward gene mutation assay.

- Chromosomal aberration assay in Chinese Hamster Ovary.

- Chromosomal aberration assay in human lymphocytes.

- Micronucleus test in naïve male mice bone marrow erythropoietic cells.

- Micronucleus test in iron-loaded and naïve male and female mice bone marrow erythropoietic cells.

APO-066 is found to be unequivocally a positive transspecies genotoxin and thus considered a transspecies carcinogen which implies a carcinogenic risk for humans.

Carcinogenicity:

Classic lifetime carcinogenicity studies have not been conducted; however, carcinogenic potential of APO-066 can be derived from the 12 month repeat dose study in rats. A high incidence of multiple metastatic tumors were observed in all groups of rats receiving APO-066, independent of whether they were naïve or iron-loaded. Organs involved were liver, lung, mammary gland, skin and thyroid. To produce these results in just 12 months, it can be concluded that APO-066 is a potent carcinogen.

The table below includes malignant tumor incidence for male and female rats at unscheduled death and terminal sacrifice intervals.

Tumor Type	Sex	Group 1 Naïve control	Group 2 Iron- loaded control	Group 3 APO-066 Iron- loaded	Group 4 APO-066 Iron- loaded	Group 5 APO-066 Iron- loaded	Group 6 APO-066 Naive
Dose APO-066 (mg/kg/day)		0	0	75	150	200	150
Hepatocellular Carcinoma	M					1	
Lung Metastasis Malignant Histiocytoma	F				1		
Lung Metastasis Hepatocellular Carcinoma	M					1	
Mammary Adenocarcinoma	F			1		1	
Mammary Carcinoma	M				1		
Mammary Fibroadenoma	F						3 (1 death)
Skin Keratoacanthoma	M				1		
Skin Malignant Fibrous Histiocytoma	F				1		
Skin Fibrocyte Fibroma	F			1			
Thyroid Follicular Cell Adenoma	F						1
Malignant Tumors	M	0	0	0	2	2	0
Malignant Tumors	F	0	0	2	2	1	4
Total Malignant Tumors	M&F	0	0	2	4	3	4
Number of animals examined	M&F	38	38	38	38	38	38

Reproductive toxicology:

Fertility and early embryonic development: Studies were not conducted

Embryofetal development:

Teratology studies were conducted in rats and rabbits and the results are in the Ciba-Geigy DMF and were previously reviewed by the agency.

From the results provided, the following can be concluded:

Rats: Doses administered were 25, 50 and 75 mg/kg/day to groups of 20 presumed pregnant rats from day 6-15 of pregnancy. The NOAEL for maternal toxicity was 75 mg/kg/day (0.2 X the MHD based on surface area). There were soft tissue and skeletal malformations at all doses given, therefore, the NOAEL for fetal malformations was not defined and was less than 25 mg/kg/day (≤ 0.06 X the MHD based on surface area).

Rabbits: Doses administered were 10, 25, 50 and 100 mg/kg/day to groups of 16 presumed pregnant rabbits from day 6-19 of pregnancy. Maternal and embryoletality were observed at 100 mg/kg/day. Drug related teratogenicity was observed at dose levels of 10, 25, 50 and 100 mg/kg/day. The NOAEL for maternal toxicity was 50 mg/kg/day (0.2 X the MHD based on surface area). The NOAEL for soft tissue and skeletal fetal malformations was not defined and was less than 10 mg/kg/day (< 0.04 X the MHD based on surface area).

Prenatal and postnatal development: Studies were not conducted.

Local tolerance: No studies were conducted.

Special toxicology studies: None

Discussion and Conclusions:

APO-066 is a positive genotoxin in multiple species, a potent teratogen and a potent carcinogen.

2.6.6.2 Single-dose toxicity

The current sponsor of Ferriprox, ApoPharma, did not conduct single-dose toxicity studies. However, there is an abundance of information in the literature and in the Ciba-Geigy DMF for single dose rodent studies that has been previously reviewed, and that information was summarized by the sponsor and is included below.

Included in the submission is a statement by the sponsor in Module 2, Section 2.6.6, Part 4, Paragraph 1, as follows:

“Pivotal general toxicology studies of 12 months duration in rats and cynomolgus monkeys, and a battery of genotoxicity studies, were sponsored by ApoPharma in order to assess the safety of deferiprone. A large amount of other information relevant to the evaluation of the toxicity of deferiprone has been published in the open literature, or is contained in a summary of studies carried out by Ciba-Geigy during their development of deferiprone.”

Elsewhere in the document it explains that when ApoPharma purchased deferiprone from Ciba-Geigy, a summary of studies was provided by Ciba-Geigy and a letter of cross reference to the DMF was provided so that the agency would have access to the previous work.

The sponsor has made the statement in numerous sections of this submission that APO-066 is relatively nontoxic with single dose maximum tolerated doses in the 500-800 mg/kg range. That statement is correct, and would normally be significant; however, with the daily maximum human dose of (b)(4) mg/kg, as indicated in the proposed labeling, the safety margin is very low, and in most cases the multiple of the MHD is less than 1.0.

Mice

Groups of naïve CD-1 mice received oral APO-066 at doses of 500 – 2000 mg/kg. At doses of 700-2000 mg/kg all animals were sacrificed in a moribund condition 3-5 hours later exhibiting lethargy, abnormal gait, labored breathing, low body temperature, ptosis and prostration. The MTD was 500 mg/kg (0.4 X the MHD based on surface area) with the animals exhibiting the same clinical signs, but less severe.

The MLD in iron-loaded male Balb c mice was ≥ 990 mg/kg (0.9 X the MHD based on surface area). In a study where Balb c mice of both sexes were given escalating doses of APO-066, the MLD was estimated at 983 mg/kg (0.9 X the MHD based on surface area). CD-1 mice were given doses of APO-066 ranging from 400-1600 mg/kg, with all animals dead at doses greater than 1000 mg/kg and the MLD was 706 mg/kg (0.7 X the MHD based on surface area). The MLD was only slightly higher in iron-loaded than in naïve animals, which indicates that iron-loading provided little if any protection against lethality in single-dose studies.

Rats

In naïve, adult albino rats, there were no deaths at doses ≤ 1000 mg/kg (1.4 X the MHD based on surface area) and all animals died at doses of ≥ 2000 mg/kg. Lethality was attributed to congestive heart damage. In weanling Sprague-Dawley rats, the MLD was calculated to be 650 mg/kg (0.8 X the MHD based on surface area).

Other Rodents

Naïve male guinea pigs showed no adverse effects of doses ranging from 100-800 mg/kg. At 1000 mg/kg single dose, eye discharge, weakness, lethargy and lethality were noted. Clinical and histopathology revealed foci of skeletal muscle necrosis at 600 mg/kg, and foci of cardiac necrosis at 800 mg/kg.

Nonrodents

Single dose toxicity studies were not conducted in nonrodent species. However, with the nonrodent repeat dose studies, it is of little value to require further single dose studies.

2.6.6.3 Repeat-dose toxicity

RAT

Study title: APO-066: 52 week oral (gavage) toxicity study in the iron-loaded and non-iron-loaded Sprague Dawley rat.

Key study findings: Treatment of rats by oral gavage with APO-066 at doses of 37.5, 75 and 100 mg/kg bid for 52 weeks did not alter iron-induced effects of hepatotoxicity and iron deposition in tissues. At all dose levels of APO-066, there was a significant increase in malignant tumors independent of whether animals were naïve or iron-loaded. Organs involved were liver, lung, mammary gland, skin and thyroid. There was evidence of iron removal from tissues at the two high doses of APO-066 of 75 and 100 mg/kg bid to naïve rats. Iron removal resulted in iron deficiency anemia that was severe enough to cause death in some animals, with hemoglobin levels as low as 1.4-2.3 g/dL. Iron loading conferred some protection against the APO-066 induced anemia effects. Treatment with APO-066 also induced serum changes indicating hepatic effects in naïve rats and at all doses caused changes in thyroid weight and morphology, and mammary gland lobular hyperplasia, independent of iron-loading. Animals did not completely recover from drug effects during the 4-week post-dose recovery period with iron deposition, bone marrow hypocellularity and thyroid effects remaining. In the naïve group receiving APO-066 at 75 mg/kg bid, three females had mammary fibroadenomas, and one of them died, with one male and many female animals displaying mammary gland lobular hyperplasia.

Study no.: PC-02-09
Volume #, and page #: Located in EDR, Module 4.
Conducting laboratory and location: (b) (4)

Report Date: January 12, 2005
Date of study initiation: October 24, 2002
Date of in-life study: December 23, 2002 –January 20, 2004
GLP compliance: Yes
QA report: Yes
Drug: Lot # IDDPC 02053, Batch GC 0499
 Assumed 100% purity

Design:

This study was designed to evaluate the toxicity of APO-066 in naïve (non-iron-loaded) and iron-loaded, (b) (4) Sprague Dawley rats after twice daily oral gavage administration for 52 weeks, followed by a 4 week post-dose observation period according to the following design;

Group No./ Treatment	Dose Level (mg/kg bid)	Dose Volume (mL/kg /dose)	Dose concentration (mg/mL)	Total Dose (mg/kg /day)	Number of animals (Tox/TK)			
					Week 52		Week 56	
					Males	Females	Males	Females
1. Control, Naive	0	5	0	0	14/6	14/6	5	5
2. Control Iron-loaded	0	5	0	0	14/6	14/6	5	5
3. Low Dose Iron-loaded	37.5	5	7.5	75	14/6	14/6	5	5
4. Mid Dose Iron-loaded	75	5	15	150	14/6	14/6	5	5
5. High Dose Iron-loaded	100	5	20	200	14/6	14/6	5	5
6. Mid-dose Naive	75	5	15	150	14/6	14/6	5	5

In each group, 6 animals/sex were selected for random blood sampling for toxicokinetics prior to treatment and at weeks 4 and 13 and were killed 13 days after the last blood sampling interval and carcasses discarded. At each treatment interval, samples were collected prior to dosing and at 0.5, 1, 2, 4 and 7 hours after dosing. In addition 9 animals/sex/group were sampled during week 51 for drug level determinations at 0.5, 1, 2, 4 and 7 hours after dosing.

At initiation of treatment, animals were 11-12 weeks old and males weighed 307-499 g and females 180-303 g. Animals were fed pelleted complete rodent diet ad libitum.

The iron-loading procedure involved twice-weekly intraperitoneal injections of 100 mg iron/kg as iron dextran at concentration of 1.02 mg/mL for 4 weeks prior to start of administration of APO-066. A single iron-loading dose of 100 mg/kg was also administered during weeks 13, 19, 31, 38, 44 and 51.

Doses of APO-066 were selected based on a 3 month study wherein 25 mg/kg was the NOEL, with the only effect in the 50 mg/kg group a minimal increase in WBC count.

Observations and intervals:

Mortality: Twice daily
Clinical signs: Daily
Body weights: At initiation and weekly with fasted weights at necropsy.

Food consumption: Weekly
EKG: Not recorded
Ophthalmoscopy: Initially and during weeks 27 and 52.

Hematology: During pre-test iron-loading period and at 5, 11, 27, 53, 54, and 57 for surviving animals. RBC, WBC, differential count, Hgb, Ht, Retic, platelet counts, mean platelet volume, PT and aPTT. MCH, MCHC and MCV were calculated.

Clinical chemistry: Same periods as hematology. ALT, AST, ALP, BUN, Na, K, Cl, Fe, Ca, P, total protein, albumin, globulin, A/G ratio, glucose, creatinine, triglycerides, cholesterol, urea, unsaturated iron binding capacity, and calculated total iron binding capacity.

Urinalysis: Same periods as hematology. Volume, specific gravity, pH, bilirubin, occult blood, glucose, ketones, protein, urobilinogen, color, appearance and microscopic sediment.

Gross pathology at necropsy: All animals, except toxicokinetics animals which were discarded without necropsy.

Organ weights: adrenals, brain, ovaries, pituitary, heart, kidneys, liver, lungs spleen, testes, thymus, thyroid/parathyroid and prostate.

Histopathology: Adequate Battery? Yes.

49 tissues and gross lesions collected and fixed in formalin. All collected tissues evaluated histopathologically in Control naïve, Control iron-loaded, 100 mg/kg iron-

loaded and 75 mg/kg naïve groups. Tissues evaluated in all groups included sternum, thyroid, mammary gland, heart, kidneys, adrenals, liver, and spleen.

Results (NNF = No Noteworthy Findings).

Mortality: Table below shows unscheduled deaths.

Group	Sex	No. Animals	Day of Death	Cause of Death
1 Control Naïve	M	0	N/A	N/A
	F	0	N/A	N/A
2 Control Iron-loaded	M	1	324	Astrocytoma
	F	3	91-173	Dosing error
3 Low Dose Iron-loaded	M	1	165	Dosing error
	F	0	N/A	N/A
4 Mid Dose Iron-loaded	M	0	N/A	N/A
	F	0	N/A	N/A
5 High Dose Iron-loaded	M	3	91-242	Iron deficiency anemia
	F	1	255	Focal ulcerative dermatitis
6 Mid Dose Naïve	M	5	105-344	Iron deficiency anemia
		1	102	Cage accident
	F	1	92	Iron deficiency anemia
		2	212-290	Focal ulcerative dermatitis
		1	322	Mammary fibroadenoma

Clinical signs: On the days following iron dextran administration, most animals exhibited swollen limbs and muzzle. On the first day of treatment with APO-066 and infrequently thereafter, essentially all rats receiving the drug exhibited excess salivation, and only very infrequently thereafter. No other treatment related effects were observed.

Anemia deaths: A significant number of deaths in the APO-066 treated groups were attributed to iron deficiency anemia and resultant effects. Hemoglobin levels ranged from 1.4 - 2.3 g/dL in the animals that died. There were 3/14 male deaths in the high dose iron-loaded group, and 5/14 male deaths and 1/14 female deaths in the mid dose naïve group. Males were more affected than females.

Body weights: Body weights were significantly reduced for males and females in the iron-loaded control group compared to the naïve control group. Generally, weights decreased with an increase in APO-066 dosage in the iron-loaded animals. There was little difference between animals in the mid dose groups, whether iron-loaded or naïve.

Body weight gains: Body weight gains were significantly reduced for males and females in the iron-loaded control group compared to the naïve control group. Generally, weight gains decreased with an increase in APO-066 dosage in the iron-loaded animals. There was little difference between animals in the mid dose groups, whether iron-loaded or naïve animals.

Food consumption: Similar for all groups.

Ophthalmoscopy: NNF

Hematology: There were no biologically significant differences between the two control groups. Iron-loading gave a certain degree of protection from the anemia effects of prolonged treatment with APO-066. For animals receiving APO-066 the effects on RBC, Hgb, and Ht were basically the known pharmacological action of an iron chelator. Males were somewhat more greatly affected than females. Effects were time dependent, and comparing the 30 and 365 day blood drawings to the pretest drawings, Hgb was 7% and 17% lower than controls for the males, and 5% and 14% lower for females. Reticulocyte counts were increased and WBC counts decreased at the 365-day period. Animals partially recovered during the 4-week post-dose observation period.

Iron deposits were observed in bone marrow smears and in macrophage cytoplasm of all animals in the groups receiving APO-066. There were no statistically significant differences in M/E ratio or percentage of lymphocyte/plasma cell between control groups and groups receiving APO-066.

Clinical chemistry: Increased total bilirubin, AST and ALT were observed for the unscheduled deaths where anemia was the cause of death. Hepatic centrolobular degeneration and necrosis were observed, histopathologically.

Generally, the administration of APO-066 along with iron-loading had no significant effect of ameliorating or exacerbating changes in clinical chemistry parameters.

The following differences in serum parameters are considered biologically significant and a result of APO-066 treatment in the naïve animals: cholesterol increased by 33-65% throughout the treatment period and BUN increased by 17-35% throughout.

Urinalysis: NNF (NNF = No Noteworthy Findings)

Gross pathology: NNF

Organ weights: NNF

Histopathology: Adequate Battery: Yes.
Peer review: Yes.

For a complete list of organs and tissues collected and organs weighed, see the table on the next page.

List of tissues collected and organs weighed in repeat dose studies.

Study	006		007	
	Rat	Rat	Cyno	Cyno
Adrenals	X	O	X	O
Bone (femur, sternum)	X/X		X/X	
Brain	X	O	X	O
Cecum	X		X	
Cervx/ Uterus/Vagina	X/X/X		X/X/X	
Colon	X		X	
Duodenum	X		X	
Epididymis	X		X	
Esophagus	X		X	
Eye/Optic nerve	X/X		X/X	
Fallopian tube	X		X	
Gall bladder			X	
Gross lesions	X		X	
Harderian gland	X		X	
Heart	X	O	X	O
Ileum	X		X	
Jejunum	X		X	
Kidneys	X	O	X	O
Liver	X	O	X	O
Lungs	X	O	X	O
Lymph node, cervical	X		X	
Lymph nodes mandibular	X		X	
Lymph nodes, mesenteric	X		X	
Mammary Gland	X		X	
Ovaries	X	O	X	O
Pancreas	X		X	
Pharynx	X		X	
Pituitary	X	O	X	O
Prostate	X	O	X	O
Rectum	X		X	
Salivary gland	X		X	
Sciatic nerve	X		X	
Seminal vesicles	X		X	
Skeletal muscle/nerve	X/X		X/X	
Skin	X		X	
Spinal cord	X		X	
Spleen	X	O	X	O
Stomach	X		X	
Testes	X	O	X	O
Thymus	X	O	X	O
Thyroid/Parathyroid	X/X	O	X/X	O
Tongue	X		X	
Trachea	X		X	
Urinary bladder	X		X	

X = histopathology performed. O = Organ weights

The fasting terminal body weights after 52 weeks of APO-066 treatment were statistically significantly lower for animals in Group 6 (Naïve/APO-066), compared to Group 1 (Naïve Control), with values that were 24% lower in males and 12% lower in females.

Fasting terminal body weights were significantly lower for rats in the iron-loaded control group (Group 2) than in the Naïve Controls (Group 1).

Thyroid, adrenal, heart, testis, ovaries, epididymides and pituitary weights were greater, with liver and brain weights lower in iron-loaded APO-066 treated groups than in iron-loaded controls.

In animals receiving 75 mg/kg/dose of APO-066 (Group 4), the naïve animals had absolute and relative liver, spleen, and kidney weights significantly lower than Naïve APO-066 treated animals (Group 6).

Microscopic findings attributed to treatment of APO-066 in Group 6 compared to Control Group 1 are as follows:

Bone Marrow: Decreased incidence and severity of cellularity in sternal and femoral bone.

Mammary Gland: Greater incidence and severity of mammary gland lobular hyperplasia in females. Three females had mammary gland fibroadenomas and one died as a result.

Spleen: Greater extramedullary hematopoiesis in spleen.

Thyroid gland: Diffuse basophilia of thyroid follicular colloid associated with diffuse hypertrophy of follicular epithelium.

Thymus: Increased severity of lymphocyte atrophy.

Terminal body weights, absolute and relative organ weights, gross observations at necropsy and histopathological findings were not different at the end of the 4-week post-dose observation period than at the end of the 52-week dosing period. There was no obvious recovery of treatment effects during the non-treatment period.

Effects of iron-loading in Group 2 compared to non-iron loading in Group 1.

Liver: Greater severity of microfoci of inflammation and bile duct hyperplasia with focal fat vacuolation in the liver of both sexes. Increased incidence of pigmented macrophages.

Thymus: Greater incidence and severity of lymphocyte atrophy in animals of both sexes.

Liver, lymph nodes and spleen: Pigment throughout tissue and increased hemosiderin.

Effects of iron-loaded Control, Group 2 compared to iron-loaded groups 3, 4 and 5.

Bone Marrow: Greater incidence and severity of decreased cellularity in sternal and femoral bone.

Mammary Gland: Greater incidence and severity of mammary gland lobular hyperplasia in females of all 3 groups receiving APO-066 with iron-loading. Effects were not dose related and there was mammary gland hyperplasia in one male of the high dose group.

Spleen: Increased level of extramedullary hematopoiesis in the high dose group males (Group 5).

Thyroid: Diffuse basophilia of follicular colloid in the majority of animals of both sexes. Females of Group 5, exhibited a greater incidence and severity of chronic nephropathy.

Toxicokinetics:

Mean APO-066 pharmacokinetic parameters for rats are shown in the table below.

Pre-treatment	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-7h} (µg.h/mL)	t_{1/2} (h)
Iron-loaded	75	M	7.9	0.5	18.9	Not collected
		F	10.6	0.5	22.3	2.5
Iron-loaded	150	M	16.6	0.5	36.7	2.7
		F	33.9	0.5	51.23	2.4
Iron-loaded	200	M	24.5	0.5	44.5	2.7
		F	27.5	0.5	46.8	3.2
Non-iron-loaded	150	M	40.1	0.5	61.5	Not collected
		F	27.7	0.5	43.6	3.0

C_{max} and AUC_{0-7h} generally increased with increasing doses and was noticeably higher in non-iron-loaded animals. T_{max} and t_{1/2} were essentially the same for all groups and were not dose dependent.

Tumorigenesis:

Although this was just a one year study in rats, there was a high incidence of malignant tumors in all groups receiving APO-066, independent of iron-loading. Of the malignant tumor types listed in the table below, there were no malignant tumors in either the naïve control group or the iron-loaded control group. The common pituitary and thyroid tumors are not included due to their frequent occurrence in all groups.

The table below includes malignant tumor incidence for rats of unscheduled death and terminal sacrifice intervals.

Tumor Type	Sex	Group 1 Naïve control	Group 2 Iron- loaded control	Group 3 APO-066 Iron- loaded	Group 4 APO-066 Iron- loaded	Group 5 APO-066 Iron- loaded	Group 6 APO-066 Naïve
Dose APO-066 (mg/kg/day)		0	0	75	150	200	150
Hepatocellular Carcinoma	M					1	
Lung Metastasis Malignant Histiocytoma	F				1		
Lung Metastasis Hepatocellular Carcinoma	M					1	
Mammary Adenocarcinoma	F			1		1	
Mammary Carcinoma	M				1		
Mammary Fibroadenoma	F						3 (1 death)
Skin Keratoacanthoma	M				1		
Skin Malignant Fibrous Histiocytoma	F				1		
Skin Fibrocyte Fibroma	F			1			
Thyroid Follicular Cell Adenoma	F						1
Malignant Tumors	M	0	0	0	2	2	0
Malignant Tumors	F	0	0	2	2	1	4
Total Malignant Tumors	M&F	0	0	2	4	3	4
Number Examined	M&F	38	38	38	38	38	38

PRIMATES

Study title: APO-066: 52 week oral (gavage) toxicity study in the iron-loaded and non-iron-loaded Cynomolgus monkey.

Key study findings: APO-066 given orally to naïve cynomolgus monkeys at 75 mg/kg twice daily for 52 weeks was without significant affect. Treatment of iron loaded monkeys by nasogastric intubation with APO-066 at doses of 37.5, 75 and 100/125 mg/kg bid for 52 weeks was without evidence of treatment related effects, except for the expected pharmacological responses due to iron loading and the administration of iron chelators. The NOAEL for naïve, non-iron-loaded animals was 75 mg/kg bid (150 mg/kg/day). The NOAEL for iron-loaded animals was 100/125 mg/kg bid (200/250 mg/kg/day). A drug effect dose was not defined. However, the top doses were near the lethal dose in a 3-month monkey study.

Since no drug effects were observed in this study it would be considered inadequate without information from a 3-month preliminary study in monkeys. In that study 6 out of 13 non-iron-loaded monkeys died from treatment with 250 mg/kg/day for 18 days, reduced to 200 mg/kg/day for 2 days and reduced to 150 mg/kg/day thereafter. The animals died of iron deficiency anemia and resultant anorexia, body weight loss, emaciation and bone marrow hypocellularity. No drug effects were observed at 100 mg/kg/day and below.

Study no.: PC-02-10
Volume #, and page #: Located in EDR, Module 4.

Conducting laboratory and location:



Report Date: January 17, 2005
Date of study initiation: November 12, 2002
Date of in-life study: November 12, 2002 –January 6, 2004

GLP compliance: Yes
QA report: Yes
Drug: Lot # IDDPC 02053, Batch GC 0499
Assumed 100% purity

Design:

This study was designed to evaluate the toxicity of APO-066 in naïve (non-iron-loaded) and iron-loaded, cynomolgus monkeys after twice daily administration by nasogastric intubation for 52 weeks, followed by a 4 week post-dose observation period according to the following design;

Group No./ Treatment	Dose Level (mg/kg bid)	Dose Volume (mL/kg/ dose)	Dose concentration (mg/mL)	Total Dose (mg/kg/day)	Number of animals			
					Week 56		Week 60	
					Male	Female	Male	Female
1. Control, Naive	0	5	0	0	4	4	2	2
2. Control Iron-loaded	0	5	0	0	4	4	2	2
3. Low Dose Iron-loaded	37.5	5	7.5	75	4	4	0	0
4. Mid Dose Iron-loaded	75	5	15	150	4	4	0	0
5. High Dose Iron-loaded	100 or 125 *	5	20 or 25*	200 or 250 *	4	4	2	2
6. Mid-dose Naive	75	5	15	150	4	4	2	2

*Dose increased during week 14.

All animals of each group were bled for sampling for toxicokinetics at the first dose on the first day of APO-066 treatment and at weeks 17 and 56. At each treatment interval, samples were collected prior to dosing and at 0.5, 1, 2, 4 and 7 hours after dosing for drug level determinations.

At initiation of treatment, animals were 24 – 30 months old and males weighed 2.2 – 3.8 kg and females 1.8-3.9 kg. Animals were fed 100 g daily of pelleted commercial primate diet ad libitum.

The iron-loading procedure involved once weekly intraperitoneal injections of 100 mg iron/kg as iron dextran for 4 weeks prior to start of administration of APO-066. A single iron-loading dose of 100 mg/kg was also administered during weeks 32 and 45. Doses of APO-066 were selected based on a 3 month study wherein 100 mg/kg/dose was the NOEL, with 200 mg/kg being a lethal dose in 18 days.

Observations and intervals:

Mortality: Twice daily
Clinical signs: Daily
Body weights: At initiation and weekly with fasted weights at necropsy.

Food consumption: Weekly
Cardiovascular: Prior to first treatment and during weeks 17, 30, 43, 56 and 60.
Systolic, diastolic and mean arterial blood pressure.
EKG leads I, II, III
Heart rate, analysis of rhythm, measurement of duration of QRS, PR and QT intervals.

Ophthalmoscopy: Initially and during weeks 17, 30, 43, 56 and 60.

Hematology: During pre-test iron-loading period and at 8, 16, 30, 41, 56, and 60 for surviving animals. RBC, WBC, differential count, Hgb, Ht, Retic, platelet counts, mean platelet volume, PT and aPTT. MCH, MCHC and MCV were calculated.

Clinical chemistry: Same periods as hematology. ALT, AST, ALP, BUN, Na, K, Cl, Fe, Ca, P, total protein, albumin, globulin, A/G ratio, total bilirubin, glucose, creatinine, triglycerides, cholesterol, urea, unsaturated iron binding capacity, and calculated total iron binding capacity.

Urinalysis: Same periods as hematology. Volume, specific gravity, pH, bilirubin, occult blood, glucose, ketones, protein, urobilinogen, color, appearance and microscopic sediment.

Gross pathology at necropsy: All animals.

Organ weights: adrenals, brain, ovaries, pituitary, heart, kidneys, liver, lungs, spleen, testes, thymus, thyroid/parathyroid and prostate.

Histopathology: Adequate Battery? Yes.

49 tissues and gross lesions collected and fixed in formalin. All collected tissues evaluated histopathologically in all animals of all groups.

Results (NNF = No Noteworthy Findings).

Mortality: All animals survived except one female in Group 3 that was sacrificed moribund during the final week of treatment due to extreme emaciation and enteritis.

Clinical signs: No unusual clinical signs except for the female in Group 3 that was sacrificed exhibiting thinness, weakness and emaciation.

Body weights: No significant treatment effects noted, except weight loss of nearly 30% in the female in Group 3 that was sacrificed.

Food consumption: Similar for all groups.

Ophthalmoscopy: NNF. (NNF = No Noteworthy Findings).

Cardiovascular: No treatment related effects on heart rate, systolic, diastolic or mean arterial blood pressure.

Electrocardiograms: There were no significant differences in duration of PR interval, QRS wave, or duration of QT interval. There were no noted differences in cardiac rhythm or wave forms. A more comprehensive cardiovascular safety study with evaluation of QTc intervals at first dose, and tracings for longer of periods would be desirable. However, with EKGs recorded after steady state was reached in this study, it is more valuable to have results of an electrophysiology study in hERG cells than to repeat the in vivo study.

Hematology: Although there were sporadic and inconsistent variations in some parameters at some intervals, there were no treatment related effects on hematology parameters as a result of iron-loading with iron dextran or treatment with APO-066 in any of the groups.

Clinical chemistry: Treatment with iron-dextran was associated with an increase in ALT in all iron-loaded groups and this effect was not noted in non-iron-loaded groups. Also, there seemed to be some protection from this effect by the administration of APO-066. Administration of iron-dextran before initiation of APO-066 treatment resulted in a long lasting increase in serum iron and a decrease in Unsaturated Iron Binding Capacity. Total Iron Binding Capacity was unaffected. Treatment with APO-066 had no effect on serum iron or UIBC levels.

Urinalysis: NNF

Gross pathology: Dark areas were observed in lymph nodes, mesentery, adipose tissue, and diaphragm of several animals of the iron-loaded groups, and by microscopy was found to be caused by the presence of pigment-laden macrophages.

Organ weights: NNF

Histopathology: Adequate Battery: Yes.
Peer review: Yes.

For a complete list of organs and tissues collected and organs weighed, see the table on the following page.

List of tissues collected and organs weighed in repeat dose studies.

Study	006		007	
	Rat	Rat	Cyno	Cyno
Adrenals	X	O	X	O
Bone (femur, sternum)	X/X		X/X	
Brain	X	O	X	O
Cecum	X		X	
Cervix/ Uterus/Vagina	X/X/X		X/X/X	
Colon	X		X	
Duodenum	X		X	
Epididymis	X		X	
Esophagus	X		X	
Eye/Optic nerve	X/X		X/X	
Fallopian tube	X		X	
Gall bladder			X	
Gross lesions	X		X	
Harderian gland	X		X	
Heart	X	O	X	O
Ileum	X		X	
Jejunum	X		X	
Kidneys	X	O	X	O
Liver	X	O	X	O
Lungs	X	O	X	O
Lymph node, cervical	X		X	
Lymph nodes mandibular	X		X	
Lymph nodes, mesenteric	X		X	
Mammary Gland	X		X	
Ovaries	X	O	X	O
Pancreas	X		X	
Pharynx	X		X	
Pituitary	X	O	X	O
Prostate	X	O	X	O
Rectum	X		X	
Salivary gland	X		X	
Sciatic nerve	X		X	
Seminal vesicles	X		X	
Skeletal muscle/nerve	X/X		X/X	
Skin	X		X	
Spinal cord	X		X	
Spleen	X	O	X	O
Stomach	X		X	
Testes	X	O	X	O
Thymus	X	O	X	O
Thyroid/Parathyroid	X/X	O	X/X	O
Tongue	X		X	
Trachea	X		X	
Urinary bladder	X		X	

X = histopathology performed. O =Organ weights

Many soft tissues from animals of all groups contained pigment-laden macrophages, with increased levels of hemosiderin deposition in the bone marrow and spleen.

In animals receiving 75 mg/kg/dose of APO-066 (Group 4), the non-iron-loaded animals had absolute and relative liver weights significantly greater than naive APO-066 treated animals (Group 6). This was attributed to the increase of iron pigment in the hepatic Kupffer's cells.

There were no microscopic findings attributed to treatment of APO-066 in Group 6 compared to Control Group 1.

Terminal body weights, absolute and relative organ weights, gross observations at necropsy and histopathological findings were not different at the end of the 4-week post-dose observation period than at the end of the 52-week dosing period.

Toxicokinetics:

Mean APO-066 pharmacokinetic parameters for monkeys are shown in the table below.

Pre-treatment	Dose (mg/kg/day)	Sex	Day of first dose		13th week of dosing		52nd week of dosing	
			C _{max} (µg/mL)	AUC _{0-7h} (µg.h/mL)	C _{max} (µg/mL)	AUC _{0-7h} (µg.h/mL)	C _{max} (µg/mL)	AUC _{0-7h} (µg.h/mL)
Iron-loaded	75	M	5.5	7.7	5.5	7.0	5.0	4.8
		F	4.3	8.4	5.0	5.5	6.1	6.4
Iron-loaded	150	M	22.0	30.6	15.3	16.5	12.0	15.6
		F	12.8	26.0	9.8	12.6	14.4	20.8
Iron-loaded	200/ 250	M	26.3	46.4	19.4	22.0	24.3	39.1
		F	23.8	39.4	21.4	27.3	35.1	50.1
Non-iron-loaded	150	M	30.0	36.7	25.0	29.2	26.6	28.4
		F	25.7	32.1	14.0	20.0	23.9	29.4

C_{max} and AUC_{0-7h} generally increased with increasing doses and was noticeably higher in non-iron-loaded animals. T_{max} was 0.5 h and t_{1/2} was in the range of 0.4 – 2.4 h and did not vary with dose, sex, iron-loading or duration of treatment.

2.6.6.4 Genetic toxicology

Study title: Ames/Salmonella-E. coli Reverse Mutation Assay with APO-066

Key study findings: Results are negative. NOEL is highest dose of 5000 ug/plate

Study Number: G96AR89.502
Report Date: October 31, 1996
Report Location: Module #4 in EDR.

Conducting laboratory and location:



Date of study initiation: May 6, 1996
In-Life Dates: May 14, 1996– June 14, 1996.
GLP compliance: Yes

QA report: Yes
Drug lot # M 0262
Purity: Assumed to be 100%.

Design: This study was designed to assess the ability of APO-066 to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes at either the histidine locus in the genome of Salmonella typhimurium or the tryptophan locus of Escherichia coli. The mutagenicity assay was performed using S. typhimurium tester strains TA98, TA100, TA 102, TA1535, TA1537, 1538 and E. coli tester strain WP2uvrA using both liquid pre-incubation and plate incorporation treatment conditions. The tester strains were exposed to APO-066 in the presence and absence of Aroclor-induced rat hepatic S9 preparation.

The dose levels selected for the confirmatory study were based on the results of the dose range-finding study, which used doses of 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg/plate. After a review of the assay results, a confirmatory assay was conducted with the same test article concentrations.

Prior to evaluation of the assay, the criteria for a valid assay were defined, and included tester strain integrity, tester strain culture density, positive control values and cytotoxicity. Criteria for a positive response in the plate incorporation assay was defined as a 2-fold increase in the mean revertants per plate of at least one of the tester strains over the mean revertants per plate of the vehicle control. The increase in the mean number of revertants required an accompanying dose response to increasing concentrations of APO-066.

Results: In the initial range-finding assay and in the confirmatory assay, no positive increases were observed with any of the tester strains with or without metabolic activation with Aroclor-induced S9 liver homogenate. Minimal cytotoxicity was observed at the highest concentration of test article of 5,000 µg/plate. Responses of vehicle control and positive control materials specific for each strain (2-nitrofluorene, sodium azide, 2 aminofluorene, 9-aminoacridine, 2-anthramine, mitomycin C, ENNG, and 2-anthramine) were as expected.

Report Conclusions: The results indicate that APO-066 is non-mutagenic in the Salmonella – E. coli/mammalian-microsome reverse mutation assay at the maximum dose of 5000 µg/plate.

Reviewer's Comment: Agree, the test is negative for evidence of reverse mutation.

Study Title: L5178Y Mouse Lymphoma Cell TK +/- Forward Gene Mutation Assay with APO-066.

Key study findings: APO-066 is positive in this assay.

Study Number: G96AR89.702
Report Date: November 18, 1996
Report Location: Module #4 in EDR

Conducting laboratory and location:

(b) (4)

Date of study initiation: May 6, 1996
In-Life Dates: May 14, 1996 – August 1, 1996
GLP compliance: Yes
QA report: Yes
Drug lot # M 0262
Purity: Assumed to be 100%.

Design: This study was designed to assess the potential of APO-066 to induce forward mutations at the thymidine kinase (TK) locus in cultured L5178Y cells in the presence or absence of mammalian microsomal enzymes (S9).

Cytotoxicity of APO-066 was estimated in a prescreen by exposing the cells to 9 concentrations of APO-066 at 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 and 5000 µg/mL in the presence and absence of Aroclor-induced rat hepatic S9 preparation.

Prior to evaluation of the assay, the criteria for a valid assay were defined, and included tester strain integrity, tester strain culture density, positive control values and cytotoxicity. Criteria for a positive response was defined as a 2-fold increase in the mean

revertants over the mean revertants of the vehicle control. The increase in the mean number of revertants required an accompanying dose response to increasing concentrations of APO-066.

The dose levels selected for the 4 hr confirmatory study were based on the results of the dose range-finding study. After a review of the assay results where there was substantial toxicity, a confirmatory assay was conducted with the concentrations of APO-066 at 1.5, 5.0, 10, 50, 100, 500, 1000 and 5000 µg/mL in the absence of Aroclor-induced rat hepatic S9 preparation, and at 5.0, 10, 50, 100, 500 and 1000 µg/mL in the presence of Aroclor-induced rat hepatic S9 preparation. All concentrations of test article were readily soluble in treatment medium with no precipitate observed.

Results: In the initial range-finding assay and in the confirmatory assays, positive increases were observed with 4 and 24 hr incubation with and without metabolic activation with Aroclor-induced S9 liver homogenate. Cytotoxicity in the cloned cultures was observed at concentrations of 15-5000 µg/mL without activation and from 25 – 250 µg/mL with S9-activation. Responses of vehicle controls and positive controls (methanesulfonate and cyclophosphamide) were as expected.

Report Conclusion: The results indicate that APO-066 was positive in the L5178Y mouse lymphoma cell TK+/- forward gene mutation assay.

Reviewer's Comment: Agree, APO-066 was found to be positive in this mutagenesis assay.

Study Title: In vitro mammalian chromosome aberration assay in Chinese Hamster Ovary with APO-066

Key study findings: APO-066 is positive for the induction of chromosomal aberrations in this assay.

Study Number: G96AR89.331
Report Date: November 14, 1996
Report Location: Module #4 in EDR

Conducting laboratory and location:

(b) (4)

Date of study initiation: May 3, 1996
In-Life Dates: May 14, 1996 – August 1, 1996
GLP compliance: Yes
QA report: Yes
Drug lot # M 0262
Purity: Assumed to be 100%.

Design: This study was designed to assess the clastogenic potential of APO-066 to induce chromosome aberrations in Chinese Hamster Ovary cells in the presence or absence of mammalian microsomal enzymes (S9).

Cytotoxicity of APO-066 was estimated in a prescreen by exposing the cells to 9 concentrations of APO-066 at 0.5, 1.0, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/mL in the presence and absence of Aroclor-induced rat hepatic S9 preparation.

Prior to evaluation of the assay, the criteria for a valid assay were defined, and included tester cell integrity, tester cell culture density, positive control values and cytotoxicity. Criteria for a positive response was defined as a statistically significant increase in the number of chromosomal aberrations in the test cultures compared to the mean chromosomal aberrations in vehicle control. The increase in the mean number of chromosomal aberrations required an accompanying dose response to increasing concentrations of APO-066.

The dose levels selected for the confirmatory study were based on the results of the dose range-finding study. After a review of the assay results where there was substantial toxicity, a confirmatory assay was conducted with the concentrations of APO-066 at 313, 625, 1250, 2500 and 5000 µg/mL in the absence of Aroclor-induced rat hepatic S9 preparation, and at 313, 625, 1250 and 2500 µg/mL in the presence of Aroclor-induced rat hepatic S9 preparation. All concentrations of test article were readily soluble in treatment medium with no precipitate observed.

Results: In the initial range-finding assay and in the confirmatory assays, positive increases in chromosomal aberrations were observed with 4 and 20 hr incubation with and without metabolic activation with Aroclor-induced S9 liver homogenate. Statistically significant increases in structural chromosome aberrations relative to negative control were observed in the non-activated groups ($p \leq 0.01$, Fisher's exact test) and in the S9 activated groups ($p \leq 0.05$, Fisher's exact test). The Cochran-Armitage test was also positive for a dose response in both the non-activated and S9-activated exposure groups ($p \leq 0.05$). Responses of vehicle controls and positive controls (mitomycin C and cyclophosphamide) were as expected.

Report Conclusion: The results indicate that APO-066 was positive in inducing chromosome aberrations in the Chinese Hamster Ovary assay.

Reviewer's Comment: Agree, APO-066 was found to be clastogenic in this assay.

Study title: In Vitro micronucleus test in male mouse bone marrow erythropoietic cells with APO-066.

Key study findings: APO-066 was clastogenic in male mice bone marrow cells.

Study no.: G96AR89.122
Report date: November 14, 1996
Report Location: Module #4 in EDR.

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: May 3, 1996
In-Life Dates: May 28 - July 18, 1996
GLP compliance: Yes
QA report: Yes
Drug lot # M 0262
Purity: Assumed to be 100%.

Design: This study was designed to assess the ability of APO-066 to induce clastogenic effects by detecting micronuclei in polychromatic erythrocyte stem cells in mouse bone marrow. Test animals were described as adult male and female mice [HSD:ICR(CD-1) from [REDACTED] (b) (4)]. They were 6-8 weeks old and weighed 24-35 grams. Five male mice were assigned to each dose group in the preliminary screen with 20 assigned in the definitive study.

In the preliminary screen, APO-066 was dosed by intraperitoneal injection at dose levels of 0, 1, 10, 100, 1000 or 2000 mg/kg body weight. Some animals in the 100 and 1000 mg/kg groups exhibited abnormal gait and lethargy in the first 24 hrs. All animals died in the 2000 mg/kg group. All mice were euthanized 72 hours after dosing for extraction of the bone marrow. Femur bone marrow smears were prepared and stained with May-

Gruenwald and Giemsa solutions. The stained slides were examined for the presence of micronuclei in 1000 erythrocytes and the ratio of polychromatic to normochromatic erythrocytes calculated.

In the definitive study, 15 male mice received sterile water for injection, which served as negative control vehicle and five mice received cyclophosphamide at 60 mg/kg which served as positive control. Three groups with 15 mice/sex each received APO-066 at 140, 280 or 560 mg/kg and 5 mice from each group were serially sacrificed at 24, 48, or 72 hrs after treatment. The positive control cyclophosphamide group was sacrificed at 24 hrs after treatment. All injections were at the volume of 10 mL/kg.

The criteria for a positive response consisted of a statistically significant increase in micronucleated polychromatic erythrocytes in at least one APO-066 group.

Results: All animals died in the 2000 mg/kg group in the prescreen study. Some animals in the 100 and 1000 mg/kg groups exhibited abnormal gait and lethargy during the first 24 hours after dosing. All vehicle and positive control animals appeared normal throughout the study observation period. In the definitive study four animals in the top dose group (560 mg/kg) were found dead on the day after dose administration and were replaced with extra animals that had been dosed with 560 mg/kg. During the first 24 hours after dosing, lethargy was observed in a majority of animals in all groups receiving APO-066.

APO-066 induced significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls at all harvest times ($p \leq 0.05$, Kastenbaum-Bowman Tables). There was a statistically significant dose related increase in the incidence of micronucleated polychromatic erythrocytes in male mice at the 24 and 48 hour collection periods, and the positive control, cyclophosphamide, produced statistically significant increases in micronucleated polychromatic erythrocytes ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Report Conclusion: The results indicate that APO-066 induced a statistically significant increase in micronucleated polychromatic erythrocytes in mouse bone marrow and therefore is clastogenic in the mouse micronucleus test.

Reviewer's Comment: Agree.

Study title: APO-066: Evaluation of micronucleus induction potential of APO-066 in iron-loaded and naïve male and female mice.

Key study findings: APO-066 was clastogenic in erythropoietic bone marrow cells of iron-loaded and naïve male and female mice. Deferoxamine did not induce micronuclei.

Study no.: 1737/4
Report date: September 9, 2004
Report Location: Module #4 in EDR.

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: February 21, 2003

In-Life Dates: March 3 – December 22, 2003.
GLP compliance: Yes
QA report: Yes
Drug lot # GC 0499
Purity: Assumed to be 100%.

Design: This study was designed to assess the ability of APO-066 and a comparator, deferoxamine, to induce clastogenic effects by detecting micronuclei in polychromatic erythrocyte stem cells in mouse bone marrow. Test animals were described as adult male and female mice CD-1 Crl:CD-1(ICR)BR from [REDACTED] (b) (4). They were 8-11 weeks old and weighed 31-40 grams at initiation of treatment. Ten animals per sex were assigned to each dose group in the preliminary screen with 150/sex assigned in the definitive study. Iron dextran served as the iron-loading agent and was given intraperitoneally at 100 mg/kg, twice a week for 4 weeks. Cyclophosphamide served as the positive control.

In the preliminary screen, APO-066 was dosed orally at dose levels of 500, 700, 1400 or 2000 mg/kg body weight. All mice were euthanized 72 hours after dosing for extraction of the bone marrow. Femur bone marrow smears were prepared and stained with May-Gruenwald and Giemsa solutions. The stained slides were examined for the presence of micronuclei in 1000 erythrocytes and the ratio of polychromatic to normochromatic erythrocytes calculated.

In the definitive study, 18 mice/sex received sterile water for injection, which served as negative control vehicle and six mice/sex received cyclophosphamide at 60 mg/kg which served as positive control. Three groups with 18 mice/sex each received oral APO-066 at 125, 250 or 500 mg/kg and three groups of the same size were iron-loaded prior to treatment with APO-066 and 6 mice from each group were serially sacrificed at 24 or 48 hrs after treatment. An additional 3 groups of the same size were dosed intraperitoneally with deferoxamine at levels of 250, 500 and 1000 mg/kg and the same number sacrificed

at the same time periods. The positive control cyclophosphamide group was sacrificed at 24 hrs after treatment. All injections were at the volume of 10 mL/kg.

The criteria for a positive response consisted of a statistically significant increase in micronucleated polychromatic erythrocytes in APO-066 or deferoxamine groups.

Results: All animals died in the 1400 and 2000 mg/kg groups in the prescreen study. Some animals in the 1000 mg/kg group exhibited abnormal gait and lethargy during the first 24 hours after dosing. In the definitive study, 3 males died in the highest dose group. All vehicle and positive control animals appeared normal throughout the study observation period. During the first 24 hours after dosing, lethargy was observed in a majority of animals in all groups receiving APO-066. There appeared to be no difference in iron-loaded and naïve animals.

APO-066 induced significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle control animals at all harvest times, in both sexes and in iron-loaded and naïve animals ($p \leq 0.05$, Kastenbaum-Bowman Tables). This was true for male and female mice at the 24 and 48 hour collection periods and for iron-loaded and naïve animals as well. The procedure of pre-treatment iron-loading had no effect on the ability of APO-066 to induce micronuclei. Deferoxamine did not induce micronucleated cells as were seen with APO-066 treatment. The positive control, cyclophosphamide, produced statistically significant increases in micronucleated polychromatic erythrocytes ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Report Conclusion: The results indicate that orally dosed APO-066 induced a statistically significant increase in micronucleated polychromatic erythrocytes in male and female mouse bone marrow and therefore is clastogenic in the mouse micronucleus test. The comparator, deferoxamine did not induce micronucleated cells.

Reviewer's Comment: Agree.

2.6.6.5 Carcinogenicity

Lifetime carcinogenicity studies have not been conducted; however, tumorigenesis data can be derived from the 12 month chronic toxicity study in rats.

Although this was just a one year study, there was a high incidence of malignant tumors in all groups receiving APO-066, independent of iron-loading. Of the malignant tumor types listed in the table below, there were no malignant tumors in either the naïve control group or the iron-loaded control group.

The table below includes malignant tumor incidence for rats from unscheduled death and terminal sacrifice intervals.

Tumor Type	Sex	Group 1 Naïve control	Group 2 Iron- loaded control	Group 3 APO-066 Iron- loaded	Group 4 APO-066 Iron- loaded	Group 5 APO-066 Iron- loaded	Group 6 APO-066 Naïve
Dose APO-066 (mg/kg/day)		0	0	75	150	200	150
Hepatocellular Carcinoma	M					1	
Lung Metastasis Malignant Histiocytoma	F				1		
Lung Metastasis Hepatocellular Carcinoma	M					1	
Mammary Adenocarcinoma	F			1		1	
Mammary Carcinoma	M				1		
Mammary Fibroadenoma	F						3 (1 death)
Skin Keratoacanthoma	M				1		
Skin Malignant Fibrous Histiocytoma	F				1		
Skin Fibrocyte Fibroma	F			1			
Thyroid Follicular Cell Adenoma	F						1
Malignant Tumors	M	0	0	0	2	2	0
Malignant Tumors	F	0	0	2	2	1	4
Total Malignant Tumors	M&F	0	0	2	4	3	4
Number Examined	M&F	38	38	38	38	38	38

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Studies were not conducted

Embryofetal development

Teratology studies were conducted in rats and rabbits and the results are in the Ciba-geigy DMF and were previously reviewed by the agency.

The sponsor provided a summary of the results and the following can be concluded:

Rats: Doses administered were 25, 50 and 75 mg/kg/day to groups of 20 presumed pregnant rats from day 6-15 of pregnancy. The NOAEL for maternal toxicity was 75 mg/kg/day (0.2 X the MHD based on surface area). There were soft tissue and skeletal malformations at all doses given, therefore, the NOAEL for fetal malformations was not defined and was less than 25 mg/kg/day (≤ 0.06 X the MHD based on surface area).

Rabbits: Doses administered were 10, 25, 50 and 100 mg/kg/day to groups of 16 presumed pregnant rabbits from day 6-19 of pregnancy. Maternal deaths and embryolethality were observed at 100 mg/kg/day. Drug related teratogenicity was observed at dose levels of 10, 25, 50 and 100 mg/kg/day. The NOAEL for maternal toxicity was 50 mg/kg/day (0.2 X the MHD based on surface area). The NOAEL for soft tissue and skeletal fetal malformations was not defined and was less than 10 mg/kg/day (< 0.04 X the MHD based on surface area).

Prenatal and postnatal development

Studies were not conducted.

2.6.6.7 Local tolerance. Studies were not conducted.

2.6.6.8 Special toxicology studies. Studies not conducted.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 Toxicology tabulated summary

OVERALL CONCLUSIONS AND RECOMMENDATIONS.

Conclusions: The information presented in this application is inadequate to support safety for an NDA for APO-066 and deficiencies are listed.

Unresolved toxicology issues: Deficiencies are presented in the “COMMENTS TO SPONSOR” section at the end of this review.

Recommendations: Based on the perspective of nonclinical pharmacology and toxicology, the information provided is inadequate to support safety. Deficiencies are presented in the “COMMENTS TO SPONSOR” section at the end of this review.

Suggested labeling: Labeling recommendations are not included in this review due to the inadequacy of the data presented.

Signatures:

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS:**COMMENTS TO SPONSOR**

We have completed our review of this RUP and have identified the following deficiencies:

1. An electrophysiology study in hERG cells.
2. Lifetime carcinogenicity studies.
3. Fertility and early embryonic development study in rats to evaluate male and female fertility and general reproductive performance.

We are providing these comments to you before we complete our review of the complete application to give you preliminary notice of issues that we have identified. These comments are being provided to you in conformance with the guidance “Continuous Marketing Applications: Pilot 1 – Reviewable Units for Fast Track Products under PDUFA” and do not reflect a final decision on the information reviewed. Issues may be added, deleted, expanded upon, or modified as we review the complete application.

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this page is the manifestation of the electronic signature.**

/s/

David Bailey
6/27/2007 02:06:46 PM
PHARMACOLOGIST

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6/27/2007 02:24:37 PM
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