

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

203585Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Synribo (omacetaxine mepesuccinate)

Date: October 9, 2012

To: File for NDA 203585

From: John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting reviews of Drs Kropp and Ricci and secondary memoranda and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Synribo may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
10/09/2012

MEMORANDUM

Date: October 7, 2012
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 203585;
Also see the Supervisory Pharmacologist memorandum under NDA 22374
Drug: SYNRIPO, omacetaxine mepesuccinate
Indication: Treatment of adult patients with chronic or accelerated phase chronic myeloid leukemia (CML) with resistance and/or intolerance to two or more tyrosine kinase inhibitors
Applicant: Cephalon Inc. (Teva Pharmaceuticals, Ltd)

Background

The mechanism of action of omacetaxine is not fully understood. Omacetaxine exerts its anticancer activity at least in part by interfering with protein elongation and inducing apoptosis. The marketing application for omacetaxine was originally submitted in 2009, under NDA # 22374. The pharmacology/toxicology team recommended approval while recognizing that the battery of genotoxicity studies was incomplete and studies needed to be conducted post-approval or as soon as feasible. Subsequently, a complete response (CR) letter was issued in 2010. The nonclinical comment for completing the battery of genetic toxicology studies was included in the CR letter among deficiencies from other disciplines.

In the current submission, the Applicant has submitted results of two genetic toxicology studies in addition to other nonclinical studies. The studies were reviewed by Dr. Stacey Ricci under NDA 203585. A previous genetic toxicology study was reviewed by Dr. Kropp under NDA 22374. The results indicate that omacetaxine was positive in the *in vitro* chromosome aberration assay and negative in the Ames test and in *in vivo* micronucleus assay.

Reviews of NDAs 22374 and 203585 will be used to revise the nonclinical sections of the label. Results of nonclinical studies are summarized in Dr. Kropp and Dr. Ricci's review and in the Supervisory Pharmacologist memorandum of March 5, 2010. Of note, since the systemic exposure (AUC) data are not available for the embryofetal reproductive toxicology study, the dose conversions will be used in the label for animal:human comparisons under Section 8.1.

Due to the lack of adequate understanding of the mechanism of action of SYNRIPO, a pharmacologic class has not been assigned to this drug.

Recommendation: I concur with Dr. Ricci that from a nonclinical perspective, SYNRIPO may be approved for the proposed indication. No additional nonclinical studies are needed to support approval of SYNRIPO for the proposed indication.

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/s/

HALEH SABER
10/07/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 203585
Supporting document/s: 1
Applicant's letter date: March 30, 2012
CDER stamp date: March 30, 2012
Product: Omacetaxine mepesuccinate
Indication: Adult patients with chronic or accelerated phase
CML with resistance or intolerance to prior
tyrosine kinase
Applicant: Cephalon, Inc. (a wholly owned subsidiary of
Teva Pharmaceuticals, Ltd.)
Review Division: Hematology and Oncology Toxicology on behalf
of the Division of Hematology Products
Reviewer: M. Stacey Ricci, M.Eng., Sc.D.
Supervisor/Team Leader: Haleh Saber, Ph.D.
Division Director: John Leighton, Ph.D.
Project Manager: Theresa Ferrara, M.P.H.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203585 are owned by Cephalon, Inc. or are data for which Cephalon, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 203585 that Cephalon, 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 reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203585.

Executive Summary

Introduction

An NDA for omacetaxine mepusuccinate (hereafter referred to as 'omacetaxine') was first submitted to FDA on Sept. 8, 2009. The Pharmacology/Toxicology data to support NDA 22374 for omacetaxine was reviewed by Dr. Timothy Kropp. From the Pharmacology and Toxicology perspective, the review team recommended approval for NDA 22374 but requested post-approval that the battery of genotoxicity studies be completed according to ICH S2. Other review disciplines identified deficiencies in the NDA and the NDA was not approved.

On March 30, 2012, Cephalon, Inc. submitted a new NDA for omacetaxine. The pharmacology and toxicology studies included in NDA 22374 were also included in NDA 203585. NDA 203585 contained additional studies, including new genotoxicity studies that were not reviewed previously. This review is an Addendum to the Pharmacology and Toxicology NDA review that was completed for NDA 22374 and archived in DARRTS on March 5, 2010 by Dr. Kropp.

Recommendation

We recommend approval of omacetaxine from the pharmacology and toxicology standpoint for the proposed indication.

Background

Studies submitted to NDA 203585 that were not submitted previously are:

Genetic Toxicology	
PTX-030	Bacterial Mutagenicity AMES Assay
PTX-031	<i>In vivo</i> mouse micronucleus assay
Pharmacology	
CS-2011-019-US	Profiling of CEP-41443 in a Kinase Panel (b) (4)
Pharmacokinetics	
PTX-029	<i>In vitro</i> assessment of protein binding for homoharringtonine (HHT) in human plasma using the ultrafiltration method
PTX-028	P-glycoprotein inhibition potential of homomharringtonine (HHT) and 4-demethyleated homoharringtonine
CLN003	<i>In vitro</i> assessment of protein binding for homoharringtonine (HHT) in human plasma
PTX-027	<i>In vitro</i> evaluation of omacetaxine as an inducer of cytochrome p450 expression in cultured human hepatocytes

The Genetic Toxicology and Pharmacology studies listed above are reviewed below; the Pharmacokinetics studies were not reviewed. Peer-reviewed literature submitted in the NDA that are pertinent to the description of omacetaxine pharmacology proposed for the Package Insert are also reviewed below.

Nonclinical Findings

- Omacetaxine did not induce genetic mutations in the Ames assay.
- Omacetaxine did not induce genetic damage using an *in vivo* mouse micronucleus assay.
- Omacetaxine did not inhibit kinase activity under conditions used to test 71 kinases using an *in vitro* screening assay.
- The mechanism of action of Omacetaxine has not been fully elucidated but includes inhibition of protein synthesis. Omacetaxine binds to the A-site cleft in the peptidyl-transferase center of the large ribosomal subunit from the *Haloarcula marismortui* archaea bacteria, which is expected to block polypeptide chain elongation. *In vitro*, omacetaxine reduced protein levels of the Bcr-Abl oncoprotein (wild type or the T315I mutant) and Mcl-1, an anti-apoptotic Bcl-2 family member. In a mouse model of Bcr-Abl-

induced CML, omacetaxine had activity against both wild-type Bcr-Abl or Bcr-Abl with the T315I kinase domain mutation.

Summary of all genotoxicity results for studies submitted in NDA 203585

Test	Study Report Number	Result
Ames Assay	PTX-004*	Negative; test was not adequate for hazard identification purposes because dosing used was insufficiently low.
Ames Assay	PTX-030	Negative
<i>In vitro</i> Chromosome Aberration Analysis in CHO cells	PTX-080*	Positive
<i>In vivo</i> Mouse Micronucleus Assay	PTX-031	Negative

*Study reviewed by Dr. Timothy Kropp for NDA 22374.

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Mutagenicity Test – Ames Assay

Study no.: PTX-030
 Study report location: NDA 203585 Section 4.2.3.3.1
 Conducting laboratory and location: (b) (4)

Date of study initiation: November 19, 2010
 GLP compliance: Yes; signed statement provided
 QA statement: Yes; signed statement provided
 Drug, lot #, and % purity: Omacetaxine Mepesuccinate (powder), Lot #12252,

Key Study Findings

Using a plate incorporation method, omacetaxine did not induce genotoxic responses in bacteria, with or without S9 metabolic activation. This study used the highest concentration recommended by ICH S2(R1) (5.0 mg/plate), and the results are considered valid and adequate.

Methods (plate incorporation assay)

Strains: *Salmonella typhimurium* strains:
TA97a, TA98, TA100, and TA1535
E. coli strain: WP2-uvrA⁻

Concentrations in definitive study: With S9:
5.0, 1.582, 0.501, 0.159, 0.050 mg/plate

Without S9:
1.582, 0.501, 0.159, 0.050, 0.016 mg/plate

Basis of concentration selection: The test article was diluted in 100% DMSO. A dose-range finding study determined that doses of 5.0 and 1.582 mg/plate with metabolic activation induced substantial toxicity in strain TA100 and resulted in no colony formation and an absent micro-colony lawn.

Negative control: DMSO

Positive control: With S9:
2-aminoanthracene (10.0 µg/plate) for all strains except TA1535 which used a concentration of 1.6 µg/plate

Without S9:
TA97a: ICR-191 Acridine (1.0 µg/plate)
TA98: 2-nitrofluorene (10.0 µg/plate)
TA1535/TA100: sodium azide (1.5 µg/plate)

Formulation/Vehicle: DMSO

Incubation & sampling time: 3 plates per treatment
Plates were incubated 48-72 hours at 37°C.
Plates were counted using an automatic image analysis system.
Negative control and test article treated plates were also examined for the presence of a bacterial lawn.

Study Validity

Bacterial strains used conform to ICH S2A recommendations. Positive and negative controls produced expected responses (see results tabulated below). Dose selection for the plate incorporation method was adequate based upon use of the limit dose (i.e., 5000 µg/plate).

Results

The following results were compiled from data tabulated in the study report:

Average colony count per plate without S9:

Dose (mg/plate)	TA97a	TA98	TA100	TA1535	WP2-uvrA
5.000	89	33	106	19	16
1.582	102	32	112	11	19
0.501	103	31	111	14	14
0.158	107	29	112	11	19
0.050	106	29	112	18	18
-control	105	26	105	14	20
+control	1107	1176	988	444	96

Average colony count per plate with S9:

Dose (mg/plate)	TA97a	TA98	TA100	TA1535	WP2-uvrA
0.501	123	34	104	15	22
0.158	119	40	107	11	19
0.050	133	37	92	18	14
0.016	127	40	92	13	18
0.005	121	37	98	15	14
-control	124	30	83	12	15
+control	2009	1254	820	181	252

Historic reversion rates for the (b) (4) were provided and the background results provided fall within the ranges for the omacetaxine treatments and negative controls.

Conclusions

The Ames assay results indicate that omacetaxine is not a bacterial mutagen under the conditions tested.

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)**Study title: In Vivo Mouse Micronucleus Assay**

Study no: PTX 031
 Study report location: NDA 203585 Section 4.2.3.3.2.1
 Conducting laboratory and location: (b) (4)

Date of study initiation: January 6, 2011
 GLP compliance: Yes, signed statement provided
 QA statement: Yes, signed statement provided
 Drug, lot #, and % purity: Omacetaxine mepesuccinate (powder), Lot #12252,

Key Study Findings

- Omacetaxine did not induce a dose-dependent increase in micronuclei formation in mouse erythrocytes derived from bone marrow.

Methods

Doses in definitive study:	0.095, 0.30, 0.95 mg/kg
Frequency of dosing:	Single administration
Route of administration:	Tail vein injection (IV)
Dose volume:	Dose volumes were not provided. Stock solution concentration = 0.3 mg/ml and lower doses were prepared by dilution immediately prior to use.
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	<i>Mus musculus</i> /CD-1
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	A dose range study demonstrated some toxicity at the high dose of 3.0 mg/kg. The next highest 3 doses were chosen for the definitive study.
Negative control:	0.9% NaCl
Positive control:	75 mg/kg cyclophosphamide

Study Validity

- A dose-range finding study was conducted in which 3 males per group received a single IV administration of 0.030, 0.095, 0.30, 0.95, 3.0 mg/kg followed by bone marrow collection 24h post-dosing. In the high dose group, 1/3 males appeared lethargic and had a spiked coat within one hour-post dose. The two other males in this group also exhibited a spiked coat four hours post-dosing. The next morning, 2/3 males “still showed some signs of toxicity” but were responsive to stimuli. All other mice appeared bright, active and responding to stimuli throughout. The sponsor chose to use 0.95 mg/kg as the maximum dose for the definitive study based on the toxicities observed in the 3.0 mg/kg group. ICH S2(R1) and Redbook 2000¹ recommends that the highest dose used produce signs of toxicity but not be expected to produce lethality. Based on this recommendation, the maximum dose used in the definitive study is low since there was no evidence of toxicity observed in the group that received 0.95 mg/kg.
- Results from an acute toxicity study using CDF₁ mice (Study Report PTX-013 conducted in 1981) identified single IV dose levels that were not lethal, but were higher than the dose levels used in the *in vivo* micronucleus study (Study PTX-013 used doses ranging from 0.80 to 10

¹<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/ucm078338.htm>

mg/kg). Clinical signs of toxicity (ruffling of the coat) were observed at 4.31 mg/kg or higher in both sexes, and lethargy at 6.56 mg/kg in females and at 10 mg/kg in males. No deaths were observed in groups receiving the 2.13 mg/kg or lower dose.

- However, results from the definitive study demonstrated a small decrease in numbers of immature erythrocytes in samples from the highest dose tested, indicating that systemic exposure to omacetaxine was sufficient to elicit toxicity.
- Bone marrow was harvested 24 and 48 hours after dosing. Immediately after harvest, three blood smears per mouse were prepared. Slides were stained and scored visually. Two thousand polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. The proportion of PCE to 500 mature erythrocytes was determined as a measure of toxicity. The preparation and analysis of cells is acceptable.
- The positive control used produced significant levels of micronuclei formation (~30 fold and ~50 fold in females and males, respectively). The number of micronuclei in the negative controls is within the historical ranges for the conducting laboratory.

Results (Data tables shown were copied from the study report)

There was no dose-dependent increase in micronuclei formation in either males or females. Curiously, the amount of micronuclei increased at the low dose of omacetaxine but the increase was not statistically significant according to the ANOVA test used. The relevance of this finding is unknown.

TABLE 4
AVERAGE MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES RATES
PER 1000 POLYCHROMATIC ERYTHROCYTES*

TREATMENT	FEMALES		MALES	
	24 HR INDUCTION	48 HR INDUCTION	24 HR INDUCTION	48 HR INDUCTION
0.950 mg/kg	0.8	0.6	1.4	1.8
0.300 mg/kg	1.6	0.7	1.3	2.4
0.095 mg/kg	2.7	2.2	3.2	2.2
Negative Control	1.4	1.2	0.9	1.5
Cyclophosphamide 75 mg/kg	35.3*	Not Tested	50.6*	Not Tested

*Micronucleus induction significantly greater than in either negative control (One-way ANOVA; $p < 0.001$)

As shown in the table below, the high dose caused a small but statistically

significant decrease in the ratio of immature to mature (PCE:RBC²) erythrocytes in males after 24h and in females after 24h and 48h. A small *increase* was observed in males treated with the mid dose (0.30 mg/kg) after 24h, but not 48h. The relevance of this increase is unknown. The positive control did not demonstrate a change in the ratio of PCE:RBC.

TABLE 5 – EFFECTS OF TREATMENT ON ERYTHROPOIESIS

TREATMENT	FEMALES		MALES	
	24 HR INDUCTION	48 HR INDUCTION	24 HR INDUCTION	48 HR INDUCTION
0.950 mg/kg	0.9*	0.5*	0.6*	1.0
0.300 mg/kg	1.0	1.0	1.5*	1.0
0.095 mg/kg	1.0	1.0	1.0	1.0
Negative Control	1.0	1.0	1.1	1.0
Cyclophosphamide 75 mg/kg	1.0	Not Tested	1.0	Not Tested

*Erythropoietic ratio was found to be significantly different compared to the negative control.

Conclusions

While the maximum dose used did not cause clinically observable signs of toxicity, it did cause a decrease in the ratio of immature to mature erythrocytes when compared to the negative control. Therefore, the assay is considered adequate and demonstrated that omacetaxine did not induce a dose-dependent increase in micronuclei formation in mouse erythrocytes derived from bone marrow. However, the lowest dose used caused a non-statistically significant increase in micronuclei, but the relevance of this finding is unknown.

Study title: Profiling of CEP-41443 (omacetaxine mepusuccinate, Blind Code IN072511) in a Kinase Panel (b) (4)

Study no.: CS-2011-0190US

Study report location: NDA 203585 Section 4.2.1.1.1

Conducting laboratory and location: (b) (4)

Date of study initiation: August 5, 2011

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Not provided

² Terminology used in the study report.

Key Study Findings

Cephalon contracted [REDACTED]^{(b) (4)} to conduct their Z'-LYTE® biochemical assay analysis to evaluate omacetaxine's ability to inhibit 71 kinases using a fluorescence-based, coupled-enzyme format.

- Omacetaxine was tested using a single concentration (1 microM). None of the kinases tested were inhibited by more than 25%, and most results were <10% inhibition.
- Study results are provided below:

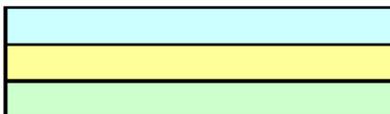
(b) (4) Kinase Profiling - Omacetaxine Mepesuccinate (CEP-41443)
Study SSBK8492_22628

Duplicate determinations at [ATP] = Km

>99% Inhibition

90-99% Inhibition

80-90% Inhibition



Kinase	Omacetaxine Mepesuccinate % Inhibition at 1 uM
ABL1	-6
ABL1 T315I	2
ACVR1B (ALK4)	11
AKT1 (PKB alpha)	4
AMPK A1/B1/G1	7
AURKA (Aurora A)	0
AURKB (Aurora B)	5
BTK	0
CDK1/cyclin B	4
CHEK1 (CHK1)	0
CLK1	0
CSNK1G2 (CK1 gamma 2)	5
CSNK2A1 (CK2 alpha 1)	-1
DAPK3 (ZIPK)	-1
DCAMKL2 (DCK2)	6
DYRK3	-5
EGFR (ErbB1)	8
EPHA2	2
EPHB1	-1
ERBB2 (HER2)	-3
FGFR1	15
FLT1 (VEGFR1)	6
FLT3	7
FLT4 (VEGFR3)	4
FRAP1 (mTOR)	3
GRK4	2
GSK3B (GSK3 beta)	-2
IGF1R	3
IKBKB (IKK beta)	0
INSR	1
IRAK4	-3
JAK3	6
KDR (VEGFR2)	1
KIT	11
LCK	5

MAP2K1 (MEK1)	22
MAP3K8 (COT)	11
MAP3K9 (MLK1)	4
MAP4K4 (HGK)	19
MAP4K5 (KHS1)	-6
MAPK1 (ERK2)	2
MAPK14 (p38 alpha)	7
MAPK8 (JNK1)	14
MAPKAPK2	-1
MARK1 (MARK)	-1
MET (cMet)	9
NEK1	-9
NEK7	-7
NTRK1 (TRKA)	20
PAK4	-19
PDGFRB (PDGFR beta)	0
PDK1 Direct	0
PHKG2	11
PIM1	6
PKN1 (PRK1)	7
PLK1	6
PRKACA (PKA)	-1
PRKCB1 (PKC beta I)	4
PRKCD (PKC delta)	-6
PRKCE (PKC epsilon)	-5
PRKG2 (PKG2)	-5
RET	1
ROCK1	-2
ROCK2	6
RPS6KA3 (RSK2)	0
RPS6KB1 (p70S6K)	9
SRC	0
STK22D (TSSK1)	2
SYK	5
TAOK2 (TAO1)	7
TEK (Tie2)	-3

Number of kinases (out of 71)

>99% Inhibition	0
90-99% Inhibition	0
80-90% Inhibition	0

S(90) 0.000

S(99) 0.000

Omacetaxine Pharmacology Published Literature Review

NDA 203585 contains four pharmacology studies, three of which were submitted previously under NDA 22374 and reviewed by Dr. Timothy Kropp. In addition, Cephalon provided literature references containing data that examined omacetaxine pharmacology. The three studies reviewed previously are:

Study Number	Study Title
TB-20081	Determination of the Relative Cytotoxicity of Homoharringtonine (HHT), 4'-DMHHT & Cephalotaxine (CTXOH) Against the Hematologic Cell Lines K-562, HL-60, Molt-4 and CCRF-CEM
TB-20084	Inhibitory Effects of Omacetaxine on Chronic Myeloid Leukemia (CML) and Bcr-Abl-Transduced Hematopoietic Stem Cells in Mice
TB-20085	Effects of Omacetaxine on the Expression of Bcl-2 family proteins in K562 Leukemia Cells

The pharmacology of omacetaxine (also known as homoharringtonine) has been investigated since the 1970s. Early work by Fresno *et al.* identified that *Cephalotaxus* alkaloids (harringtonin, homoharringtonine and isoharringtonine) inhibit the elongation phase of protein translation using ribosomes isolated from eukaryotic cells.³

A more recent study evaluated the crystal structure of homoharringtonine bound to the large ribosomal subunit from *H. marismortui* in an effort to understand its antibiotic properties.⁴ *Haloarcula marismortui* is a halophilic red Archaeon (from the Halobacteriaceae family) found in the Dead Sea, a high saline, low oxygen solubility, and high light intensity environment. Homoharringtonine competes with incoming aminoacyl-tRNAs for binding to the A-site cleft in the peptidyl transferase center. This paper cites other work that describes the ribosomal RNA sequences of archaea bacteria as being more closely related to eukaryotes than to eubacteria.

Study Report TB-20085 (conducted by ChemGenex) contains results from western blot analysis of protein lysates collected from Bcr-Abl positive-K562 cells that were treated with omacetaxine. Mcl-1 and Bim levels decreased in a dose-dependent manner, while Bax increased. Puma and Bcl-XI were not affected. The concomitant increase in pro-apoptotic Bax and decrease of Mcl-1 levels support the observation of increased apoptosis following omacetaxine treatment, while the significance of the decrease in Bim (a pro-apoptotic Bcl-2 family member) is unknown.

³ Fresno M, Jimenez A, Vazquez D. Inhibition of translation in eukaryotic systems by harringtonine. *Eur J Biochem.* 1977;72(2):323-330.

⁴ Gurel G, Blaha G, Moore PB, Steitz TA. U2504 determines the species specificity of the A-site cleft antibiotics: the structures of tiamulin, homoharringtonine, and bruceantin bound to the ribosome. *J Mol Biol.* 2009;389(1):146-156.

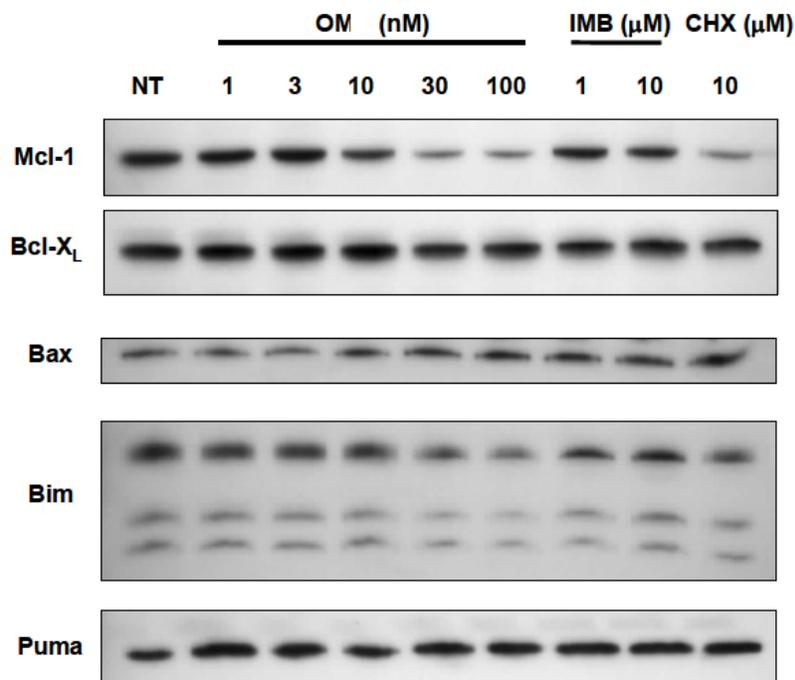
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Figure 2: Dose response analysis of the effect of omacetaxine on bcl-2 family protein expression

K562 cells were treated with omacetaxine (OM), imatinib (IM), cyclohexamide (CHX) or vehicle (not treated, NT) for 4 hours and protein lysates were prepared. 20ug of total protein was analyzed by PAGE and western blotting with antibodies specific for bcl-2 family proteins.

Data published by Chen et al.⁵ demonstrated that omacetaxine treatment of K562 cells resulted in a decrease of Bcr-Abl levels.

⁵ Chen R, Gandhi V, Plunkett W. A sequential blockade strategy for the design of combination therapies to overcome oncogene addiction in chronic myelogenous leukemia. *Cancer Res.* 2006;66(22):10959-10966

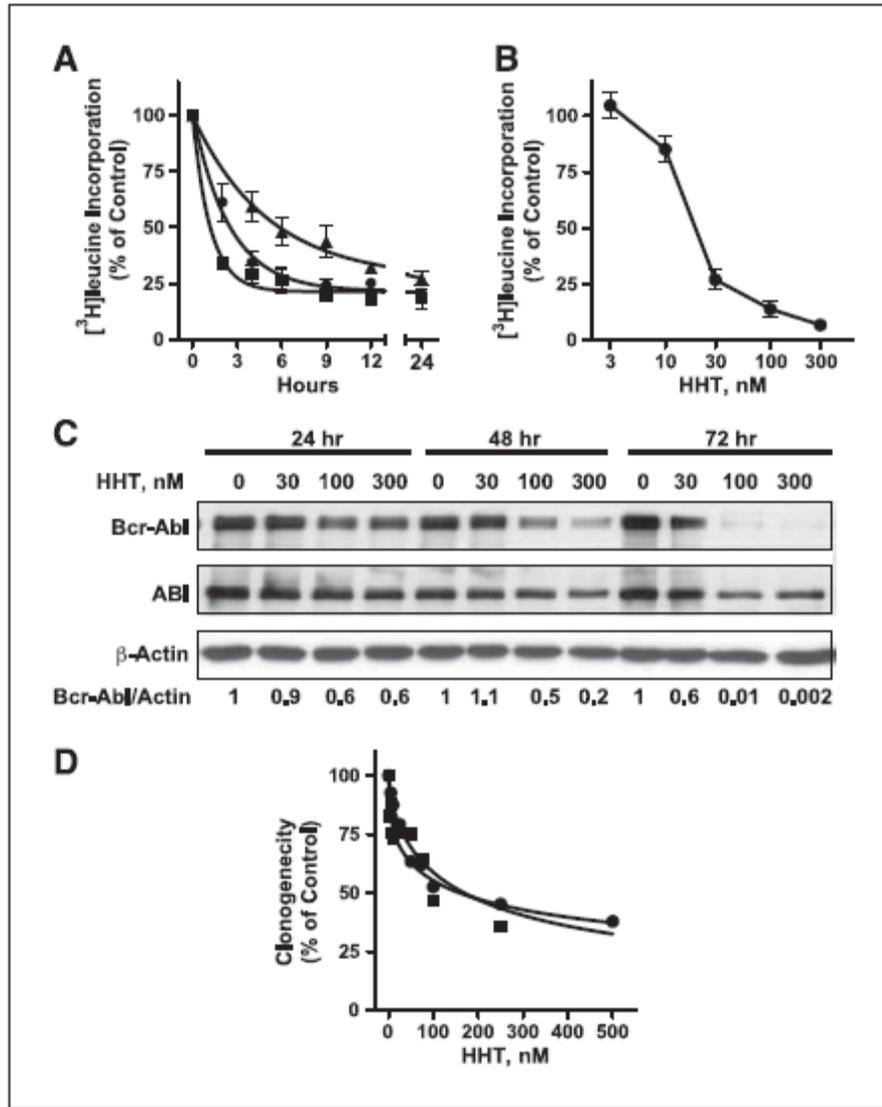


Figure 3. HHT inhibited protein synthesis and reduced the Bcr-Abl protein levels in K562 cells. **A**, time-dependent inhibition of protein synthesis by HHT. K562 cells were incubated with HHT [100 nmol/L (●) or 300 nmol/L (■)] or 2.5 μg/mL puromycin (▲) for the indicated time and then labeled with 1 μCi/mL [³H]leucine for 30 minutes. *Points*, mean percentage of protein synthesis compared with untreated controls of three independent experiments done in triplicate; *bars*, SE. **B**, concentration-dependent inhibition of protein synthesis by HHT. K562 cells were incubated with increasing concentrations of HHT for 24 hours and then pulse labeled with 1 μCi/mL [³H]leucine for 30 minutes. *Points*, protein synthesis expressed as the mean percentage of radioactivity compared with controls of triplicate data; *bars*, SD. **C**, Bcr-Abl protein decreased after incubation with HHT. K562 cells were incubated with 30, 100, or 300 nmol/L HHT or solvent for 24, 48, and 72 hours. Levels of Bcr-Abl and Abl protein were detected by immunoblot. Results were calculated as the ratio of the relative film density of Bcr-Abl and β-actin. β-Actin was used as a loading control. **D**, inhibition of clonogenicity by HHT in K562 cells after 24 hours (●) and 48 hours (■) of incubation. Data represent the percentage of control colonies. The IC₅₀ of inhibition of clonogenicity by HHT was 168 nmol/L for 24 hours and 158 nmol/L for 48 hours in experiments done in triplicate.

Data from a different laboratory illustrated that omacetaxine treatment of cell lines derived from primary mouse pre-B cells that had been retrovirally transduced with wt-Bcr-Abl or T315I-mutant Bcr-Abl resulted in decreased Bcr-Abl protein levels regardless of mutation status.⁶

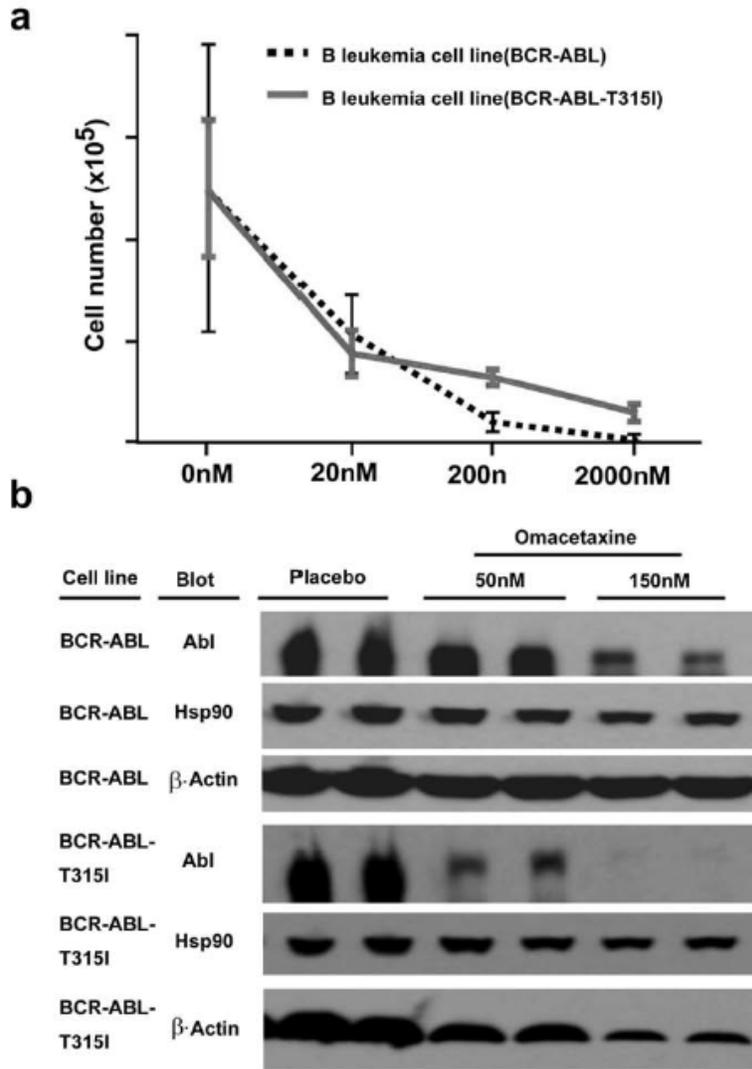


Figure 6. Omacetaxine inhibits B-ALL cells by suppressing BCR-ABL without affecting HSP90
a. Omacetaxine inhibited pre-B cells expressing BCR-ABL or BCR-ABL-T315I associated with drug concentration. The number of viable cells at the indicated drug concentrations was determined by trypan blue.
b. Omacetaxine inhibited the expression of ABL in pre-B cells expressing BCR-ABL or BCR-ABL-T315I. These pre-B cells were treated with omacetaxine (50 nM, 150 nM) for 48 hours. Protein lysates were analyzed by Western blotting using antibodies indicated.

⁶ Chen Y, Hu Y, Michaels S, Segal D, Brown D, Li S. Inhibitory effects of omacetaxine on leukemic stem cells and BCR-ABL-induced chronic myeloid leukemia and acute lymphoblastic leukemia in mice. *Leukemia*. 2009;23(8):1446-1454.

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Section 12.1 of the package insert submitted in the NDA states that c-Myc and Cyclin D1 levels are decreased following omacetaxine treatment, but data to support this claim was not provided in the NDA nor is there mention of omacetaxine action on c-Myc or Cyclin D1 levels in the Pharmacology Written Review.

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/s/

M S RICCI

09/05/2012

This review is an Addendum to the Pharmacology and Toxicology Review for NDA 22374 by Dr. Timothy Kropp.

HALEH SABER

09/05/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203585

Applicant: Cephalon, Inc. (wholly owned subsidiary of Teva Pharmaceuticals) **Stamp Date:** Mar. 30, 2012

Drug Name: Omacetaxine mepesuccinate

NDA/BLA Type: 505(b)(1) NDA;
Previously submitted as NDA 22374, which was not approved

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		6 month tox studies used DP lot #05D08; DS lot #07758 was used to make this DP
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	This will determined during the review cycle. No impurity issues were identified during NDA 22374 review that needed toxicology safety evaluation. Since the formulation has changed, the impurity profile may be different.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

M. Stacey Ricci, M.Eng., Sc.D.
Reviewing Pharmacologist

May 11, 2012
Date

Haleh Saber, Ph.D.
Team Leader/Supervisor

May 11, 2012
Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
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/s/

M S RICCI
05/11/2012

HALEH SABER
05/11/2012

MEMORANDUM

Omapro (omacetaxine mepesuccinate)

Date: March 5, 2010

To: File for NDA 22374

From: John K. Leighton, PhD, DABT

Associate Director for Pharmacology/Toxicology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review by Dr. Kropp and supervisory memorandum provided by Dr. Saber. I concur with their conclusions that Omapro may be approved but that an additional genotoxicity study(ies) should be conducted. A labeling review will need to be conducted when appropriate.

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22374

ORIG-1

CHEMGENEX
PHARMACEUTICA
LS

Omapro

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/s/

JOHN K LEIGHTON

03/05/2010

MEMORANDUM

Date: March 5, 2010
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Drug Oncology Products
Re: Approvability for Pharmacology and Toxicology
NDA: File for NDA # 22,374
OMAPRO™ (omacetaxine mepesuccinate)
Indication: Treatment of adults with chronic myeloid leukemia (CML) who have failed prior therapy with imatinib and have the Bcr-Abl T315I mutation

As reviewed by Dr. Timothy Kropp, OMAPRO (omacetaxine mepesuccinate) is an anticancer drug, which exerts its activity at least in part by blocking aminoacyl tRNA binding to the acceptor site on the 60S ribosome subunit and interfering with protein elongation. Anticancer effects observed with omacetaxine may be also due to increased apoptosis. In a pharmacology study, treatment with omacetaxine resulted in decreased expression of Bcl-2 family of proteins, Mcl-1 and Bim, and increased expression of pro-apoptotic protein Bax. The term used for a pharmacologic class of a drug should provide scientifically valid information that will be meaningful to prescribers. Due to the lack of adequate understanding of the mechanism of action of this drug, a pharmacologic class cannot be established at this time. The Applicant is seeking approval for treatment of adults with chronic myeloid leukemia (CML) who have failed prior therapy with imatinib and have the Bcr-Abl T315I mutation. In pharmacology studies, the anticancer activity of omacetaxine was demonstrated in cultured cells and in a mouse model of CML. Omacetaxine had anticancer activity against both wild-type and T315I mutant cells with no clear increased activity against T315I mutation.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. With regard to animal toxicology studies, only studies in which drug was given subcutaneously have been reviewed, because this is the proposed route of administration in patients. Based on the general toxicology studies, toxicities associated with omacetaxine include effects in the hematopoietic system, heart, liver, gastrointestinal tract, kidneys, skin, and male reproductive organs. Hemorrhage was observed in multiple organs and tissues, including the brain, and may be at least partially due to the reduced platelet counts. Hyperglycemia was observed in few studies; however, there was no clear correlating effect on pancreas upon histopathology examination. Based on the summary information presented by the Applicant, hyperglycemia and cardiovascular toxicities were more prominent in studies where drug was administered intravenously. Toxicities in animals were generally a good predictor of toxicities reported in patients. Clinical adverse reactions based on a small number of patients include bone marrow suppression and subsequent hematologic effects, cardiac rhythm abnormality and hypotension, hyperbilirubinemia, hyperglycemia, and skin reactions. Of note, additional clinical safety data will become available in the near future

and the safety profile of omacetaxine may need to be updated. We recommend that drug-induced liver toxicity in patients be re-evaluated in future based on findings in animals which consisted of the following: increased weight, increased bilirubin, hepatocellular necrosis/degeneration, centrilobular hepatocyte hypertrophy, centrilobular cytoplasmic eosinophilia, hepatocyte vacuolation, and glycogen depletion. Presently a significant number of patients experienced hyperbilirubinemia.

Based on the findings in the general toxicology studies (e.g. reduced testicular weight, seminiferous tubular epithelial degeneration, and hypospermia/aspermia) male fertility may be compromised by omacetaxine treatment. Additionally, omacetaxine can cause embryofetal lethality. In a pilot study in mice, the omacetaxine dose of 0.835 mg/kg/day (~2.5 mg/m²/day) resulted in 100% embryo-fetal mortality when given subcutaneously during the period of organogenesis. This dose is equivalent to the human recommended dose of 2.5 mg/m²/day, based on the body surface area (BSA). In a pivotal reproductive toxicology study in mice, a lower dose of omacetaxine, i.e. 0.41 mg/kg/day (approximately half the recommended human daily dose on the BSA-basis) caused embryo-fetal toxicities including pre- or post-implantation loss, increased resorption, reduced fetal weight and presence of unossified bones. Embryo-fetal toxicities were seen in the absence of significant maternal toxicities. Based on these findings, a pregnancy Category D is recommended for this drug.

Omacetaxine was positive for clastogenicity in Chinese hamster ovary (CHO) cells. The Ames test conducted is deemed inadequate because of low concentrations used in the assay. The Applicant should conduct the Ames assay according to ICH S2. According to ICH S9, if a drug is positive in 2 *in vitro* genotoxicity studies, the *in vivo* assay might not be warranted. The *in vivo* study will be necessary to fully elucidate the genotoxicity potential of the drug, if the result of Ames test is negative. As the mechanism of action of omacetaxine is not fully elucidated, there is a potential for the Ames assay to be irrelevant (due to differences in bacterial versus eukaryotic protein synthesis). Of note, the *in vivo* genotoxicity of omacetaxine, with sister chromatid exchange (SCE) as the endpoint, has been assessed in 3 patients. The negative result obtained in this study was likely due to the sub-optimal duration and/or sub-optimal doses used, as indicated by lack of drug activity in 2 patients.

The nonclinical studies were reviewed by Dr. Kropp. The nonclinical findings are summarized in the “Executive Summary”, and “Discussion and Conclusions” of the review. A separate labeling review will be conducted when appropriate.

Recommendation: I concur with Dr. Kropp that from a nonclinical perspective, omacetaxine may be approved for the proposed indication. The Ames assay conducted by the Applicant was inadequate and needs to be conducted according to ICH S2. If negative mutagenicity is obtained in the Ames test, the *in vivo* genetic toxicity assay will be needed for a complete assessment of genotoxicity associated with the drug. While the genotoxicity assay(s) could be conducted post-marketing, the Applicant is encouraged to start the study(ies) as soon as feasible.

Application
Type/Number

Submission
Type/Number

Submitter Name

Product Name

NDA-22374

ORIG-1

CHEMGENEX
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Omapro

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/s/

HALEH SABER
03/05/2010



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: **22374**

DATE RECEIVED BY CENTER: **8 September 2009**

PRODUCT: **OMAPRO™ (omacetaxine mepesuccinate)**

INTENDED CLINICAL POPULATION: **Adults with chronic myeloid leukemia who have failed prior therapy with imatinib and have the T315I Bcr-Abl mutation**

APPLICANT: **Chemgenex Pharmaceuticals**

REVIEW DIVISION: **Division of Drug Oncology Products**

PHARM/TOX REVIEWER: **Timothy Kropp, Ph.D.**

PHARM/TOX SUPERVISOR: **Haleh Saber, Ph.D**

DIVISION DIRECTOR: **Robert Justice, MD**

PROJECT MANAGER: **Allison Adams-McLean**

Date of review submission to DARRTS: **5 March 2010**

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EXECUTIVE SUMMARY

I. Recommendations

We recommend approval of omacetaxine from a pharmacology/toxicology standpoint for the proposed indication.

II Additional studies needed

Based on the life expectancy of a significant proportion of the imatinib-resistant chronic phase CML patient population, a full battery of genotoxicity studies should be performed per ICH S2. These studies are necessary to adequately inform patients of the possible risks of omacetaxine and to determine the possible need for carcinogenicity studies.

A separate labeling review will be conducted if necessary.

II. Summary of nonclinical findings

Omacetaxine shows activity in various anti-leukemic cells lines but the mechanism of action of omacetaxine as related to anti-cancer activity has not been fully elucidated. There is no evidence that hematological malignancies that contain the T315I mutation of the Bcr-Abl fusion gene are more sensitive to omacetaxine than wild type Bcr-Abl; in fact, pharmacology studies submitted by the applicant demonstrate that omacetaxine activity is similar in cells with or without the T315I mutation of Bcr-Abl fusion gene.

In mice and dogs, omacetaxine was the primary exposure moiety and was absorbed quickly and cleared quickly through the bile and urine with peak concentration occurring at 0.5 h and washing out by approximately 12 hours. Dose proportionality varied over the course of the study for mice but for dogs exposure was generally greater than dose proportional. In both species, induction of metabolism or clearance appears to occur over the course of the study but this finding was not consistent. Omacetaxine did not appear to be accumulative over the course of the 6-month studies; however, for certain cycles accumulation did appear to minimally occur. Omacetaxine appears to preferentially distribute to the bone marrow (in rats), liver, and kidneys in rodents. By 24 h post dose, omacetaxine was only detected in the liver and that was eliminated by 72 h post dose but does not appear to be metabolized significantly by CYP-mediated oxidative metabolism or by esterases in human liver microsomes.

In general toxicology studies, omacetaxine showed a steep dose-response relationship in mice and dogs with the primary toxicities being on the bone marrow with platelets particularly affected. Other targets of omacetaxine toxicity included the heart, skin, and possibly the kidney and liver. Serious hemorrhage and lymphoid depletion are also related to omacetaxine but it is unclear if the finding of hemorrhage is solely due to the severe drop in platelets. The hemorrhaging due to omacetaxine-treatment is significant due to the widespread nature, seriousness of this endpoint, and the non-reversibility of this finding after a month of recovery in dogs.

There was little histopathological correlation for many findings in the general toxicology studies. Most strikingly, the pale organs such as the brain and adrenal glands, the increased heart weights, and the black contents of the stomach and intestines of early death animals had no histopathological correlates. Red foci, striations, and general color

were also noted and may be related to hemorrhage although, these gross pathology findings effected more organs than the histopathology findings can confirm.

In a small pilot embryofetal developmental study in mice, omacetaxine appeared to be 100% embryofetolethal at approximately the proposed human dose on a mg/m^2 basis. At levels were no significant maternal toxicity is noted (approximately half the proposed human dose), fetal weights are decreased and ossification was lacking in some sternebra. If ossification continues to be absent in the tissues noted, malformations will occur as fetal development continues.

Omacetaxine was positive for clastogenicity in Chinese hamster ovary (CHO) cells in the only adequately performed genetic toxicology study submitted with this NDA.

APPEARS THIS WAY ON ORIGINAL

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-374

Review number: 1

Sequence number/date/type of submission: 006/8 Sept 2009

Applicant and/or agent: Chemgenex Pharmaceuticals

Reviewer name: Timothy Kropp, Ph.D.

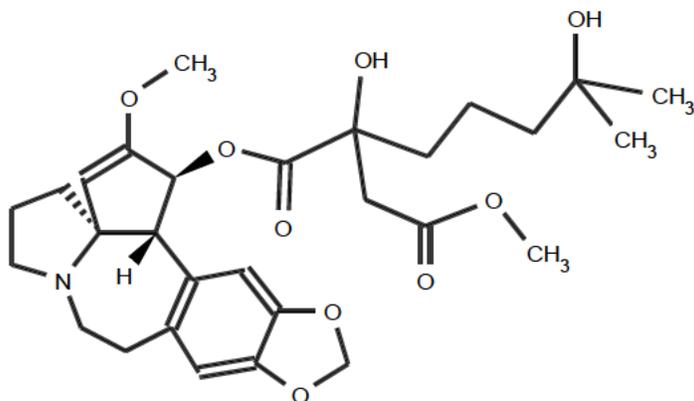
Division name: Division of Drug Oncology Products

HFD #: 150

Review completion date: 1 March 2010

Drug:

Trade name:	OMAPRO™
Generic name:	Omacetaxine mepesuccinate
Other names:	homoharringtonine, HHT, omacetaxine
Chemical name:	cephalotaxine, 4'-methyl(2'R)-hydroxyl-2'- 4''-hydroxyl-4''- methylpentylbutanedioate(ester),[3(R)]
CAS registry number:	26833-87-4
Molecular weight:	545.625
Structure:	



Relevant INDs/NDAs/DMFs: IND 62384, IND (b) (4), DMF 20542 Type II, DMF (b) (4), DMF (b) (4), DMF (b) (4) Type V

Pharmacologic class: None can be determined at this time

Intended clinical population: adults with chronic myeloid leukemia who have failed prior therapy with imatinib and have the T315I Bcr-Abl mutation

Proposed dose: 1.25 mg/m²

Clinical formulation: 0.9% saline for injection

Route of administration: subcutaneous injection

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

Studies reviewed within this submission:

	Study Title	Study number
Pharmacology		
1	Determination of the Relative Cytotoxicity of Homoharringtonine (HHT), 4'-DMHHT & Cephalotaxine (CTXOH) Against the Hematologic Cell Lines K-562, Molt-4, HL-60, CCRF-CEM.	TB-20081
2	Inhibitory Effects of Omacetaxine on Chronic Myeloid Leukemia (CML) and Bcr-Abl-Transduced Hematopoietic Stem Cells in Mice. (Report)	TB-20084
3	Study Title: Effects of Omacetaxine on the Expression of Bcl-2 family proteins in K562 Leukemia Cells. (Report)	TB-20085
Safety Pharmacology		
1	Effect of HHT on hERG Tail Current Recorded from Stably Transfected HEK293 Cells	PTX009
2	Effects of HHT on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers.	PTX010
Pharmacokinetics		
1	Stability of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples	PTX024
2	Stability of Homoharringtonine in Mouse Plasma Samples	PTX026
3	Protein Binding of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples	PTX006
4	Protein Binding of Homoharringtonine in Mouse Plasma Samples	PTX007
5	Study to Investigate the Permeability and P-gp mediated efflux of Homoharringtonine and 4-Demethylated Homoharringtonine in MDR1-MDCK cells.	PTX001
6	Stability of Homoharringtonine (HHT) in Human Liver Microsomes: Assessment of the Effect of the Esterase Inhibitor, Phenylmethanesulfonyl Fluoride	PTX002

	(PMSF).	
7	Study to Investigate the Potential of Homoharringtonine and 4-Demethylated Homoharringtonine to Inhibit or Induce Human Cytochrome P450.	PTX003
General Toxicology		
1	Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in CD-1 Mice	PTX019, PTX022
2	Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in Beagle Dogs	PTX018a, PTX023a
Genetic Toxicology		
1	Bacterial Mutagenicity Test – Ames Assay	PTX004
2	<i>In Vitro</i> chromosome aberration analysis in Chinese hamster ovary (CHO) cells.	PTX008
Reproductive and Developmental Toxicity		
1	Developmental Toxicity Study of Homoharringtonine (HHT) in Mice: Pilot Study	PTX011
2	Study title: Developmental Toxicity Study of Homoharringtonine (HHT) in Mice Following Twice Daily Subcutaneous Administration.	PTX012

Studies not reviewed within this submission:

	Study Title	Study number
General Toxicology (b) (4)		
1		
2		
3		
4		
5		

ABBREVIATIONS USED

- VC – Vehicle Control
- LD – Low Dose
- MD – Mid Dose
- HD – High Dose

SD – Standard Deviation

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2.6.2 PHARMACOLOGY

2.6.2.2 Primary pharmacodynamics

Mechanism of action: The mechanism of action of HHT as related to anti-cancer activity has not been fully elucidated. HHT is an inhibitor of protein elongation¹; decreasing incorporation of [³⁵S]methionine but not [³H]thymidine or [³H]uridine in TCA precipitable counts precipitates in HL-60 cells². HHT prevents protein elongation by blocking aminoacyl tRNA binding to the acceptor site on the ribosome 60S subunit³.

Drug activity related to proposed indication:

Study Title: Determination of the Relative Cytotoxicity of Homoharringtonine (HHT), 4'-DMHHT & Cephalotaxine (CTXOH) Against the Hematologic Cell Lines K-562, Molt-4, HL-60, CCRF-CEM. (Report TB-20081)

IC₅₀ values for HHT and its metabolites (4'-DMHHT and CTXOH) against a variety of human leukemia cell lines (K-562, myeloid leukemia; Molt-4, acute lymphoblastic leukemia; HL-60, promyelocytic leukemia; CCRF-CEM, T cell lymphoblast-like) were determined and are listed below.

IC ₅₀ values (μM)			
Cell Line	HHT	4'-DMHHT	CTXOH
K562	0.058	33	360
Molt-4	0.034	39	150
HL-60	0.042	23	310
CCRF-CEM	0.027	13	77

¹ Tujebajeva RM, Graifer DM, Karpova GC, Ajtkhozina NA. 1989. Alkaloid homoharringtonine inhibits polypeptide chain elongation on human ribosomes on the step of peptide bond formation. *FEBS Lett.* 257(2): 254-256.

Tang R, Faussat AM, Majdak P, Marzac C, Dubrulle S, Marjanovic, Legrand O Marie JP. 2006. Semisynthetic homoharringtonine induces apoptosis via inhibition of protein synthesis and triggers rapid myeloid cell leukemia-1 down-regulation in myeloid leukemia cells. *Mol Cancer Ther.* 5(3): 723-731.

² Zhou JY, Chen DL, Shen ZS, Koeffler HP. 1990. Effect of homoharringtonine on proliferation and differentiation of human leukemic cells in vitro. *Cancer Res.* 50(7): 3826-3832.

³ Fresno M, Jimenez A, Vazquez D. 1977. Inhibition of translation in eukaryotic systems by harringtonine. *Eur J Biochem.* 72(2): 323-330.

Tujebajeva RM, Graifer DM, Karpova GC, Ajtkhozina NA. 1989. Alkaloid homoharringtonine inhibits polypeptide chain elongation on human ribosomes on the step of peptide bond formation. *FEBS Lett.* 257(2): 254-256.

Tujebajeva RM, Graifer DM, Matasova NB, Fedorova OS, Odintsov VB, Ajtkhozina NA, Karpova GG. 1992. Selective inhibition of the polypeptide chain elongation in eukaryotic cells. *Biochim Biophys Acta.* 1129(2): 177-182.

Study Title: Inhibitory Effects of Omacetaxine on Chronic Myeloid Leukemia (CML) and Bcr-Abl-Transduced Hematopoietic Stem Cells in Mice. (Report TB-20084)

The activity of HHT in a mouse model of chronic myeloid leukemia was investigated. Bone marrow cells were transfected with wildtype or T315I mutant Bcr-Abl-containing cDNA in a retrovirus. Cells were subsequently injected by tail vein into irradiated BABL/c and C57BL/6 mice.

Eight days after bone marrow transplant, control, 0.5 mg/kg HHT once per day or 100 mg/kg imatinib twice/day was administered by oral gavage.

Control group animals all died by day 17 (Bcr-Abl WT) or day 18 (Bcr-Abl T315I) while HHT animals all survived to day 30. No information on survival of imatinib-treated animals is given.

C57BL/6 (B6) mice were treated at the above schedule for 6 days, 8 days after transplantation. The number of leukemic stem cells from treated mice was significantly lower ($p < 0.002$) for HHT-treated animals but not for imatinib- nor control-treated animals (values not given).

Study Title: Effects of Omacetaxine on the Expression of Bcl-2 family proteins in K562 Leukemia Cells. (Report TB-20085)

The effects of HHT on Bcl-2 family protein expression in K562 cells were investigated by western blot analysis.

The IC_{50} value for effect on cell viability was reported as 20 nM for a 24 h incubation.

For dose response studies, 1, 3, 10, 30 nM HHT; 1, 10 μ M imatinib; vehicle or 10 μ M cycloheximide were incubated with K562 cells for 4 h. Vehicle not defined. In response to HHT treatment, Mcl-1 and Bim levels were decreased dose-dependently, the level of Bax was increased dose-dependently, and Puma and Bcl-X_L levels were not changed by HHT. Imatinib increased Bax. Cycloheximide decreased Mcl-1 and increased Bax.

For time course studies of Mcl-1, 30 nM HHT or 10 μ M imatinib, were incubated with K562 cells for 1, 2, 4, or 6 h. Cycloheximide (10 μ M), or vehicle were incubated with K562 cells for 6 h only. Mcl-1 was decreased after 2 h treatment with HHT with a continued decrease through 6 h. Mcl-1 was decreased after 6 h treatment with imatinib.

For 2 and 10 μ M proteasome inhibitor MG132 and 10 μ M caspase inhibitor zVAD-FMK pre-treatments, 30 nM HHT, 10 μ M imatinib, or vehicle were incubated with K562 cells for 4 h to determine if loss of Mcl-1 in the presence of HHT was due to proteasomal degradation or caspase activity. MG-132 prevented Mcl-1 decrease in the presence of HHT, while zVAD-FMK had no effect suggesting that the Mcl-1 decrease seen in response to HHT treatment of K562 cells is due to proteasome degradation.

2.6.2.4 Safety pharmacology

An ECG evaluation was done as part of the 6-month repeat-dose study in Beagle dogs (study # PTX018a). See review of that study in Section 2.6.6.3

Study title: Effect of HHT on hERG Tail Current Recorded from Stably Transfected HEK293 Cells

Key study findings: HHT decreased hERG tail current 19% (significant from control at $P < 0.1$ but not $P < 0.05$) at 50 μM .

Study no.: PTX009 (ZNA19021.001)

Volume #, and page #: Vol 1, pg 28

Conducting laboratory and location: (b) (4)

Date of study initiation: 24 Jan 2008

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: HHT, 07758, 99.1%

Methods

Doses: 0.1% DMSO (vehicle), 50 μM HHT, 100 nM E-4031 (positive control)
4 cells/treatment were used in a standard hERG assay

Results

The decreases in tail current were 9.8%, 18.6%, and 91.9% for DMSO, HHT, and E-4031, respectively. The decreases for DMSO and HHT were not significantly different at $P < 0.05$ but was at $P < 0.1$ ($P = 0.071$, unpaired, 2-tail, student's t-test).

Study title: Effects of HHT on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers.

Key study findings: Changes suggest sodium or calcium channel blockage.

Study no.: PTX010 (ZNA19021.002)

Volume #, and page #: Vol 1, pg 52

Conducting laboratory and location: (b) (4)

Date of study initiation: 18 Feb 2008

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: HHT, 07758, 99.1%

Methods

- 8 cardiac Purkinje fibers isolated from 4 female Beagle dogs.
- Doses: 0.1% v/v DMSO, 0.5, 5, 50 μM HHT, 50 μM sotalol (positive control)

- Fibers were electrically paced at 0.5 and 1 Hz and action potential duration (APD) at 60% and 90% repolarisation, maximum rate of depolarization (MRD), upstroke amplitude (UA), resting membrane potential (RMP) were measured. At 50 μ M HHT and control, MRD was also measured at 3 Hz stimulation.
- Fibers were exposed to either repeated vehicle treatment or sequential HHT treatment. Fibers exposed to vehicle treatments were exposed lastly to a positive control treatment. Each treatment lasted for 30 mins/treatment

Results

- 5 μ M
 - 27% \downarrow APD60 and 20% \downarrow APD90 at 0.5 Hz
 - 24% \downarrow APD60 and 17% \downarrow APD90 at 1 Hz
 - 25% \downarrow triangulation at 0.5 Hz and 15% \downarrow triangulation at 1 Hz.
- 50 μ M
 - 27 mV \downarrow UA at 0.5 Hz and 26 mV \downarrow UA at 1 Hz.
 - 32% \downarrow MRD at 0.5 Hz and 33% \downarrow MRD at 1 Hz.
 - 58% \downarrow APD60 and 42% \downarrow APD90 at 0.5 Hz
 - 49% \downarrow APD60 and 35% \downarrow APD90 at 1 Hz
 - 54% \downarrow MRD at 3 Hz

Conclusion

Changes suggest sodium or calcium channel blockage further suggesting that contractility and conduction velocity may be decreased. Furthermore, QRS complex measurement may increase while QT might decrease due to a shorter S-T.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Study Title	Study Number	Test system	Results
Determination of the Relative Cytotoxicity of Homoharringtonine (HHT), 4'-DMHHT & Cephalotaxine (CTXOH) Against the Hematologic Cell Lines K-562, Molt-4, HL-60, CCRF-CEM.	TB-20081	Human Leukemic Cell Lines K-562, Molt-4, HL-60, CCRF-CEM	IC ₅₀ values ranged from 0.027-0.058 μ M for HHT against the various cells lines. Activity is orders of magnitude greater than the metabolites of HHT.
Inhibitory Effects of Omacetaxine on Chronic Myeloid Leukemia (CML) and Bcr-Abl-Transduced Hematopoietic Stem Cells in Mice	TB-20084	BABL/c or C57BL/6 mice containing the Bcr-Abl wild-type or Bcr-Abl-T3151 gene	All HHT-treated animals survived to day 30; all control animals died by day 18. Leukemic stem cells from HHT-treated mice were significantly lower.
Effects of Omacetaxine on the Expression of	TB-20085	Human Leukemic Cell Line K526	HHT treatment led to decreased Bim and Mcl-1 and increased Bax. Mcl-1

Bcl-2 family proteins in K562 Leukemia Cells			decrease was affected by 2 hr treatment and appears to be due to proteasome degradation.
Effect on HHT on hERG Tail Current Recorded from Stably Transfected HEK293 Cells	ZNA19021.001	transfected (hERG) HEK293 cells	50 µM HHT decreased hERG tail current 19% (significant at P<0.1 but not P<0.05)
Effects of HHT on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres	ZNA19021.002	Purkinje fibers isolated from Beagle dogs.	Evidence of sodium and/or calcium channel blockage.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Toxicokinetic sections of submitted toxicology studies are reviewed with the toxicology study in section 2.6.6 Toxicology. No absorption studies were submitted.

2.6.4.2 Methods of Analysis

See method validation sections of individual study reviews under section 2.6.6 Toxicology

2.6.4.4 Distribution

Study title: Stability of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples

Key study findings: HHT appears to be very unstable in rat plasma. Stability was greatest in dog, then monkey, then human plasma. HHT concentrations for all timepoints for dog, monkey, or human plasma were within 20% of the initial concentration.

Study no.: PTX024

Volume #, and page #: Vol 1, pg 215

Conducting laboratory and location: (b) (4)

Date of study initiation: not given; report signed 15 April 2008

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, 040601, purity unknown

Methods

- Stability of HHT at 1 or 10 µM in commercially available human, cynomolgus monkey, dog (breed not given), and rat (strain not given) plasma was analyzed “initially”, 30, and 60 min at 37°C for 3 samples/species.
- It is unclear what “initially” means. The report notes that “a 0.3 mL sample was taken from each sample at 1, 0.5 and 1 hr”. This is likely a typo and the first ‘1’ refers to 1 min.

- For analyzation, blank plasma was added, vortexed, internal standard added ((b) (4)), 80:20 acetonitrile/methanol added and solids pelleted at 4000 rpm for 10 min (rotor size not given so g value is unknown). Supernatant was mixed in 0.1% formic acid centrifuged in duplicate manner and sample was analyzed by LC/MS/MS.

Results

- HHT was below LOQ (175 nM) even at the initial timepoint for rat plasma samples.

Stability of HHT at 1 μM (% of initial)

	dog	monkey	human
30 min	98	93	86
60 min	86 ⁴	86	82

APPEARS THIS WAY ON ORIGINAL

⁴ The results for the 60 min timepoint for the third sample for 1 μM HHT is listed as “N/A” without explanation. The value calculated here is the average of the remaining two samples.

Stability of HHT at 10 µM (% of initial)

	dog	monkey	human
30 min	99	96	84
60 min	94	82	80

Conclusion

HHT appears to be very unstable in rat plasma. However it is possible that the method for rat plasma was not validated as no validated assay for rat plasma has been submitted nor referred to. Additionally, it is unclear whether the lack of HHT found at the initial timepoint is due to lack of stability or complete protein binding since the protocol also listed “Centrifree® Ultrafiltration Devices, Millipore” as a supply. Such devices are to be used with a centrifuge to remove protein from samples. It is unclear if these products were used but would additionally impact the interpretation of the results in rats.

Because the results in the other samples are taken as relative to the initial sample, the interference of protein binding should be minimal unless time to equilibrium of protein-drug binding was very long.

Stability was greatest in dog, then monkey, then human plasma. All timepoints for dog, monkey, or human plasma were within 20% of the initial timepoint.

Study title: Stability of Homoharringtonine in Mouse Plasma Samples

Key study findings: HHT concentration was decreased 51% and 39% at 60 min in mouse plasma at 1.5 and 8 µM, respectively.

Study no.: PTX026

Volume #, and page #: Vol 1, pg 211

Conducting laboratory and location: (b) (4)

Date of study initiation: not given; report signed 30 May 2008

GLP compliance: No

QA report: yes () no ()

Drug, lot #, and % purity: HHT, 040601, purity unknown

Methods

- Stability of HHT at 1.5 or 8 µM in commercially available mouse (strain not given) plasma was analyzed at 0, 30, and 60 min at 37°C for 3 samples.
- For analyzation, blank plasma was added, vortexed, internal standard added ((b) (4) 80:20 acetonitrile/methanol added and solids pelleted at 4000 rpm for 10 min (rotor size not given so g value is unknown). Supernatant was mixed in 0.1% formic acid centrifuged in duplicate manner and sample was analyzed by LC/MS/MS.

Results

Stability of HHT at 1.5 μ M (% of initial)

	mouse plasma
30 min	69
60 min	49

Stability of HHT at 8 μ M (% of initial)

	mouse plasma
30 min	81
60 min	61

Conclusion

HHT concentration was decreased 51% and 39% at 60 min in mouse plasma at 1.5 and 8 μ M, respectively.

Study title: Protein Binding of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples

Key study findings: The amount of HHT that was protein bound was low, with binding in dog plasma < monkey plasma < human plasma. HHT was not detected in rat plasma indicating near complete protein binding, instability in this matrix, or an assay problem.

Study no.: PTX006

Volume #, and page #: Vol 1, pg 227

Conducting laboratory and location: (b) (4)

Date of study initiation: not given; report signed 13 April 2007

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, 040601, purity unknown

Methods

- Fraction of HHT binding to plasma proteins following 1 hour of incubation of HHT at 1.5 or 8 μ M in commercially available rat (strain not given), dog (strain not given), or human plasma was analyzed at 37°C for 2 samples/dose level/species.

Results**Protein binding of HHT (% Bound)**

	dog	monkey	human
1 μ M	1.7	19.1	49.7
10 μ M	2.9	20.3	40.7

- HHT was below LOQ (175 nM) for all rat plasma samples.

- The standard deviation was 0.001 at 1 μ M and 0.013 at 10 μ M for human plasma.

Conclusion

The amount of HHT that was protein bound was low, with binding in dog plasma < monkey plasma < human plasma. Any differential in drug degradation among sample types would affect both the absolute binding percentage but also possibly the order (although changes in the order would be unlikely given the magnitude of the difference). It is unclear if HHT is completely protein-bound, very unstable in rat plasma, or if the assay was invalid for this matrix.

The decrease in the amount bound in human plasma at 10 μ M compared to 1 μ M may suggest that binding sites are beginning to be saturated in this concentration range.

Study title: Protein Binding of Homoharringtonine in Mouse Plasma Samples

Key study findings: The amount of HHT that was protein bound was 33 and 24% in 1 and 10 μ M HHT, respectively.

Study no.: PTX007

Volume #, and page #: Vol 1, pg 222

Conducting laboratory and location: (b) (4)

Date of study initiation: not given; report signed 8 May 2007

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, 040601, purity unknown

Methods

- Fraction of HHT binding to plasma proteins following 1 hour of incubation of HHT at 1.5 or 8 μ M in commercially available mouse plasma was analyzed at 37°C for 2 samples/dose level/species.

Results

Protein binding of HHT (% Bound)	
	mouse plasma
1 μ M	32.5
10 μ M	24.0

- The standard deviation was 0.013 at 1 μ M and 0.076 at 10 μ M.

Conclusion

The amount of HHT that was protein bound was 33 and 24% in 1 and 10 μ M, respectively. The decrease in the amount bound at 10 μ M may suggest that binding sites are beginning to be saturated in this concentration range.

Study title: Study to Investigate the Permeability and P-gp mediated efflux of Homoharringtonine and 4-Demethylated Homoharringtonine in MDR1-MDCK cells.

Key study findings: HHT appears to have low permeability and is subject to P-gp efflux. DHHT has no measureable permeability nor is subject to P-gp efflux.

Study no.: PTX001

Volume #, and page #: Vol 1, pg 298

Conducting laboratory and location: (b) (4)

Date of study initiation: unknown, experimental start date was 19 Dec 2007.

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, 07758, 99.1%; 4-demethylated homoharringtonine (DMHHT), 20060321, 99.6%

Methods

- MDR1-MDCK monolayers were incubated at 37°C in 5% CO₂
- The final concentration of test compound was 1 µM; vehicle final was 1% DMSO.
- Studies of basical to apical transport and *vice versa* were performed in the absence and presence of 10 µM cyclosporine A (cA) on the apical side.
- Integrity of the monolayers was checked by monitoring lucifer yellow permeation.
- Propranolol was used as a highly permeable control
- Prazosin was used as a positive P-gp substrate control

Results

- DMHHT was not detectable in receiver compartments in any assays.
- Monolayer was intact as assessed by lucifer yellow.
- Permeability coefficients (rate of permeation/(donor compartment concentration initially • area of the cell monolayer)) and the efflux ratio (ratio of permeability coefficients: B to A / A to B) are in the table below:

	cA	Direction*	Permeability coefficients	Efflux ratio
HHT	-	A to B	0.22	52.8
		B to A	12.1	
	+	A to B	0.621	2.10
		B to A	1.30	
Prazosin	-	A to B	2.94	19.1
		B to A	56.2	
	+	A to B	31.8	0.233
		B to A	7.41	

Propranolol	-	A to B	62.8	0.439
		B to A	27.6	
	+	A to B	66.1	0.408
		B to A	27.0	

*A=apical; B=basal.

Conclusion

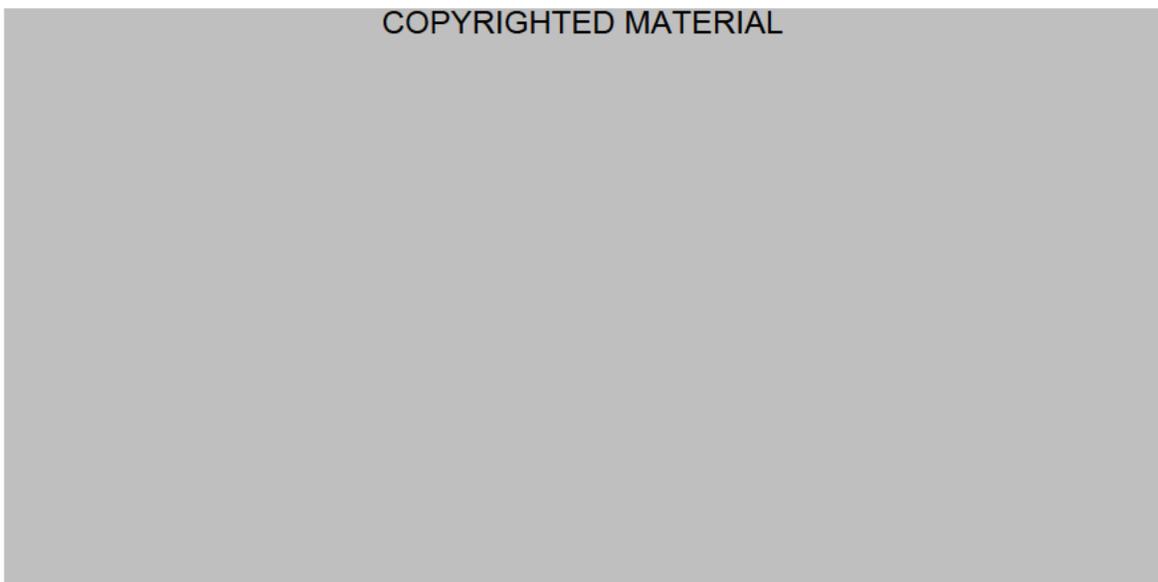
HHT appears to have low permeability and is subject to P-gp efflux. DMHHT has no measureable permeability nor appears to be is subject to P-gp efflux.

Information on Distribution from the Published Literature.

Two papers have been published investigating the distribution of HHT in animal models. These studies are not GLP. Results pertinent to distribution are discussed below.

Ji X, Liu Y, Lin H, Liu Z. 1982. [Metabolism of Homoharringtonine in rats and mice.] Yao xue xue bao [Acta Pharmaceutica Sinica].17(12): 881-888.

(note: translation assumed to have been performed by applicant) 200 µCi/kg ³H-HHT (unknown purity) was given by IV in normal rats and tumor bearing mice. Radioactivity was determined in individual rat tissues at 15 min, 2 h, 24 h post IV infusion (2 animals/group). Specific values are not reported but a bar graph is shown (from original paper):



The y-axis is cpm·g/tissue x 10⁴. The x-axis is tissue type (from left to right: marrow, kidney, liver, lung, spleen, heart, GI tract, muscle, blood, brain).

Values in tumor bearing mice (2 animals/group) after IV injection are given in a translated table (from applicant's submission):

Tu-mor	Time after drug given	Radioactivity of the drug (10 ⁴ cpm/g or ml of wet tissue)													
		Liv-er	Kid-ney	Slv-gld	Stm and int	Mar-row	Lng	Spln	Hrt	Bld	Mus-cle	Tu-mor	Brn	Stm and int.	Content of stm and int**
Lws src	15 min	12.1	11.1	7.3	5.8	5.5	4.4	3.8	3.7	3.5	2.9	2.1	1.0	7.5	23.7
	2 g	2.7	2.1	1.8	2.0	3.5	1.1	1.2	1.0	1.6	1.2	1.2	0.3	3.6	40.7
180 lng cnc	15 min	10.9	8.6	4.7	1.8	9.7	6.0	5.2	3.9	3.1	3.5	2.6	1.3	4.0	30.7
	2 h	2.0	1.8	2.1	1.1	3.4	2.1	1.5	0.5	0.5	0.7	0.9	0.3	1.6	40.4

* 10⁴ cpm/total tissues; ** 10⁴ cpm/total contents

Abbreviations: Lws scr = Lewis sarcoma; 180 lng cnc = 180 lung cancer; Stm and int = stomach and intestines; Lng = lung; Spln = spleen; Hrt = heart; Bld = blood; Brn = brain

Distribution was also determined in tumor-bearing mice by whole body radiography with the following results (from the applicant's submission):

Tumor	Time after drug given	OD (average value ± SD)						
		Liver	Intestines (contents)	Kidneys	Heart	Salivary gland	Spleen	Bladder (urine)
Lewis lung cancer	15 min	3.06±0.10	2.27±0.04	1.87±0.15	0.58±0.14	0.49±0.01	0.31±0.02	-
	2h	0.41±0.01	4.02±0.13	-	-	0.36±0.03	-	3.68±0.04
Sarcoma 180	15 min	1.81±0.21	1.41±0.12	-	0.77±0.13	1.27±0.05	0.47±0.04	-
	2h	0.49±0.06	2.47±0.06	-	-	0.73±0.09	-	3.48±0.12

HHT appears to preferentially distribute to the bone marrow (in rats, at least), liver, and kidneys in rodents

Lu K, Savaraj N, Feun LG, Zhengang G, Umsawasdi T, Loo TL. 1988. *Pharmacokinetics of homoharringtonine in dogs. Cancer Chemother Pharmacol. 21: 139-142*

200 µCi/kg ³H-HHT (radiochemical purity 85%, overall purity unknown) was given by IV in 5 mongrel dogs of either sex. Radioactivity was determined in individual tissues at 5 h post IV infusion. The following values (% of total radioactivity) were reported:

- 7.4% - liver
- 2.5% - small intestine
- 1% - stomach
- 0.8% - pancreas
- 0.8% - kidney

- 0.7% - lung
- < 0.5% - heart, spleen, large intestine, brain

At 24 h only the liver contained more than 100 ng/g HHT (calculated). At 72 h no HHT was found in the tissues. It does not appear that intestinal contents were analyzed.

2.6.4.5 Metabolism

Study title: Stability of Homoharringtonine (HHT) in Human Liver Microsomes: Assessment of the Effect of the Esterase Inhibitor, Phenylmethanesulfonyl Fluoride (PMSF).

Key study findings: HHT does not appear to be metabolized quickly by CYP-mediated oxidative metabolism. HHT does not appear to be metabolized by liver microsome esterases.

Study no.: PTX002

Volume #, and page #: Vol 1, pg 232

Conducting laboratory and location: (b) (4)

Date of study initiation: 2 Aug 2007

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, HHT0505001, 99.8%; 4-demethylated homoharringtonine (DMHHT), 20060321, 99.6%; cephalotaxine (CTX), 9619E, 99.5%

Methods

- Liver, microsomes from male and female human donors were pooled.
- HHT was incubated with microsomes with and without the esterase inhibitor, PMSF.
- Procaine (with and without PMSF), dextromethorphan and verapamil (without PMSF) were used as positive controls.
- NADPH was added to all samples at 0 min. A NADPH-negative control was also used.
- Incubations were for 0, 5, 15, 30, and 45 min (45 min only for control)
- 4 samples for each treatment were used and pooled for analysis

Results

- Results from control incubations indicate that the microsomes contain esterase activity and are otherwise, and generally, metabolically active.
 - Procaine concentration was 56.5% of initial without PMSF and 94.8% of initial with PMSF at 45 min.
 - Clearance of procaine was 26 $\mu\text{L}/\text{min}/\text{mg}$ protein without PMSF and 8 $\mu\text{L}/\text{min}/\text{mg}$ protein with PMSF.

- Clearance of dextromethorphan was 35 $\mu\text{L}/\text{min}/\text{mg}$ protein.
- Clearance of verapamil was 192 $\mu\text{L}/\text{min}/\text{mg}$ protein.
- Clearance of HHT was 3.36 and 7.12 $\mu\text{L}/\text{min}/\text{mg}$ protein in the absence and presence of PMSF, respectively.
- The percentage of test compound after 45 min incubation was 105% with or without PMSF.
- There appears to be a slight increase in the amount of DMHHT in the HHT-without-PMSF treated sample. The ratio of DMHHT peak area to internal standard peak area was 0.00229 at 0 min and 0.00574 at 45 min.

Conclusion

HHT does not appear to be metabolized quickly by CYP-mediated oxidative metabolism. HHT does not appear to be metabolized by liver microsome esterases.

Study title: Study to Investigate the Potential of Homoharringtonine and 4-Demethylated Homoharringtonine to Inhibit or Induce Human Cytochrome P450.

Key study findings: HHT showed weak inhibition of 1A and 2D6 isozymes and strong inhibition of the 3A4 isozyme; DMHHT showed weak inhibition of 1A, 2C19, and 2D6 isozymes. HHT showed possible weak induction of the 1A isozyme. DMHHT also appeared to weakly induce the 3A4 isozyme.

Study no.: PTX003

Volume #, and page #: Vol 1, pg 247

Conducting laboratory and location: ^(b)

Date of study initiation: 12 Sept 2007

GLP compliance: No

QA report: yes () no (x)

Drug, lot #, and % purity: HHT, 07758, 99.8%; 4-demethylated homoharringtonine (DMHHT), 20060321, 99.6%

Methods

- For IC_{50} determination
 - 0.02, 0.1, 0.2, 1, 2, and 10 μM HHT or DMHHT were incubated with human liver microsomes (0.25 mg/mL) and NADPH (1 mM) in the presence of the appropriate substrate for CYP1A, CYP2C9, CYP2C19, CYP2D6, for CYP3A4 (substrates were, respectively, 0.5 μM ethoxyresorufin for 5 min, 120 μM tolbutamide for 60 min, 25 μM S-mephenytoin for 60 min, 5 μM dextromethorphan for 30 min, or 2.5 μM midazolam for 5 min) at 37°C.
 - number of samples not given for test substance samples. Positive controls used n=7.

- reactions were stopped by methanol and internal standard (except for CYP1A where the metabolite was determined by fluorescence) and analyzed by LC/MS/MS.
- The following positive controls were used:

	Control for Inhibition
CYP1A	a-naphthoflavone
CYP2C9	sulphaphenazole
CYP2C19	tranylcypromine
CYP2D6	quinidine
CYP3A4	ketoconazole

- For mechanism based inhibition
 - Substrates were the same as those used for IC₅₀ determination except diclofenac was used for CYP2C9.
 - human liver microsomes were pre-incubated with 10 μM test compound or DMSO. After 30 min, dilutant, substrate and NADPH were added and sample analyzed.
 - The following comparative inhibitors were used at 10 μM:

	Control for Inhibition
CYP1A	furaflyline, resveratrol
CYP2C9	tienilic acid
CYP2C19	ticlopidine
CYP2D6	paroxetine
CYP3A4	mibefradil hydrochloride, mifepristone

- For induction
 - Only CYP1A and CYP3A4 were investigated.
 - Substrates were the same as those used for IC₅₀ determination..
 - Isolated human hepatocytes from the (b) (4) were seeded and cultured before incubation with the treatment. Replacement of medium occurred every 24 h. Substrate was added after 72 h for 30 for CYP3A4 and 1 h for CYP1A.
 - HHT and DMHHT were incubated at 0.01, 0.1, and 1 μM (n=3)
 - The following comparative inhibitors were used:

	Control for Inhibition
CYP1A	omeprazole (50 μM)
CYP3A4	dexamethasone (50 μM), rifampicin (1 μM)

Results

- Results from control incubations indicate that the microsomes/hepatocytes were responding normally.
- IC₅₀ values for HHT or DMHHT were all above 10 μM
 - 37.5% inhibition at 10μM for CYP2C19

- 27.3% inhibition at 10µM for CYP2C19
 - 30.4% inhibition at 10µM for CYP2C19
 - no inhibition for CYP1A and CYP2C9
- % inhibition of CYP isozymes by HHT from the mechanism based inhibition assay are below

	% Inhibition by HHT	SD
CYP1A	10.7	2.5
CYP2C9	1.30	8.2
CYP2C19	-0.159	N/A (n=1)
CYP2D6	18.2	6.2
CYP3A4	74.3	1.3

- % inhibition of CYP isozymes by DMHHT from the mechanism based inhibition assay are below

	% Inhibition by DMHHT	SD
CYP1A	6.89	1.3
CYP2C9	0.581	9.8
CYP2C19	16.9	3.2
CYP2D6	12.6	7.6
CYP3A4	-2.27	3.1

- Induction of CYP1A by HHT, DMHHT, and omeprazole is below

	Concentration (µM)	Fold induction
omeprazole	50	5.210
HHT	0.01	0.914
HHT	0.1	0.835
HHT	1	1.200
DMHHT	0.01	0.900
DMHHT	0.1	0.932
DMHHT	1	0.901

- Induction of CYP3A4 by HHT, DMHHT, and omeprazole is below

	Concentration (µM)	Fold induction
dexamethasone	50	3.56
rifampicin	1	5.16
HHT	0.01	1.05
HHT	0.1	0.194
HHT	1	0.0084
DMHHT	0.01	1.41
DMHHT	0.1	1.26

DMHHT	1	1.30
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Conclusion

Neither HHT nor DMHHT showed strong inhibition of the CYP isozymes assayed in the reversible inhibition assay.

In the irreversible inhibition assay with preincubation HHT showed weak inhibition of 1A and 2D6 isozymes and strong inhibition of the 3A4 isozyme; DMHHT showed weak inhibition of 1A, 2C19, and 2D6 isozymes.

HHT showed possible weak induction of the 1A isozyme at 1 μ M. The decreased values for both 1A and 3A4 by HHT (at the lower concentrations for 1A) correlate with the information on irreversible inhibition above. DMHHT also appeared to weakly inhibit 1A and weakly induce 3A4 in the induction assay, consistent with the irreversible inhibition assay results.

2.6.4.6 Excretion

Information on Excretion from the Published Literature.

Two papers have been published investigating the excretion of HHT in animal models. These studies are not GLP. Results pertinent to distribution are discussed below.

Ji X, Liu Y, Lin H, Liu Z. 1982. [Metabolism of Homoharringtonine in rats and mice.] Yao xue xue bao [Acta Pharmaceutica Sinica].17(12): 881-888.

(note: translation assumed to have been performed by applicant) 200 μ Ci/kg 3 H-homoharringtonine (unknown purity) was given by IV in normal rats. Excretion in urine and feces was investigated. Bile was also collected by catheterization of the bile duct and analyzed. Total radioactivity recovered in the urine and feces by 24 h was 42.2 and 6.3%, respectively, of the administered dose. The amount of radioactivity as HHT in the urine and feces was 14.8 and 1.1%, respectively, of the total dose. At 48 h, bile excreted accounted for 57.7% of the total administered dose with and 20.2% of the administered dose as HHT. 41% of the dose excreted in the bile was excreted in the first 30 min. Original figures with translation are below:

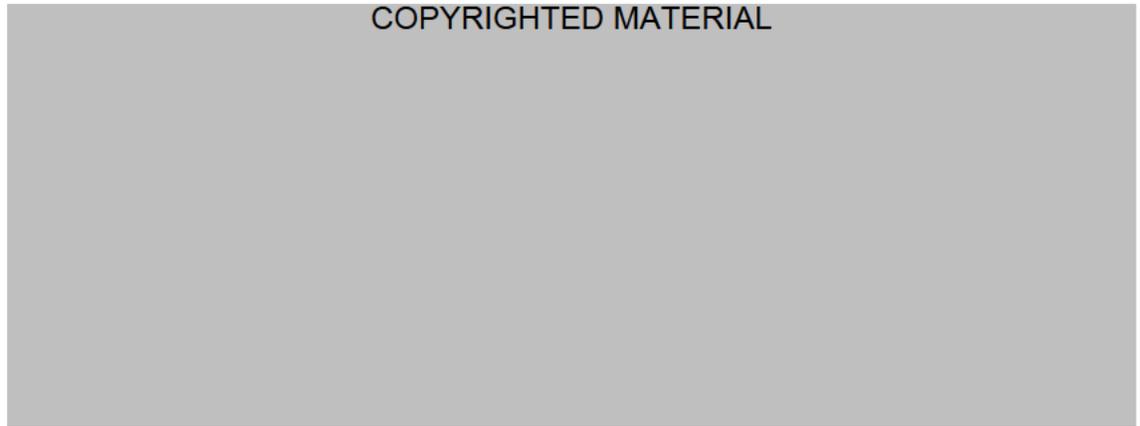


Figure 4. Excretion of Intravenously Injected ³H-Homoharringtonine in Rat Urine and Feces

x-----x	total radioactivity in urine
o o	radioactivity of original from of drug in urine
Δ Δ	total radioactivity in feces
• •	radioactivity of original from of drug in feces

[vertical axis]: Radioactivity corresponding injected drug %
 [horizontal axis]: Time after injection (h)

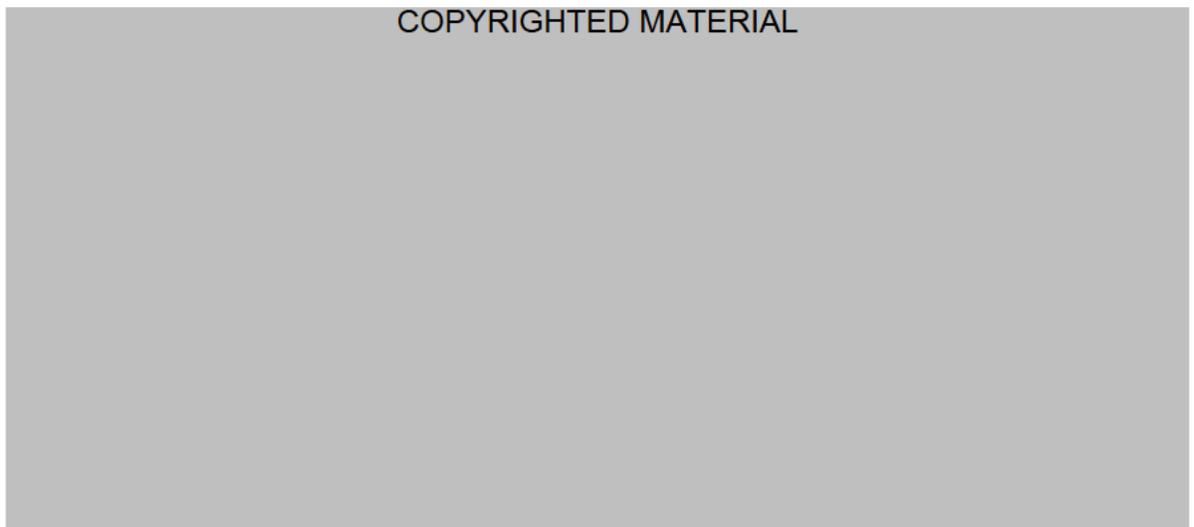


Figure 5. Excretion of Intravenously Injected ³H-Homoharringtonine in Rat Bile

x-----x	total radioactivity in bile
o o	radioactivity of original from of drug in bile

[vertical axis]: Radioactivity corresponding injected drug %
 [horizontal axis]: Time after injection (h)

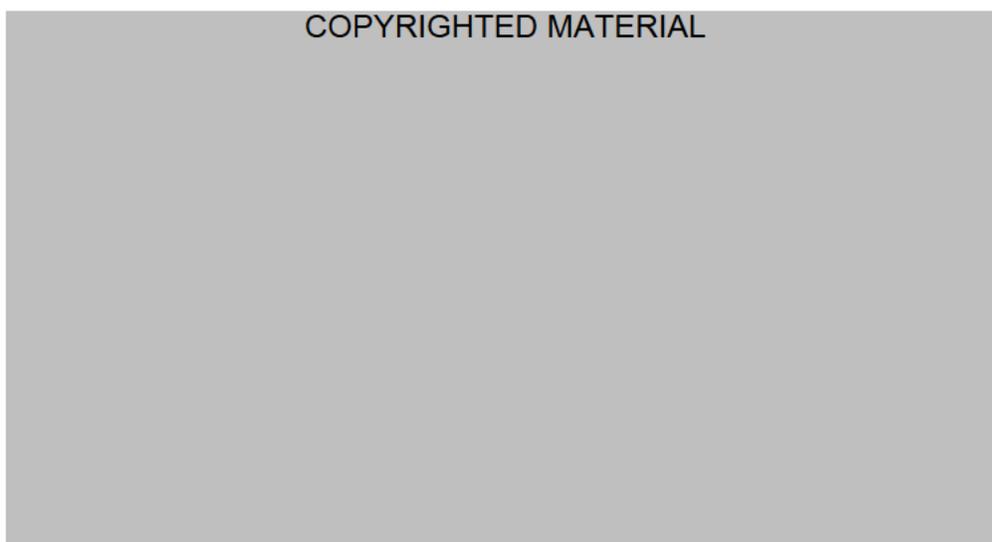
HHT and metabolites appear to be excreted significantly by both the urine and the bile. The amounts found in the intestines and in the bile at early timepoints

suggest that excretion by way of the liver and into the bile is a rapid process in the rat. The majority of radioactivity excreted was in a metabolized form.

Lu K, Savaraj N, Feun LG, Zhengang G, Umsawasdi T, Loo TL. 1988. Pharmacokinetics of homoharringtonine in dogs. Cancer Cehmother Pharmacol. 21: 139-142

200 µCi/kg ³H-HHT (radiochemical purity 85%, overall purity unknown) was given by IV to 5 mongrel dogs of either sex. Radioactivity was determined over 72 h post IV infusion. The following values were reported:

- 40% of the radioactive dose was excreted in urine over 72 hr.
- 18% of the radioactive dose was excreted as HHT in urine over 72 hr.
- The value of radioactive dose excreted in the bile was not reported but appeared to be similar to that of non-metabolized HHT in the urine according to the following figure from the paper:



Excretion was primarily by urine but excretion through bile was also significant. Metabolites made up the largest fraction of excreted material.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Study Title	Study Number	Test system	Results
Toxicokinetic Report: Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in CD-1 Mice	PTX022	CD-1 mice	day 14 AUCs for males: 0.29 mg/kg/dose – 100 h·ng/mL 0.585 mg/kg/dose – 93.4 h·ng/mL 1.165 mg/kg/dose – 209 h·ng/mL day 14 AUCs for females: 0.29 mg/kg/dose – 99.3 h·ng/mL 0.585 mg/kg/dose – 110 h·ng/mL

			<p>1.165 mg/kg/dose – 230 h·ng/mL</p> <p>T_{max} at 0.5 h. Washout by 12 h.</p> <p>Possible induction of metabolism at high doses.</p>
<p>Toxicokinetic Report: Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in Beagle Dogs</p>	PTX018a	Beagle dogs	<p>day 14 AUCs for males: 0.0125 mg/kg/dose – 5.48 h·ng/mL 0.0250 mg/kg/dose – 19.3 h·ng/mL 0.0500 mg/kg/dose – 37.7 h·ng/mL day 14 AUCs for females: 0.0125 mg/kg/dose – 0.633 h·ng/mL 0.0250 mg/kg/dose – 5.11 h·ng/mL 0.0500 mg/kg/dose – 22.5 h·ng/mL</p> <p>T_{max} 0.5-2 h. Washout by 12 h.</p> <p>Possible induction of metabolism initially but increased exposure likely due to decreased metabolism/clearance after day 70.</p>
<p>Stability of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples</p>	PTX024	<i>in vitro</i>	<p>Stability in plasma: rat << dog < monkey < human</p> <p>HHT in dog, monkey, and human plasma within 20% of initial after 1 h. HHT not detected in rat serum.</p>
<p>Stability of Homoharringtonine in Mouse Plasma Samples</p>	PTX026	<i>in vitro</i>	<p>HHT in mouse plasma decreased 39-51% after 1 h</p>
<p>Protein Binding of Homoharringtonine in Human, Monkey, Dog and Rat Plasma Samples</p>	PTX006	<i>in vitro</i>	<p>HHT protein binding in plasma: dog (~2% bound) monkey (~20% bound) human (~45% bound)</p>
<p>Protein Binding of Homoharringtonine in Mouse Plasma Samples</p>	PTX007	<i>in vitro</i>	<p>HHT protein binding in plasma: dog (~30% bound)</p>
<p>Study to Investigate the Permeability and P-gp mediated efflux of Homoharringtonine and 4-Demethylated Homoharringtonine in MDRI-MDCK cells.</p>	PTX001	<i>in vitro</i>	<p>HHT - low permeability, P-gp efflux. DHHT - no measureable permeability, no P-gp efflux.</p>
<p>Metabolism of Homoharringtonine in rats and mice</p>	Ji <i>et al.</i> 1982	rats and tumor bearing mice (<i>in vivo</i>)	<p>Highest HHT concentrations in bone marrow, kidney, liver.</p> <p>Excretion by urine and bile with metabolites the largest fraction of excreted material</p>
<p>Pharmacokinetics of homoharringtonine in dogs</p>	Lu <i>et al.</i> 1988	mongrel dogs (<i>in vivo</i>)	<p>Highest HHT concentration was in the liver, small intestine, stomach and kidney. By 72 h no HHT was found in the tissues.</p>

			Excretion by urine (primary) and bile with metabolites the largest fraction of excreted material
Stability of Homoharringtonine (HHT) in Human Liver Microsomes: Assessment of the Effect of the Esterase Inhibitor, Phenylmethanesulfonyl Fluoride (PMSF).	PTX002	human liver mcirosomes	HHT not quickly metabolized by CYP isozymes nor at all by liver microsome esterases.
Study to Investigate the Potential of Homoharringtonine and 4-Demethylated Homoharringtonine to Inhibit or Induce Human Cytochrome P450.	PTX003	human liver microsomes	HHT: weak inhibitor of 1A, 2D6; strong inhibitor of 3A4; weak induction of 1A DMHHT: weak inhibitor of 1A, 2C19, 2D6; DMHHT weak induction of 3A4

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

No studies reviewed. Findings in single dose studies were limited to findings also noted in repeat dose studies reviewed elsewhere in this review. The only exception were additional cardiac findings in Beagle dogs (Study PTX013) after single 0.32 mg/kg I.V. dosing of HHT in 5% ethanol. Findings included rapid heart beats, weak pulse, sinus arrhythmia, ventricular and sinus tachycardia, and premature ventricular contractions. All dogs at 0.32 mg/kg died prematurely or were sacrificed moribund.

2.6.6.3 Repeat-dose toxicity

Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in CD-1 Mice

Key study findings: HHT dose-response relationship for most toxicities was steep, particularly for males. Bone marrow depletion appears to be the primary toxicity but HHT also targeted gastrointestinal organs, skeletal muscle, spleen, thymus, adrenals, skin, pancreas and liver. Hyperglycemia was also noted. Some findings, including increased heart weight, black stomach and intestinal contents in early death animals, and BUN/creatinine variations had no correlates in this study.

Study no.: PTX019 and PTX022 (PTX022 is an interim report)

Volume #, and page #: Vol 4, pg 2 and Vol 2, pg 11, for PTX019 and PTX022, respectively

Conducting laboratory and location:

(b) (4)

Date of study initiation: 28 Jun 2007

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Homoharringtonine, 05D08, 99.9%

Methods

Doses: vehicle, 0.29, 0.585, 1.165 mg/kg/dose (0.58, 1.17, 2.33 mg/kg/day).

Species/strain: mouse/Hsd:ICR (CD-1[®])

Number/sex/group: 30 mice/sex/dose

Route, formulation, volume: subcutaneous injection in the back (injections rotated over 4 sites), 0.9% NaCl, 5 mL/kg

Satellite groups used for toxicokinetics or recovery: 12/sex/group extra animals were used for TK.

Age: 5-8 wks

Weight: 19.8-32.8 g

Schedule: six cycles of twice daily (~12 h between doses) for 14-days with 14 subsequent days of non-dosing between dosing periods. 5/sex/dose were euthanized on days 15, 30 and 216. 15/sex/dose were euthanized over days 155 and 156.

TK sampling times: 3 mice/sex in HHT treatment groups on days 1 and 14 at pre-dose, 0.5, 1, 2, 4, 8, 24 h post-dose. 3 mice/sex from the vehicle group on days 1 and 14 at pre-dose and 2 h post-dose.

Unique study design or methodology (if any): The HD was lowered to 1.0 mg/kg/dose from the P.M. dose on day 35 (the 7th day of dosing in the 2nd cycle). The HD was further lowered to 0.835 mg/kg/dose from the P.M. dose on day 93 to the end of study (the 9th day of dosing in the 4th cycle to the end of study). The changes were due to signs of general toxicity and unscheduled deaths. The metabolites demethylhomoharringtonine (DMHHT) and cephalotaxine (CTXOH) were also assayed for as part of TK analysis.

Method validation:

The validation of the methods for determination of HHT, demethylhomoharringtonine, and cephalotaxine in dog plasma samples was reported in the applicant's GLP study # PTX016, *An LC/MS/MS method for the determination of homoharringtonine, demethylhomoharringtonine, and dephalotaxine in mouse sodium heparin plasma samples*. No interference was seen in the assays. The following are the results of that validation:

	homoharringtonine	demethylhomoharringtonine	cephalotaxine
Validated Range (ng/mL)	1.00-128	0.500-64.0	0.300-38.4

Linearity (r ²)	0.9976-0.9994	0.9978-0.9990	0.9986-0.9994
Precision for samples low, med., high (% coefficient of variation)	intra-assay 0.710-6.55	intra-assay 0.922-8.47	intra-assay 1.39-13.4
	inter-assay 1.77-5.78	inter-assay 4.40-9.77	inter-assay 2.00-10.3
Accuracy for samples low, med., high (% of nominal)	intra-assay 97.5-105	intra-assay 90.4-109	intra-assay 99.0-108
	inter-assay 99.5-103	inter-assay 95.3-104	inter-assay 100-103
Mean Recovery	100%	101%	102%
Stable in dog sodium heparin plasma at room temperature for 6 h	✓	✓	✓
Stable in dog sodium heparin plasma for 3 freeze-thaw cycles	✓	✓	✓

Observations and times:

Mortality: twice daily
Clinical signs: twice daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: pre-study and before necropsy
Hematology: before necropsy for 3/sex/group on days 14, 30, and at least 60 days after the last cycle. 10/sex/group on day 155-6. Before necropsy if possible for unscheduled sacrifices.
Clinical chemistry: in fasted state before necropsy for 2/sex/group on days 14, 30, and at least 60 days after the last cycle. 5/sex/group on day 154. Before necropsy if possible for unscheduled sacrifices.
Urinalysis: not done
Gross pathology: at necropsy
Organ weights: at necropsy
Histopathology: for days 15 and 30, at necropsy for all tissues (see histopathology table) for control animals, HD animals, and unscheduled deaths. Target tissues as determined by study pathologist and those with gross lesions were also inspected in LD and MD animals.

for days 155/156 and 216, at necropsy for all tissues (see histopathology table) for control animals, HD animals, and unscheduled deaths. Gross lesions in LD and MD animals were also inspected.

adequate battery: yes
peer review: no

Results

Mortality:

- One control female died of accidental causes on day 2 and one on 153. The accidental deaths are not further characterized.
- One HD female died of accidental causes on day 155 (4F221). The accidental death is not further characterized.
- The cause of death for animal 2M56 (LD group) is unknown.
- See table below in clinical signs section for details of other deaths. There were 16 deaths in HD males and 6 deaths in HD females (excluding 4F221).

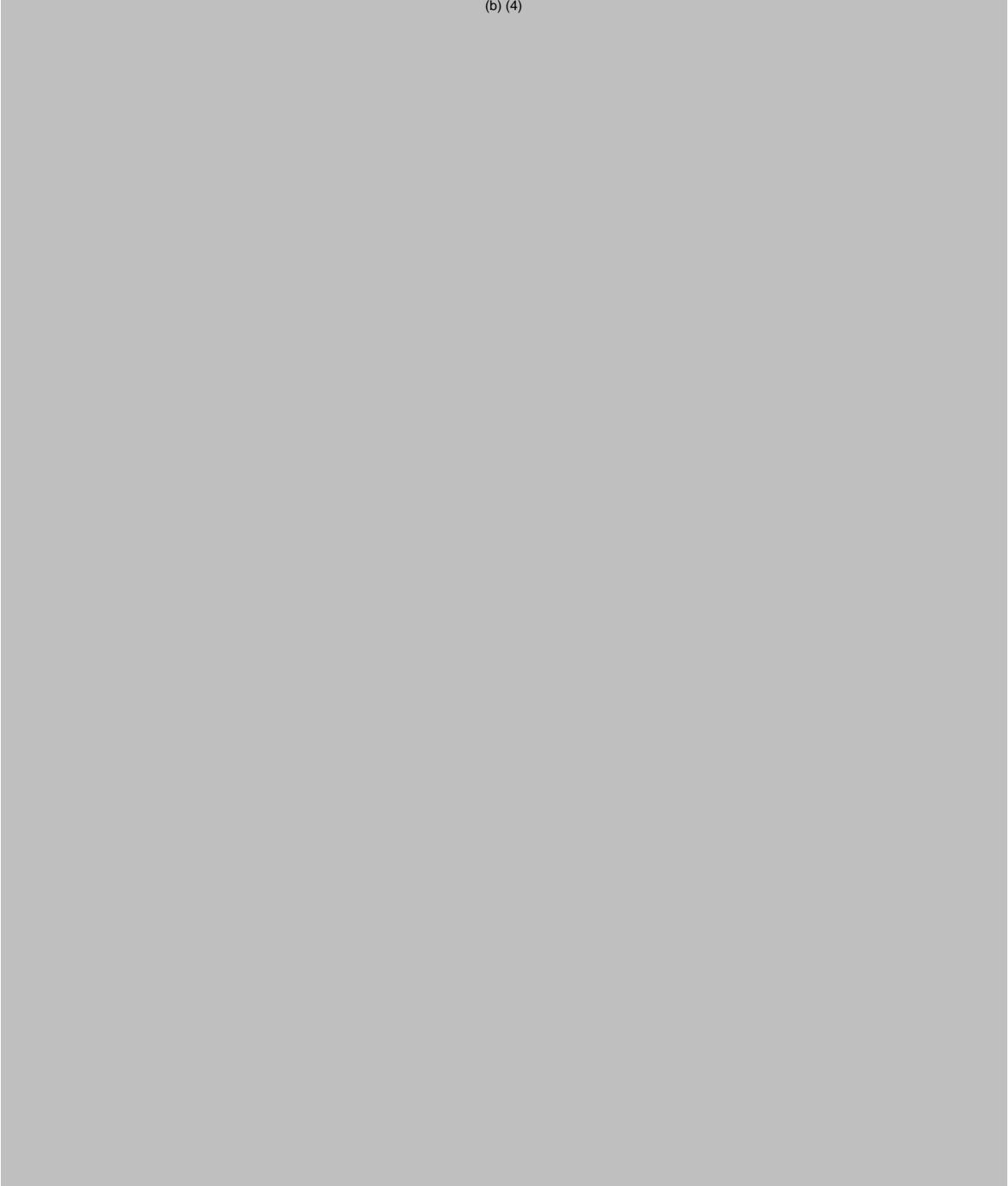
Clinical signs: unkempt appearance and dehydration were noted sporadically but more often in HHT treated animals. Other significant signs were only seen more often in animals with unscheduled deaths. Details are in the table below. Dosage refers to the last dosage before death.

Animal #	Signs	Dose (mg/kg/dose)	Death type	Day
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(b) (4)



Body weights: unremarkable

Food consumption: unremarkable

Ophthalmoscopy: unremarkable

EKG: not done

Hematology:

% Changes in hematology parameters in males compared to control

Parameter	LD Day 15	LD Day 30	LD Day 155	MD Day 15	MD Day 30	MD Day 155	HD Day 15	HD Day 30	HD Day 155
WBC	-23		-37	-40		-56	-78		-67
Neut	-41		-37	-35		-59	-79		-56
Lymph	-22	-21	-38	-45	-50	-55	-80	-75	-69
Eos	100		-50	60		-70	-40		-70
RBC	-18		-21	-25		-33	-28		-41
HGB	-21		-23	-27		-35	-29		-43
HCT	-18		-18	-30		-33	-33		-42
Retic	+120	-3	+86	-55	+44	-46	-98	+64	-86

*Note: Day 30 is the end of the first cycle treatment free period. Day 155 is the end of study dosing.

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% Changes in hematology parameters in females compared to control

Parameter	LD			MD			HD		
	Day 15	Day 30	Day 155	Day 15	Day 30	Day 155	Day 15	Day 30	Day 155
WBC	-45	-9		-50	-39		-76	-45	
Neut	-27	+38		-33	-32		-82	-30	
Lymph	-47	-14		-56	-40		-77	-49	
Eos	-75	-37		-33	-39		-88	-96	
RBC	-13		-13	-34		-24	-42		-33
HGB	-11		-17	-37		-32	-43		-39
HCT	-10		-11	-34		-27	-44		-36
Retic	+85		+168	+101	+16	+198	-98	+47	+7
MCH			-4			-9			-9
MCHC			-6			-7			-5

- Findings at the end of final recovery (day 216) were unremarkable
- A reticulocyte response to decreased RBC/HB/HCT is seen at lower doses immediately but at higher doses only after recovery.
- All types of WBCs were recoverable in males with the exception of lymphocytes which were only recoverable after the final recovery period.
- All types of decreased WBCs were not recoverable in females in the first cycle but were normal by the end of the last cycle.
- The combination of slightly lowered MCH and MCHC in females at the end of the last cycle of dosing suggests decreased HGB production from slight iron deficiency or other cause.

Clinical chemistry:**% Changes in clinical chemistry parameters in males compared to control***

Parameter	LD	MD
	Day 155	Day 155
BUN	-32	-36
TP	-2	-9
GLOB	-5	-14
TBILI	+41	+29

*There were no reported numbers for the one surviving HD male (4M105). The reason is not given.

% Changes in clinical chemistry parameters in females compared to control

Parameter	LD Day 155	MD Day 155	HD Day 155	LD Day 216	MD Day 216	HD Day 216
BUN	-7	-57	-43			
CREAT	+7	+13	+20			
GLU	+16	+71	+127	+33	+62	+111

Urinalysis: not done

Gross pathology:

- **Unscheduled sacrifices**
 - Common for animals with unscheduled deaths were findings of black stomach and intestinal contents, pale brain, some discoloration at treatment sites, and decreased thymus and testes size.

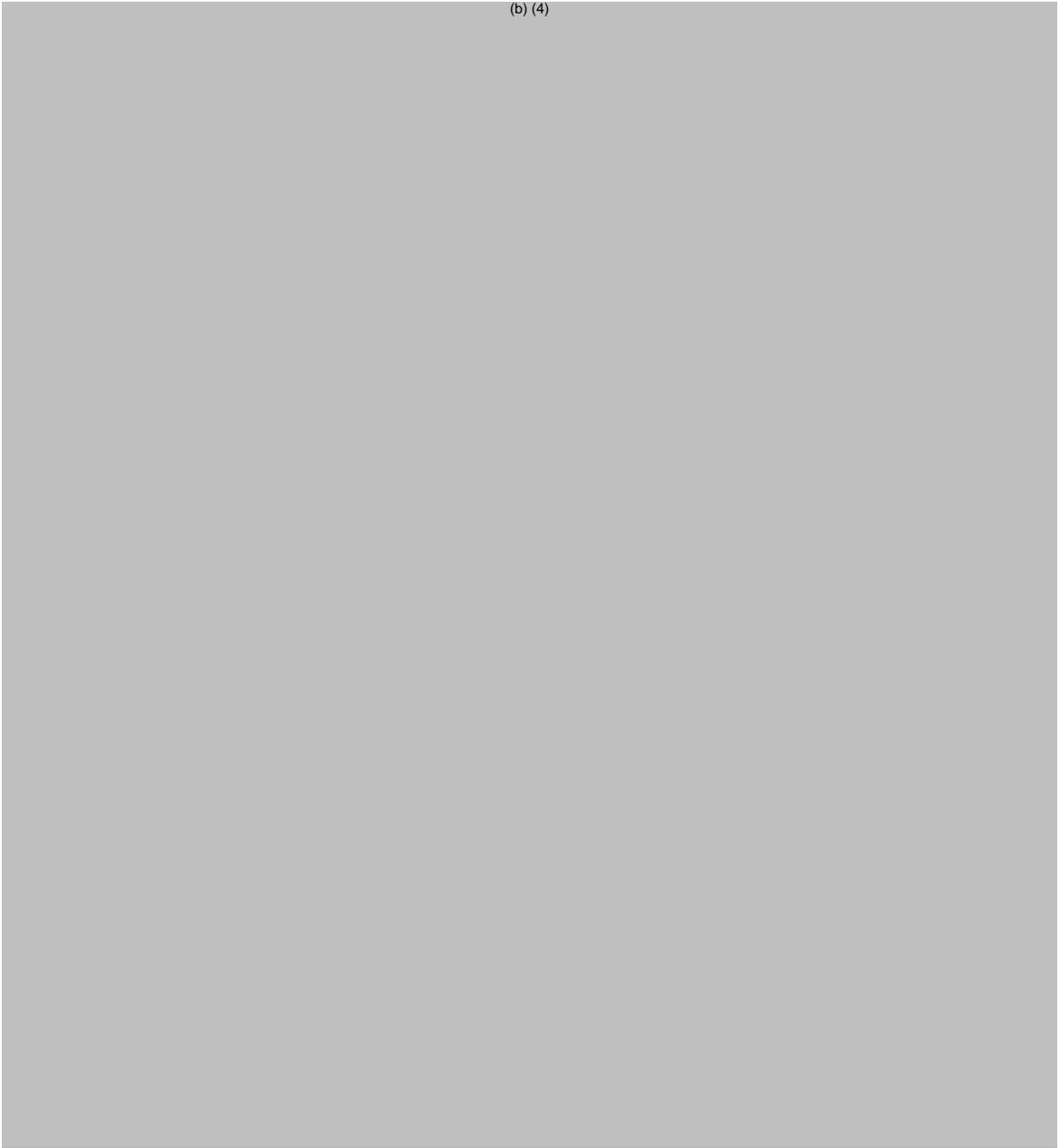
Detailed gross pathology findings for unscheduled deaths

Animal #	Findings	Dose (mg/kg/dose)	Death type	Day
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(b) (4)



(b) (4)



- Scheduled deaths
 - Day 15
 - large spleen in 2/5 MD females
 - pale kidney in 1/5 MD females
 - Day 30
 - small testes in 1/5 MD male
 - small thymus in 1/5 HD male
 - Day 155 (males)
 - unremarkable
 - Day 156 (females)
 - large spleens

- 1/15 in LD
- 8/15 in MD
- 5/8 in HD
- correlates to increased reticulocytes; see clinical chemistry results.
- large thymus
 - 1/15 in LD correlating to
- Day 216
 - unremarkable

Organ weights:

Significant changes in organ weights on Day 15, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart	+21	+45	+34	+7	+18	+17
Kidney					+11	+14
Liver					+20	+29
Testes			-29			
Thymus	-26	-70	-76	-22	-55	-54

Significant changes in organ weights on Day 30, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart	+24	+10	+12		+5	+5
Kidney				+18	+26	+20
Testes		-10	-40			
Thymus		-14	-31	+35	+16	-19

Significant changes in organ weights on Day 155/6, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart	+11	+25	+15	+15	+18	+30
Kidney	+11	+23	+28			+12
Liver	+17	+33	+61	+15	+22	+30
Spleen	+60	+27	-27	+96	+180	+176
Thymus	-20	-36	-29	+92	-42	-51
Testes	-8	-12	-39			
Uterus				-28	-41	-47

Significant changes in organ weights on Day 216, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart	+2	+8	+12		+17	+8
Liver		+12	+16		+12	+17

Spleen			+33		+23	+49
Thymus				-18	-7	-35

- It appears that there is a negative dose-response relationship between HHT and thymus weight and the increased weight seen in the LD average is due to lymphoma of the thymus in one female noted below (and correlated to the large thymus noted under gross pathology above).

Histopathology:

- One low dose female was found to have lymphoma of the spleen and thymus after investigation of gross lesions.
- No correlating lesions were found to explain black contents of the stomach and intestines nor the pale organs (adrenals, brain, kidneys, liver, thyroid/parathoid).
- The results from the necropsy of 4M101 (early death on day 9) are as follows (with severity):
 - Decreased cellularity of the sternum bone marrow (5)
 - Bacteria in the duodenum
 - Lobular hemorrhage in the lung (4)
 - Chronic inflammation (1) and hyperplasia (3) in the non-glandular mucosa of the stomach.
 - Bilateral seminiferous tubular epithelial degeneration (2)
 - Thymic atrophy/involution (5)
- Because of the large numbers of deaths animals with both scheduled and unscheduled deaths are combined in the tables with the exception of animal 4M101 (see above)
- Severity codes in this section have the following correspondence:
 - 1=minimal
 - 2=slight/mild
 - 3=moderate
 - 4=moderately severe
 - 5=severe/high
- VC = vehicle control

Incidence and grade of histopathology findings at day 15 in males

	Type	VC	LD	MD	HD
Number examined		5	5	5	5
Bone marrow (sternum) – decreased cellularity	# affected				5
	avg grade				3.6
Liver – increased centrilobular, hepatocellular cytoplasmic eosinophilia	# affected				4
	avg grade				2.5
Spleen – red pulp cell degeneration/necrosis	# affected				4
	avg grade				1.75
Spleen – lymphoid depletion	# affected				4
	avg grade				2.5
Spleen – increased hematopoiesis	# affected	2	4	4	
	avg grade	1.5	2.3	2.3	
Thymus – atrophy/involution	# affected			5	5
	avg grade			3.8	3.6
Testes – bilateral seminiferous tubular epithelial degeneration	# affected				1
	avg grade				3

Incidence and grade of histopathology findings at day 15 in females

	Type	VC	LD	MD	HD
Number examined		5	5	5	5
Bone marrow (sternum) – decreased cellularity	# affected				4
	avg grade				2.75
Spleen – lymphoid depletion	# affected		2	5	1
	avg grade		2.5	3.4	4
Spleen – red pulp cell degeneration/necrosis	# affected				1
	avg grade				3
Spleen – increased hematopoiesis	# affected		2	5	
	avg grade		2.5	3.4	
Thymus – atrophy/involution	# affected			5	5
	avg grade			3.4	4
Liver – hematopoiesis	# affected			5	
	avg grade			1	
Liver – centrilobular hepatocellular hypertrophy	# affected			2	3
	avg grade			1	1.7

Incidence and grade of histopathology findings at day 30 in males

	Type	VC	LD	MD	HD
Number examined		5	5	5	5
Epididymis – cell detritus in duct lumina, bilateral	# affected				4
	avg grade				2.3
Epididymis – hypospermia/aspermia	# affected			3	2
	avg grade			1	3
Spleen – increased hematopoiesis	# affected		1	5	5
	avg grade		1	2	2.4
Thymus – atrophy/involution	# affected	1			2
	avg grade	2			2.5
Testes – bilateral seminiferous tubular epithelial degeneration	# affected			3	4
	avg grade			1	1.5

Incidence and grade of histopathology findings at day 30 in females

	Type	VC	LD	MD	HD
Number examined		5	5	5	5
Lung – bronchoalveolar cell hyperplasia	# affected				1
	avg grade				2

Incidence and grade of histopathology findings at day 155 in males

	Type	VC	LD	MD	HD
Number examined		15	1		17
Adrenal gland – hypertrophy in the cortical epithelium	# affected				2
	avg grade				2
Bone Marrow (sternum) – decreased cellularity	# affected	1			16 ⁵
	avg grade	5			4.3
Epididymis – cell detritus in duct lumina	# affected				17
	avg grade				3
Epididymis – hypospermia/aspermia	# affected	1			17
	avg grade	4			3.7
Cecum – necrosis/degeneration, mucosal epithelium	# affected				2
	avg grade				2
Colon – necrosis/degeneration, mucosal epithelium	# affected				1
	avg grade				2
Liver – bacterial colonies present	# affected				5
	-				

⁵ For one animal, no section present

Liver – necrosis/degeneration, hepatocellular	# affected				9
	avg grade				1.3
Lung – hemorrhage	# affected	1			5
	avg grade	1			1.2
Lung – bacterial colonies present	# affected				2
	-				
Lymph node (cervical) – erythrocytosis	# affected				3
	avg grade				2.7
Lymph node (mesenteric) – sinus histiocytosis	# affected				6
	avg grade				2.7
Pancreas – necrosis/degeneration, acinar epithelium	# affected				2
	avg grade				1.5
Skeletal muscle (biceps fermoris) – myofiber degeneration	# affected				2
	avg grade				2
Skin – acanthosis	# affected				3
	avg grade				1.7
Skin – atrophy of sebaceous glands	# affected				13
	avg grade				3.6
Skin (treatment site) – atrophy of sebaceous glands	# affected				14
	avg grade				3.8
Skin (treatment site) – hemorrhage, subcutaneous	# affected				3
	avg grade				2.3
Spleen – lymphoid depletion	# affected				16
	avg grade				3
Spleen – increased hematopoiesis	# affected				1
	avg grade				3
Stomach – ulceration	# affected				7
	avg grade				3.1
Stomach – bacterial colonies present	# affected				5
	-				
Stomach – abnormal content, blood	# affected				1
	-				
Testes – degeneration,	# affected	2			17

seminiferous tubular epithelium	avg grade	1.5			2.6
Thymus – involution/atrophy	# affected	7			11
	avg grade	1.4			4.1

Incidence and grade of histopathology findings at day 156 in females

	Type	VC	LD	MD	HD
Number examined		15		8⁶	15
Adrenal gland – vacuolization, cortex	# affected				3
	avg grade				1.7
Bone marrow (sternum) – decreased cellularity	# affected				8 ⁷
	avg grade				3.5
Kidney – bacterial colonies	# affected				1
	-				
Liver – bacterial colonies present	# affected				1
	-				
Liver – hypertrophy, hepatocellular, centrilobular	# affected				11
	avg grade				6
Lung – hemorrhage	# affected	2			1
	avg grade	1			3
Lung – bacterial colonies present	# affected				1
	-				
Skeletal muscle (biceps femoris) – myofiber degeneration	# affected				5
	avg grade				1.4
Skin (treatment site) – hemorrhage, subcutaneous	# affected				2
	avg grade				2.5
Spleen – lymphoid depletion	# affected				6
	avg grade				3
Spleen – increased hematopoiesis	# affected				7
	avg grade				3.1
Stomach – ulceration	# affected				1
	avg grade				4
Stomach – bacterial colonies present	# affected				2
	-				

⁶ Only spleen analyzed for these animals

⁷ Incidence and severity was much greater in animals with unscheduled deaths. For scheduled death animals incidence (severity) was 2 (2). For unscheduled death animals incidence (severity) was 6 (4).

Thymus – involution/atrophy	# affected	3			16 ⁸
	-	2			3.9
Vagina – inflammation	# affected	8			6 ⁹
	avg grade	2.1			1.8

Incidence and grade of histopathology findings at day 216 in males

	Type	VC	LD	MD	HD
Number examined		5			2
Liver – intracytoplasmic brown pigment, centrilobular	# affected				1
	avg grade				2
Liver – necrosis/degeneration, hepatocellular	# affected				1
	avg grade				1

Incidence and grade of histopathology findings at day 216 in females

	Type	VC	LD	MD	HD
Number examined		5			5
Lymph node (cervical) – erythrocytosis	# affected				1
	avg grade				3

Toxicokinetics:

- The HHT metabolite, DMHHT was found at lower levels than HHT (roughly 1/3) with a similar TK profile. DMHHT, however, was found at the 24 h timepoint in two high dose females at 0.551 and 0.665 ng/mL on day 1 and one LD female at 0.976 ng/mL on day 14.
- The HHT metabolite, CTXOH was found sporadically at timepoints ≤ 4 h at levels less than 1 ng/mL

Data on HHT toxicokinetic parameters are below in tabular and graphical form.

HHT Toxicokinetic Parameters

Dose (mg/kg/dose)	Day	Sex	T _{max} (h)	C _{max} (ng/mL)	AUC _{all} (h·ng/mL)	C _{max} /dose	AUC _{all} /dose
0.290	1	M	0.5	30.3	84.2	104	290
0.585	1	M	0.5	72.4	166	124	284
1.165	1	M	0.5	215	343	185	294
0.290	1	F	0.5	32.2	91.3	111	315
0.585	1	F	0.5	88.7	157	152	268

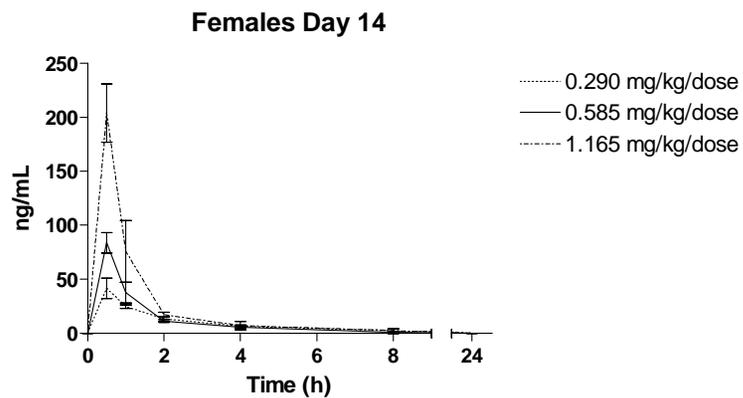
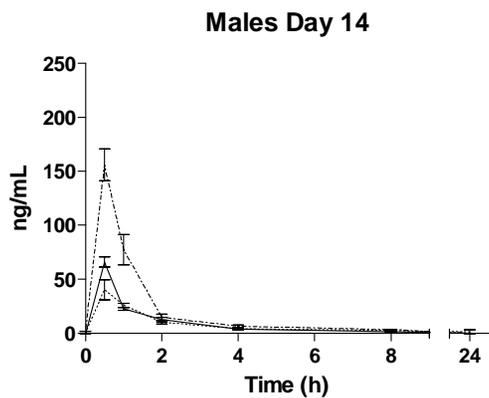
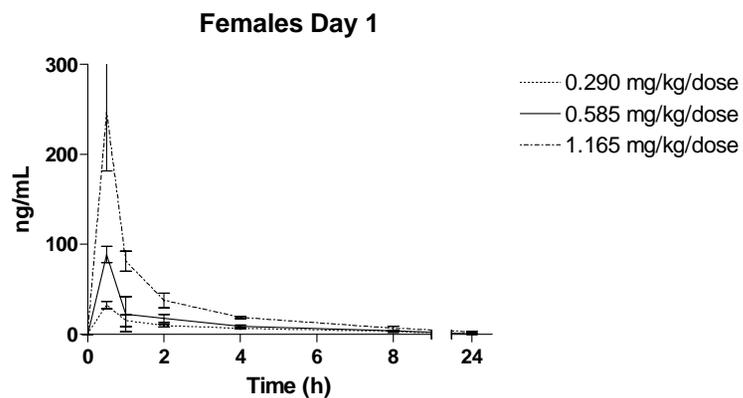
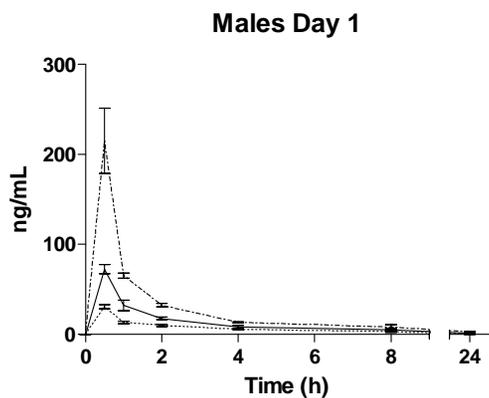
⁸ For one animal, no section present.

⁹ While less than that seen in controls, vaginal inflammation was seen exclusively in HD animals with early deaths.

1.165	1	F	0.5	247	380	212	326
0.290	14	M	0.5	40.4	100	139	345
0.585	14	M	0.5	66.0	93.4	113	160
1.165	14	M	0.5	156	209	133	179
0.290	14	F	0.5	41.5	99.3	143	342
0.585	14	F	0.5	83.9	110	143	188
1.165	14	F	0.5	204	230	175	197

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HHT Plasma Levels



Conclusion:

CD-1 mice were treated for 6 cycles of 14 days twice daily subcutaneous treatment and 14 days without treatment with the last dosing period followed by 61 days of recovery. Only 3/20 HD males and 12/20 HD females survived the 6 cycles of dosing. The cause of death is not obvious for most early death animals but all had marked bone marrow depletion. Some early deaths had lung hemorrhage and/or bacteria present in multiple organs with no evidence of an inflammatory response, likely due to the bone marrow suppression. There was little histopathological correlation for other findings in the study. Most strikingly, the pale organs such as the brain and adrenal glands, the increased heart weights, and the black contents of the stomach and intestines of early death animals had no histopathological correlates. There is evidence of an iron deficiency, but based on the hematology information the deficiency is minimal and limited to HD females, which does not explain the black contents. Ulceration of the stomach was also found in treated males and females but there was no significant correlation between the animals with ulcerations and black stomach contents. Clinical chemistry suggests impaired glomerular filtration (decreased BUN and increased creatinine), possible cholestasis (increased bilirubin in the absence of hemolysis), but these can not be further characterized with the information from these studies. Hemolysis is ruled out as a cause of bilirubin increase because it is not consistent with the pattern of red blood cell and bilirubin fluctuations. Hyperglycemia did have a correlate in HD females with necrosis/degeneration found in the pancreas but this finding was not also found in male and since no histopathology was performed in the LD and MD for this organ at the end of study, it is unclear if this is the cause of the hyperglycemia seen at all treatment levels. Thymus and spleen were also targeted by HHT. There were adaptive responses in the spleen (increased hematopoiesis) as well as adverse responses (red pulp degeneration and lymphoid depletion). Thymus atrophy/involution is a natural process but it appears that HHT is accelerating the development of this process. There was some evidence of hepatotoxicity but this was minor and was accompanied by possible adaptive changes (e.g. hypertrophy). Other targets included the skin independent from injection sites (acanthosis and atrophy of sebaceous glands) and skeletal muscle (degeneration). Some injection sites also were associated with hemorrhage along with the findings in general skin sections.

Based upon the clinical signs, histopathology at 15 and 30 days as well as the clinical chemistry and hematology results, it appears that a HHT dose-response curve for most toxicities, including death, is steep. The most significant characterized toxicity, bone marrow depletion is moderate to severe at the HD, but does not occur at dose levels half or less than HD the amount.

Toxicokinetic conclusions

At similar dose levels female exposure was generally slightly greater than male exposure and exposure on day 14 was generally lower than on day 1. HHT is absorbed quickly and cleared quickly with the peak concentration occurring at 0.5 h and returning to base line, presumably, by 12 h (see issues regarding interpretation of 24 hr time points below). HHT C_{max} was greater than dose-proportional on day 1 but approximately dose-proportional on day 14. HHT total exposure, as measured by AUC, was approximately dose-proportional on day 1, but less than dose-proportional on day 14. This, combined

with information that day 14 exposure was generally lower than on day 1, may indicate an induction of metabolism by higher doses of HHT.

The AUC values can not be used for cross-species comparisons on a total daily dose basis due to an issue with study design. Namely, the collection of samples was at pre-dose and 0.5, 1, 2, 4, 8, 24 h post-dose while the dosing was twice daily with approximately 12 hr in between dosing. Because an additional dose was given in a large gap in the sampling times, the calculations are likely to slightly overestimate the AUC on a per dose basis at the low dose and severely underestimate the AUC on a per day basis. The AUC value is likely inaccurate because the model used assumes a linear decrease between 8 h and 24 h, a near impossible situation given another dose at ~12 hr. In essence, the 24 h time point is informative only for data on accumulation after 2 doses and not as a time point related to the initial dosing at time 0.

It is not specified in the protocol whether the sampling on day 14 was after the AM or PM dose so while it is acceptable to compare sampling from pre-dose to 8 hours between days, the 24 h time point is additionally unhelpful because it is unknown whether there was a second dosing at 12 h post-dose for day 14 as there was on day 1, regardless of whether time 0 was the AM or PM dosing on day 1. The impact of this unknown could only make it appear that less accumulation was seen at the 24 h time point after 14 days.

The data is appropriate, however, for approximating AUC values on a mg/kg/dose basis because the values at 24 h are almost uniformly low and, importantly, very low relative to values ≤ 4 h.

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Study title: Six Month (Six Cycle) Subcutaneous GLP Toxicity Study of Homoharringtonine (HHT) in Beagle Dogs

Key study findings: The target organs of HHT toxic action were primarily the bone marrow, cardiovascular system (as related to widespread hemorrhage and increases in heart weight), and the lymphoid tissues. The widespread hemorrhage is at least partially due to drastically decreased platelets but does not appear to be wholly responsible. The dose-response relationship of HHT in dogs is relatively steep.

Study no.: PTX018a, PTX023a (PTX023a is the one month interim report for this study)

Volume #, and page #: Vol 11, pg 2

Conducting laboratory and location:

(b) (4)

Date of study initiation: 02 May 2007

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Homoharringtonine, 05D08, 99.9%

Methods

Doses: vehicle, 0.0125, 0.025, 0.05 mg/kg/dose (0.025, 0.05, 0.1 mg/kg/day). 0.05 mg/kg/dose lowered to 0.0375 mg/kg/dose and then 0.03125 mg/kg/dose (see unique study design)

Species/strain: dog/Beagle

Number/sex/group: 10 dogs/sex/group

Route, formulation, volume: subcutaneous injection in the back (injections rotated over 7 sites), 0.9% NaCl, 0.1 mL/kg (0.083 mg/kg/dose for P.M. dose on day 63 through day 70 due to dose reduction. subsequent dosing was at 0.1 mL/kg with reformulated HHT)

Satellite groups used for toxicokinetics or recovery: no additional animals

Age: 5-7 months

Weight: 5.1-8.4 kg

Schedule: six cycles of twice daily (~12 h between doses) for 14-days with 14 subsequent days of non-dosing between dosing periods. 3/sex/dose were euthanized on days 15 and 155. 2/sex/dose were euthanized on days 29 and 187.

TK sampling times: the last 3 dogs/sex in HHT treatment groups on days 1, 14, 57, 70, 141, 154 (first and last dose for 1st, 3rd and last dosing cycles) at pre-dose, 0.5, 1, 2, 4, 8, 12, 24 h post-dose. the last 3 dogs/sex in vehicle group on days 57, 70, 141, 154 (first and last dose for 2nd and last dosing cycles) at pre-dose and 1 h post-dose. See review of the toxicokinetic report from study report PTX018a in the Absorption section 2.6.4.3 for results.

Unique study design or methodology (if any):

3 animals/sex/dose were scheduled for necropsy at the end of the first and last dosing period and 2 animals/sex/dose were scheduled for necropsy at 14 days after the first dosing period and 33 days after the last dosing period. The HD was lowered to 0.0375 mg/kg/dose beginning with the second dosing cycle (day 29) through the AM dose of day 63 (dosing day 7 of cycle 3). The HD was further lowered to 0.03125 mg/kg/dose from the P.M. dose on day 63 through the end of the study. The changes were due to signs of general toxicity and unscheduled deaths. The metabolites demethylhomoharringtonine (DMHHT) and cephalotaxine (CTXOH) were also analyzed for the toxicokinetics section.

Method validation:

The validation of the methods for determination of HHT, demethylhomoharringtonine, and cephalotaxine in dog plasma samples was reported in the applicant's GLP study # PTX014, *An LC/MS/MS method for the determination of homoharringtonine, demethylhomoharringtonine, and cephalotaxine in dog sodium heparin plasma samples*. No interference was seen in the assays.

The following are the results of that validation:

	homoharringtonine	demethylhomoharringtonine	cephalotaxine
Validated Range (ng/mL)	1.00-128	0.500-64.0	0.300-38.4
Linearity (r ²)	0.9984-0.9998	0.9948-0.9998	0.9932-0.9992
Precision for samples low, med., high (% coefficient of variation)	intra-assay 0.420-9.21	intra-assay 1.63-7.76	intra-assay 1.95-6.26
	inter-assay 3.05-6.74	inter-assay 2.92-8.73	inter-assay 3.28-7.71
Accuracy for samples low, med., high (% of nominal)	intra-assay 97.1-107	intra-assay 89.9-104	intra-assay 89.6-109
	inter-assay 101-103	inter-assay 94.5-103	inter-assay 96.4-107
Mean Recovery	98.5%	103%	108%
Stable in dog sodium heparin plasma at room temperature for 6 h	✓	✓	✓
Stable in dog sodium heparin plasma for 3 freeze-thaw cycles	✓	✓	✓

Observations and times:

Clinical signs: twice daily
Body weights: weekly
Food consumption: daily
Ophthalmoscopy: pre-study and before scheduled sacrifice. It was not reported for last cycle recovery animals due to lost reporting forms
ECG: pre-study, day 1, end of dosing and recovery periods. On days 1, 14, 154, ECGs were performed 0.5 h post A.M. dose
Hematology: pre-study, end of dosing and recovery periods, and, if possible, before unscheduled sacrifices
Clinical chemistry (including cardiac troponin I): pre-study, end of dosing and recovery periods, and, if possible, before unscheduled sacrifices
Urinalysis: pre-study and before scheduled sacrifice
Gross pathology: at necropsy
Organ weights: at necropsy
Histopathology: at necropsy for all tissues (see histopathology table) for control animals, HD animals, and unscheduled deaths. Target tissues as determined by study pathologist and with gross lesions were also inspected in LD and MD animals. Determined target tissues were spleen, thymus, cervical and mesenteric lymph nodes, sternal bone marrow, stomach, duodenum, jejunum, ileum, cecum, colon, urinary bladder, liver, heart, lungs, brain, adrenals, pancreas, esophagus, and treatment site.

adequate battery: yes
peer review: no

Results

Mortality:

- There were unscheduled deaths for 2 MD males, 3 HD males, and 4 HD females. The deaths appear, ultimately, to be due to immunosuppression (see other **Results** sections)
- See details on days and types of deaths in the table below in clinical signs.

Clinical signs:

- Clinical signs in HHT treated animals were similar to GI-related signs in unscheduled sacrificed animals but did not occur as often or for as long.

Clinical signs in animals with unscheduled deaths

Animal	Signs	Dose	Death type	Day
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#		(mg/kg/dose)		
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Body weights: unremarkable

Food consumption: occasionally minimally decreased at the high dose

Ophthalmoscopy: unremarkable

EKG: on day 14, MD and HD females had decreased QTc intervals of 8 and 10%, respectively.

Hematology:

% Change from controls for hematology parameters are in the tables below.

Day 84 was included for females as it was the timepoint of greatest change for multiple parameters. Parameters in males followed a roughly linear path downwards over time. For some parameters however, HD male values plateau somewhat; this may be due to the animals with the largest changes at the beginning of the study dying early. See representative graphs below tables for males and females using WBC for illustration.

There were no males for MD recovery analysis as both animals scheduled for recovery had unscheduled deaths.

Low-dose males

Day	13	28	154	187
cycle/period ending	1/dosing	1/recovery	6/dosing	6/recovery
WBC	-12		-25	-14
Neut			-23	-15
Lym	-18		-36	
Mono			-22	-31
Eos		-24	-99	-19
Retic	+12	+20	-28	+66
PLT			-38	-21
MCHC				-3

Mid-dose males

Day	13	28	154
cycle/period ending	1/dosing	1/recovery	6/dosing
WBC	-19		-72
Neut	-100	-100	-71
Lym	-47	-22	-73
Mono	-25	-8	-80
Eos	-17	-36	-100
Bas	-20		-50
RBC	-11		-32
HB	-11		-30
HCT	-12		-27
Retic	-30	+63	+45
PLT			-93
MCHC			-4

High-dose males

Day	13	28	154	187
cycle/period ending	1/dosing	1/recovery	6/dosing	6/recovery
WBC	-38	-56	-61	-64
Neut	-14	-48	-55	-70
Lym	-73	-61	-70	-47
Mono	-58	-74	-67	-70
Eos	-30	-76	-98	90
Bas	-60	-25	-25	-100
RBC	-13	-16	-15	-19
HB	-16	-20	-19	-23
HCT	-17	-18	-15	-17
Retic	-86	+103	-12	
PLT			-96	-88
MCHC			-5	-8

Low-dose females

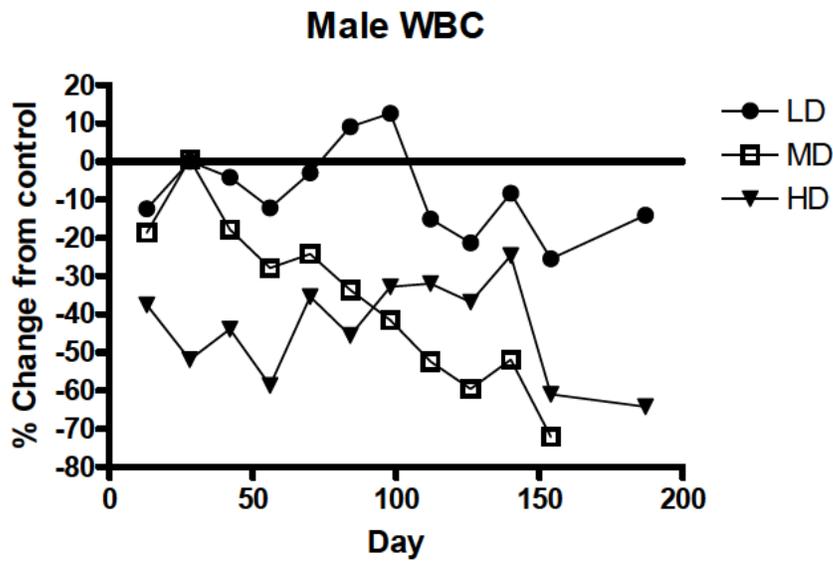
Day	13	28	84	154	187
cycle/period ending	1/dosing	1/recovery	3/recovery	6/dosing	6/recovery
WBC			-18	-12	
Neut			-19		
Lym		-13	-18	-31	
Mono			-22		
Eos	+50			+65	+21
Bas	-14	-14		-25	
Luc	-33			-50	
Retic	-5	+36		+38	
PLT	-20		-20	-35	-30

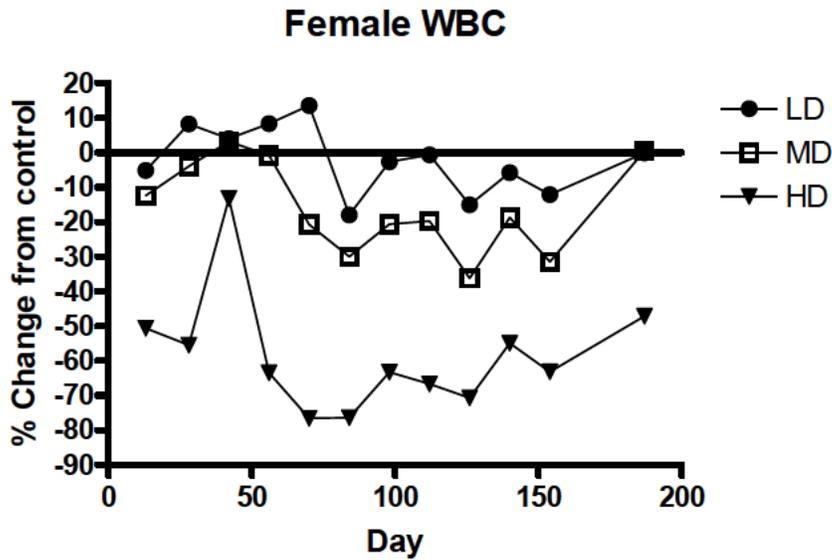
Mid dose females

Day	13	28	84	154	187
cycle/period ending	1/dosing	1/recovery	3/recovery	6/dosing	6/recovery
WBC	-12		-30	-31	
Neut	-4		-28	-23	
Lym	-34	-13	-32	-47	
Mono	-15		-27	-27	
Eos	+133	-17		-35	-61
Bas	-29			-25	
Luc	-33			-50	
RBC	-5		-5		+18
HB	-9		-10		+11
HCT	-9		-8		+12
Retic	-27	+122		+64	+33
PLT	-50		-33	-68	-49

High dose females

Day	13	28	84	154	187
cycle/period ending	1/dosing	1/recovery	3/recovery	6/dosing	6/recovery
WBC	-51	-52	-76	-63	-47
Neut	-40	-44	-78	-61	-48
Lym	-74	-60	-70	-67	-37
Mono	-73	-74	-91	-59	-61
Eos		-50		-69	-89
Bas	-57	-57		-63	
Luc	-67	-50		-75	-50
RBC	-7		-23		+13
HB	-10		-25		+8
HCT	-11		-20		+12
Retic	-85	+170		-20	-21
PLT	-89	-61	-95	-89	-88
MCH	-3	-4		-8	-5
MCHC	1	-3		-4	-4





Clinical chemistry:

While within normal ranges, glucose levels in males were higher at the end of treatment by 17 to 22% at the top two dose levels. Creatinine levels were consistently higher in the treated male groups in a dose responsive manner throughout the study but recovered by day 187. See table below:

% change in creatinine compared to control (males)

Day	42	56	70	84	98	112	126	140	154
LD	-3	-4	-1	-3	-1	1	-5	-5	-7
MD	-5	0	-3	-2	-2	0	-10	-9	-14
HD	-9	-7	-9	-10	-13	-8	-14	-13	-16

% change in BUN compared to control (males)

Day	42	56	70	84	98	112	126	140	154
LD	-15	-13	0	0	-8	-7	-13	0	0
MD	-38	-20	-36	-21	-31	0	-33	-13	-27
HD	-31	-7	-14	-7	-23	0	-27	-7	-27

Findings in females were unremarkable.

Urinalysis: unremarkable

Gross pathology:

Findings in animals with unscheduled deaths

Animal #	Findings	Dose (mg/kg/dose)	Death type	Day
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Organ weights:

Significant changes in organ weights on Day 15, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart					+7	+12
Spleen			-23	-30	-22	-41

Significant changes in organ weights on Day 30, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart						
Spleen	-40	-30	-30		-19	-32

Significant changes in organ weights on Day 155/6, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart	+14	+14	+11		+14	+11
Spleen	-25	-28	-50	-20	-29	-76
Thymus		-23	-33			

Significant changes in organ weights on Day 216, relative to body weight at the end of dosing are displayed in the table below.

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Heart						+25
Spleen			+124		-13	-18
Thymus				-10	-37	-23

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

- At all timepoints, treated animals had fibrosis, inflammation, and hemorrhage in treatment site epidermis and subcutis (and fibrosis and inflammation in the

dermis) with severity increasing with dose. Control animals has some similar findings with much reduced incidence and severity.

- 7 HD males and all but one of the HD females were considered sexually immature at time of necropsy (LD and MD were not examined).
- Severity codes in this section have the following correspondence:
 - 1=minimal
 - 2=slight/mild
 - 3=moderate
 - 4=moderately severe
 - 5=severe/high
 - NE=not examined
- VC = vehicle control

Incidence and grade of histopathology findings at day 15 in males

Type	VC	LD	MD	HD
Number examined	3	3	3	3
Bone marrow (sternum) – depletion	# affected			2
	avg grade			2.5
Cecum – depletion of lymphoid follicles	# affected		1	2
	avg grade		1	1
Cecum – dilation of mucosa glands	# affected	1	2	1
	avg grade	1	1	1
Ileum – lymphoid depletion of Peyer’s patch	# affected	1	1	3
	avg grade	1	2	2
Jejunum – lymphoid depletion of Peyer’s patch	# affected		1	2
	avg grade		1	1
Liver – diffuse, glycogen depletion in hepatocytes	# affected			2
	avg grade			1
Spleen – lymphoid depletion	# affected		3	3
	avg grade		2	2.7
Stomach – increased mucosal goblet cells	# affected		2	1
	avg grade		1	1

Incidence and grade of histopathology findings at day 15 in females

Type	VC	LD	MD	HD
Number examined	3	3	3	3
Bone marrow (sternum) – depletion	# affected		1	3
	avg grade		1	3
Cecum – depletion of lymphoid follicles	# affected		1	1
	avg grade		2	1

Cecum – increased mucosal goblet cells	# affected				3
	avg grade				1
Colon – increased mucosal goblet cells	# affected				2
	avg grade				1
Liver – diffuse, glycogen depletion in hepatocytes	# affected		2	1	1
	avg grade		2	3	2
Lung – chronic/chronic active inflammation	# affected		2	3	3
	avg grade		1	2	1
Lymph node (cervical) – lymphoid depletion	# affected				
	avg grade				
Lymph node (mesenteric) – lymphoid depletion	# affected		2	2	2
	avg grade		1	1	1
Pancreas – zymogen depletion in acinar cells	# affected		1	1	
	avg grade		4	3	
Spleen – lymphoid depletion	# affected		2	1	2
	avg grade		1.5	1	1.5

Incidence and grade of histopathology findings at day 30 in males

	Type	VC	LD	MD	HD
Number examined		2	2	2	3
Bone marrow (sternum) – depletion	# affected				2
	avg grade				3
Brain – hemorrhage, gray matter	# affected				1
	avg grade				1
Brain – hemorrhage, meninges	# affected				1
	avg grade				2
Esophagus – mucosal atrophy	# affected				1
	avg grade				1
Duodenum – mucosal gland dilation	# affected		1	1	1
	avg grade		1	1	1
Ileum – mucosal gland atrophy	# affected				1
	avg grade				2
Ileum – lymphoid depletion of Peyer’s patch	# affected		1	2	2
	avg grade		1	1	2
Jejunum – lymphoid depletion of Peyer’s patch	# affected		1	2	
	avg grade		1	1	
Liver – diffuse, glycogen depletion in hepatocytes	# affected				1
	avg grade				4

Lung – hemorrhage in alveolus	# affected				1
	avg grade				2
Lymph node (cervical) -- congestion	# affected				1
	avg grade				3
Lymph node (cervical) – lymphoid depletion	# affected				2
	avg grade				2
Lymph node (cervical) -- plasmacytosis	# affected				1
	avg grade				2
Lymph node (cervical) – sinus histiocyte pigmentation	# affected				1
	avg grade				2
Lymph node (mesenteric) -- congestion	# affected				2
	avg grade				2.5
Lymph node (mesenteric) – lymphoid depletion	# affected		1	1	2
	avg grade		1	1	2.5
Lymph node (mesenteric) -- hyperplasia	# affected			1	
	avg grade			2	
Lymph node (mesenteric) -- plasmacytosis	# affected				1
	avg grade				2
Lymph node (popliteal) -- congestion	# affected		NE	NE	1
	avg grade				4
Lymph node (popliteal) – lymphoid depletion	# affected		NE	NE	1
	avg grade				3
Lymph node (popliteal) -- plasmacytosis	# affected		NE	NE	1
	avg grade				2
Pancreas – zymogen depletion in acinar cells	# affected				3
	avg grade				1.3
Skin (non-treatment site) – chronic dermal inflammation	# affected				2
	avg grade				1
Stomach – increased mucosal goblet cells	# affected				1
	avg grade				2
Thymus -- hemorrhage	# affected				1 ¹⁰
	avg grade				1
Trachea – chronic	# affected				1

¹⁰ No section for early death animal 4M36

inflammation of the lamina propria	avg grade				1
Spleen – hematopoiesis	# affected				1
	avg grade				2
Spleen – lymphoid depletion	# affected			1	1
	avg grade			1	3

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Incidence and grade of histopathology findings at day 30 in females

	Type	VC	LD	MD	HD
Number examined		2	2	2	3
Adrenals – congestion	# affected				1
	avg grade				1
Bone marrow (sternum) – depletion	# affected				2
	avg grade				5
Bone marrow (sternum) – congestions	# affected				2
	avg grade				3
Brain – hemorrhage, gray matter	# affected				1
	avg grade				1
Esophagus – mucosal atrophy	# affected				1
	avg grade				1
Esophagus – hemorrhage, adventitia	# affected			1	
	avg grade			2	
Gallbladder – autolysis	# affected				1
Heart – myocardial fibrosis	# affected				1
	avg grade				1
Heart – myocardial hemorrhage	# affected				1
	avg grade				1
Heart – pericardial fibrosis	# affected				1
	avg grade				1
Cecum – lymphoid follicle depletion	# affected				1
	avg grade				3
Cecum – increased mucosal goblet cells	# affected				1
	avg grade				2
Cecum – mucosal hemorrhage	# affected				1
	avg grade				2
Colon – lymphoid follicle depletion	# affected				1
	avg grade				3
Colon – increased mucosal goblet cells	# affected				1
	avg grade				2
Colon – mucosal hemorrhage	# affected				2
	avg grade				1.5
Ileum – mucosal congestion	# affected				1

	avg grade				2
Ileum – mucosal gland atrophy	# affected				1
	avg grade				2
Ileum – mucosal hemorrhage	# affected				2
	avg grade				1
Ileum – Peyer’s patch lymphoid depletion	# affected		2	1	3
	avg grade		1	1	3.3
Ileum – villous atrophy	# affected				2
	avg grade				4.5
Jejunum – mucosal gland atrophy	# affected				1
	avg grade				2
Jejunum – mucosal hemorrhage	# affected				1
	avg grade				2
Jejunum – Peyer’s patch lymphoid depletion	# affected		2	1	1
	avg grade		1	1	1
Liver – diffuse glycogen depletion in hepatocytes	# affected			2	2
	avg grade			3.5	4
Liver – diffuse vacuolization of hepatocytes	# affected				1
	avg grade				3
Lung – edema in alveoli	# affected				2
	avg grade				5
Lung – hemorrhage in alveoli	# affected				2
	avg grade				2.5
Lung – bacteria	# affected				2
	avg grade				3.5
Lung – lobe necrosis	# affected				1
	avg grade				5
Lymph node (cervical) -- congestion	# affected				2
	avg grade				5
Lymph node (cervical) – lymphoid depletion	# affected			2	2
	avg grade			1.5	4.5
Lymph node (mesenteric) -- congestion	# affected				2
	avg grade				2.5
Lymph node (mesenteric) – lymphoid depletion	# affected			2	3
	avg grade			1	2.7

Lymph node (mesenteric) -- hyperplasia	# affected			2	
	avg grade			2.5	
Lymph node (mesenteric) -- plasmacytosis	# affected				1
	avg grade				2
Pancreas – zymogen depletion in acinar cells	# affected			1	2
	avg grade			3	3.5
Spleen – lymphoid depletion	# affected			1	2
	avg grade			1	4.5
Stomach – increased mucosal goblet cells	# affected				1
	avg grade				3
Stomach – mucosal hemorrhage	# affected				2
	avg grade				1
Thymus – hemorrhage	# affected			1	1
	avg grade			5	3
Thymus – lymphoid depletion	# affected	1	2	2	3
	avg grade	1	1	1	3
Urinary bladder – mucosal hyperplasia	# affected				1
	avg grade				2
Urinary bladder – diffuse mucosal vacuolization	# affected				2
	avg grade				3.5
Urinary bladder – lamina propria hemorrhage	# affected	1		1	2
	avg grade	1		1	2

Incidence and grade of histopathology findings at day 155 in males

	Type	VC	LD	MD	HD
Number examined		3	3	3	3
Bone marrow (sternum) – depletion	# affected			3	3
	avg grade			2.7	4.3
Brain – hemorrhage, gray matter	# affected				3
	avg grade				1
Heart – epicardial hemorrhage	# affected				2
	avg grade				1
Heart – myocardial hemorrhage	# affected				1
	avg grade				1
Heart – chronic active myocardial inflammation	# affected				1
	avg grade				1

Heart – pericardial fat hemorrhage	# affected				2
	avg grade				1.5
Heart – chronic active pericardial cardiac inflammation	# affected				1
	avg grade				1
Heart – pericardial fat necrosis	# affected				1
	avg grade				2
Cecum – lymphoid follicle depletion	# affected			2	1
	avg grade			1	1
Cecum – increased mucosal goblet cells	# affected				1
	avg grade				1
Colon – lymphoid follicle depletion	# affected				2
	avg grade				2.5
Colon – mucosal erosion/ulceration	# affected				1
	avg grade				1
Colon – mucosal gland dilation	# affected				2
	avg grade				1
Colon – increased mucosal goblet cells	# affected				1
	avg grade				1
Colon – mucosal hemorrhage	# affected				1
	avg grade				2
Duodenum – mucosal edema	# affected				1
	avg grade				2
Duodenum – mucosal gland dilation	# affected				2
	avg grade				1
Duodenum – increased mucosal goblet cells	# affected				1
	avg grade				3
Duodenum – Peyer’s patch lymphoid depletion	# affected			1	1
	avg grade			1	1
Ileum – mucosal edema	# affected				1
	avg grade				1
Ileum – Peyer’s patch lymphoid depletion	# affected			2	3
	avg grade			1	2.7
Jejunum – mucosal edema	# affected				1
	avg grade				2
Jejunum – mucosal gland	# affected				2

dilation	avg grade				1
Jejunum – increased mucosal goblet cells	# affected				1
	avg grade				3
Jejunum – Peyer’s patch lymphoid depletion	# affected			1	1
	avg grade			1	2
Kidney – hemorrhage	# affected				1
	avg grade				1
Kidney – mononuclear cell infiltrate	# affected				1
	avg grade				1
Liver – diffuse glycogen depletion in hepatocytes	# affected				2
	avg grade				5
Liver – necrosis, random, hepatocytes	# affected				1
	avg grade				2
Lung – hemorrhage in alveoli	# affected				1
	avg grade				1
Lung – congestion	# affected		2	1	
	avg grade		2	2	
Lung – interstitium chronic active inflammation	# affected				1
	avg grade				3
Lymph node (cervical) – pigmentation of sinus histiocytes	# affected				3
	avg grade				2.7
Lymph node (mesenteric) -- congestion	# affected			1	3
	avg grade			3	2.7
Lymph node (mesenteric) – lymphoid depletion	# affected		2	3	3
	avg grade		1.5	2	3
Pancreas – zymogen depletion in acinar cells	# affected				1
	avg grade				2
Prostate – hemorrhage	# affected			1	
	avg grade			2	
Prostate – suppurative inflammation	# affected				1
	avg grade				3
Salivary gland (mandibular) – atrophy of acinar cells	# affected				1
	avg grade				1
Spleen – diffuse decreased congestion	# affected				2
	avg grade				4.5

Spleen – lymphoid depletion	# affected		2	3	3
	avg grade		1	2	2
Stomach – increased mucosal goblet cells	# affected				1
	avg grade				1
Stomach – mucosal hemorrhage	# affected			3	1
	avg grade			1	1.5
Stomach – serosal hemorrhage	# affected		1		
	avg grade		2		
Thymus – hemorrhage	# affected				1
	avg grade				1
Thymus – lymphoid depletion	# affected	2	2	3	3
	avg grade	1	2	2	3
Thyroid – decreased diameter of follicles	# affected			1	3
	avg grade			1	1.3
Urinary bladder – lamina propria edema	# affected				1
	avg grade				2
Urinary bladder – lamina propria hemorrhage	# affected				2
	avg grade				1.5

- Additionally, one HD male had bacteria, moderately severe ulceration, and moderate chronic inflammation in the oral cavity along with moderate to moderately severe edema and hemorrhage in the lamina propria, and mild depletion of the lymphoid follicles. This male also had a bacterial infection in the jugular groove with the mild to moderately severe findings of edema, hemorrhage, inflammation, ulceration, fibrosis in the dermis, epidermis, and subcutis.

Incidence and grade of histopathology findings at day 156 in females

	Type	VC	LD	MD	HD
Number examined		3	3	3	3
Adrenals – focal vacuolization in cortex	# affected		1	1	1
	avg grade		2	3	2
Adrenals – hemorrhage	# affected				1
	avg grade				2
Bone marrow (sternum) – congestion	# affected				1
	avg grade				3
Bone marrow (sternum) – depletion	# affected			1	2
	avg grade			3	4

Cervix – hemorrhage	# affected				1
	avg grade				2
Esophagus – mucosa atrophy	# affected				1
	avg grade				2
Heart – epicardial hemorrhage	# affected				1
	avg grade				1
Heart – endocardial hemorrhage	# affected				1
	avg grade				1
Heart – myocardial hemorrhage	# affected				1
	avg grade				1
Heart – pericardial fat hemorrhage	# affected				1
	avg grade				1
Cecum – lymphoid follicle depletion	# affected			1	3
	avg grade			1	3
Cecum – increased mucosal goblet cells	# affected				1
	avg grade				2
Colon – luminal hemorrhage	# affected				1
	avg grade				2
Colon – lymphoid follicle depletion	# affected			1	1
	avg grade			1	5
Colon – increased mucosal goblet cells	# affected				1
	avg grade				3
Colon – serosal hemorrhage	# affected				1
	avg grade				2
Colon – submucosal hemorrhage	# affected				1
	avg grade				3
Duodenum – mucosal erosion/ulceration	# affected				1
	avg grade				1
Duodenum – mucosal gland dilation	# affected	1			3
	avg grade	1			1
Duodenum – mucosal hemorrhage	# affected				1
	avg grade				2
Ileum – mucosal gland dilation	# affected				1
	avg grade				1
Ileum – Peyer’s patch lymphoid depletion	# affected		1	3	3
	avg grade		1	1	3

Ileum – submucosal hemorrhage	# affected				2
	avg grade				2
Liver – bile retention in bile canaliculi	# affected				1
	avg grade				2
Liver – diffuse glycogen depletion in hepatocytes	# affected	1	3	2	2
	avg grade	3	2.3	3	4.5
Liver – necrosis, random, hepatocytes	# affected				1
	avg grade				1
Liver – mononuclear cell periportal infiltrate	# affected			2	
	avg grade			1	
Lymph node (cervical) -- congestion	# affected				2
	avg grade				3.5
Lymph node (cervical) – lymphoid depletion	# affected		3	3	3
	avg grade		1	1.3	1.3
Lymph node (cervical) – pigmentation of sinus histiocytes	# affected				2
	avg grade				3
Lymph node (mesenteric) -- congestion	# affected			1	2
	avg grade			3	2.5
Lymph node (mesenteric) – lymphoid depletion	# affected			2	3
	avg grade			2	3.5
Mammary gland – hemorrhage, subcutis	# affected				1 ¹¹
	avg grade				3
Omentum – colon adhesions	# affected				1
	avg grade				2
Omentum -- hemorrhage	# affected				1
	avg grade				4
Ovary – hemorrhage	# affected				1
	avg grade				2
Pancreas – zymogen depletion in acinar cells	# affected				1
	avg grade				2
Spleen – diffuse decreased congestion	# affected				3
	avg grade				5
Spleen – lymphoid depletion	# affected				2

¹¹ No section for one HD female

	avg grade				3.5
Stomach – increased mucosal goblet cells	# affected				2
	avg grade				2
Stomach – mucosal hemorrhage	# affected			1	1
	avg grade			1	1
Stomach – serosal hemorrhage	# affected		1		
	avg grade		2		
Stomach – submucosal hemorrhage	# affected				1
	avg grade				1
Thymus – hemorrhage	# affected				1
	avg grade				5
Thymus – lymphoid depletion	# affected	1		2	2
	avg grade	1		2	2.5
Thyroid – hemorrhage	# affected				1
	avg grade				4
Uterus – hemorrhage	# affected				1
	avg grade				1
Urinary bladder – lamina propria edema	# affected	1	1		1
	avg grade	1	1		2
Urinary bladder – lamina propria hemorrhage	# affected				2
	avg grade				2
Urinary bladder – mucosal erosion/ulceration	# affected				1
	avg grade				2

Incidence and grade of histopathology findings at day 187 in males

	Type	VC	LD	MD	HD
Number examined		2	2	2	1
Adrenals – diffuse cytoplasmic alteration, cortex	# affected			2	1
	avg grade			2	3
Bone marrow (sternum) – congestion	# affected			1	
	avg grade			1	
Bone marrow (sternum) – depletion	# affected			2	
	avg grade			4.5	
Brain – hemorrhage, gray matter	# affected			2	
	avg grade			1	
Brain – hemorrhage,	# affected			1	

meninges	avg grade			1	
Heart – epicardial fibrosis	# affected			2	
	avg grade			1	
Heart – chronic active myocardial inflammation	# affected			2	
	avg grade			1	
Heart – pericardial fat hemorrhage	# affected			2	
	avg grade			1	
Cecum – lymphoid follicle depletion	# affected			2	1
	avg grade			4	1
Cecum – mucosal gland dilation	# affected			2	
	avg grade			1	
Colon – lymphoid follicle depletion	# affected			1	1
	avg grade			4	1
Colon – mucosal gland dilation	# affected			1	
	avg grade			1	
Duodenum – luminal hemorrhage	# affected			1	
	avg grade			2	
Duodenum – mucosal gland atrophy	# affected			2	
	avg grade			2.5	
Ileum – mucosal gland atrophy	# affected			1	
	avg grade			3	
Ileum – Peyer’s patch lymphoid depletion	# affected			2	1
	avg grade			4	1
Ileum – submucosal hemorrhage	# affected			1	
	avg grade			3	
Jejunum – mucosal gland atrophy	# affected			2	
	avg grade			2	
Jejunum – Peyer’s patch lymphoid depletion	# affected				1
	avg grade				1
Kidney – hemorrhage	# affected			1	
	avg grade			1	
Liver – centrilobular hemorrhage	# affected			1	
	avg grade			2	
Liver – centrilobular hepatocyte degeneration	# affected			2	
	avg grade			2	

Liver – diffuse glycogen depletion in hepatocytes	# affected			2	
	avg grade			5	
Lung – hemorrhage in alveoli	# affected			1	
	avg grade			2	
Lymph node (cervical) -- congestion	# affected			2	
	avg grade			4	
Lymph node (cervical) – lymphoid depletion	# affected			2	1
	avg grade			3.5	3
Lymph node (cervical) – pigmentation of sinus histiocytes	# affected			2	1
	avg grade			3	1
Lymph node (mesenteric) -- congestion	# affected			2	
	avg grade			1.5	
Lymph node (mesenteric) – lymphoid depletion	# affected			2	1
	avg grade			4	1
Lymph node (mesenteric) – pigmentation of sinus histiocytes	# affected			1	
	avg grade			1	
Pancreas – zymogen depletion in acinar cells	# affected			1	
	avg grade			2	
Skin – subcutis hemorrhage	# affected			1	
	avg grade			5	
Stomach – increased mucosal goblet cells	# affected			2	
	avg grade			2	
Testes – hemorrhage	# affected			1	
	avg grade			2	
Testes – sertoli cell vacuolization	# affected			1	
	avg grade			2	
Thymus – hemorrhage	# affected			1	1
	avg grade			2	1
Thymus – lymphoid depletion	# affected	1	2	2	1
	avg grade	1	1.5	4	1
Thyroid – decreased diameter of follicles	# affected			1	1
	avg grade			3	2
Urinary bladder – lamina propria hemorrhage	# affected			1	
	avg grade			1	

- Additionally, one HD male had bacteria, severe ulceration, and moderate chronic inflammation in the oral cavity along with moderate to moderately severe edema and hemorrhage in the lamina propria, and mild depletion of the lymphoid follicles. This male also had mild edema and hemorrhage of the tongue lamina propria along with minimal ulceration of the tongue mucosa

Incidence and grade of histopathology findings at day 187 in females

	Type	VC	LD	MD	HD
Number examined		2	2	2	1
Bone marrow (sternum) – depletion	# affected				1
	avg grade				1
Brain – mononuclear cell infiltrate	# affected				1
	avg grade				1
Cecum – lymphoid follicle depletion	# affected				1
	avg grade				1
Duodenum – Peyer’s patch lymphoid depletion	# affected				1
	avg grade				1
Jejunum – Peyer’s patch lymphoid depletion	# affected				1
	avg grade				1
Lung – chronic interstitial inflammation	# affected			2	
	avg grade			1	
Lymph node (cervical) – lymphoid depletion	# affected				1
	avg grade				1
Lymph node (mesenteric) – lymphoid depletion	# affected		1		1
	avg grade		1		1
Stomach – serosal hemorrhage	# affected				2
	avg grade				1

- The cause of death for several of the HD animals was clear with serious lesions in the oral cavity, tongue, omentum, and those with serious necrosis or hemorrhage in the thymus or lung. These lesions were likely due to lymphoid and bone marrow changes. The proximate causes of other deaths, while related to HHT were not clear.

Toxicokinetics:

- Due to mortality, only 2 males and 2 females at the top dose on day 57 and only 1 male and 2 females at the top dose on subsequent sampling days were sampled for TK analysis.
- AUC values are for 0-12 h

- CTXOH was not detected at any timepoint.
- DMHHT was detected at lower levels than HHT but did not vary significantly from timepoint to timepoint with only slight increases after dosing (< 2-fold). Levels of exposure with regards to the day followed the same pattern as HHT exposure but DMHHT was generally at similar levels. Most but not all animals had washout of DMHHT by the beginning of the 3rd dosing cycle but none was found at the beginning of the final cycle.
- Data on HHT toxicokinetic parameters are below in tabular and graphical form.

HHT Toxicokinetic Parameters

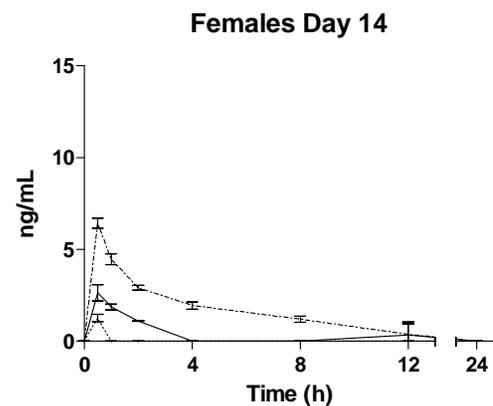
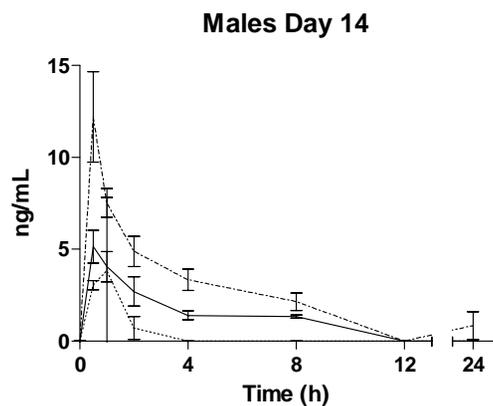
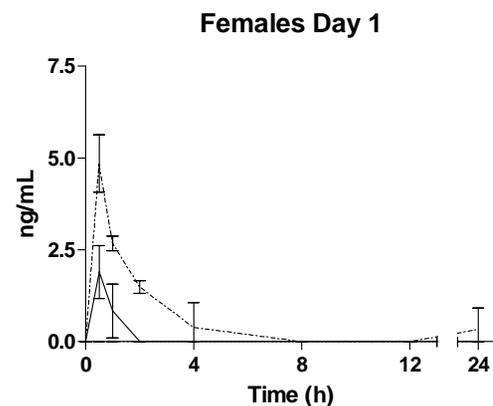
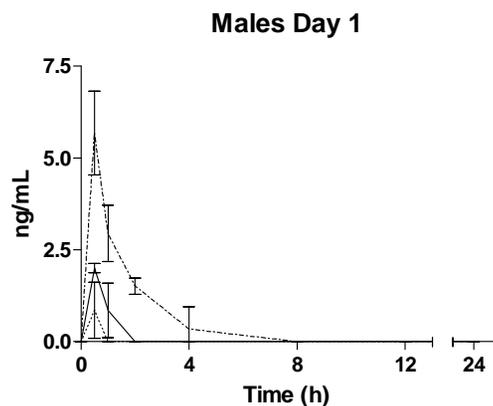
Dose (mg/kg/dose)	Day	Sex	T _{max} (h)	C _{max} (ng/mL)	AUC _{all} (h·ng/mL)	C _{max} /dose	AUC _{all} /dose
0.0125	1	M	0.5	0.857	0.428	68.6	34.2
0.0250	1	M	0.5	2.01	1.65	80.4	66
0.0500	1	M	0.5	5.68	8.37	114	167
0.0125	1	F	*	*	*	*	*
0.0250	1	F	0.5	1.9	1.58	76	63.2
0.0500	1	F	0.5	4.85	7.86	97	157
0.0125	14	M	0.7	4.85	5.48	388	438
0.0250	14	M	0.7	5.29	19.3	212	772
0.0500	14	M	0.5	12.2	37.7	244	754
0.0125	14	F	0.5	1.27	0.633	102	50.6
0.0250	14	F	0.5	2.65	5.11	106	204
0.0500	14	F	0.5	6.43	22.5	129	450
0.0125	57	M	0.5	1.21	0.603	96.8	48.2
0.0250	57	M	0.5	2.53	3.07	101	123
0.0375	57	M	0.5	3.36	7.04	89.6	188
0.0125	57	F	0.5	1.25	0.627	100	50.2
0.0250	57	F	0.5	2.26	2.71	90.4	108
0.0375	57	F	0.5	3.80	14	101	373
0.0125	70	M	*	*	*	*	*
0.0250	70	M	0.5	1.57	2.71	62.8	108
0.03125	70	M	0.5	1.95	1.84	62.4	58.9
0.0125	70	F	*	*	*	*	*
0.0250	70	F	0.7	1.64	1.36	65.6	54.4
0.03125	70	F	0.5	2.57	3.38	82.2	108
0.0125	141	M	*	*	*	*	*
0.0250	141	M	0.5	1.56	1.10	62.4	44
0.03125	141	M	2.0	2.42	5.46	77.4	175
0.0125	141	F	0.7	1.14	0.955	91.2	76.4

0.0250	141	F	0.5	2.98	4.09	22+	164
0.03125	141	F	0.5	4.16	10.4	133	333
0.0125	154	M	0.7	1.4	1.5	112	120
0.0250	154	M	0.5	4.23	8.61	169	344
0.03125	154	M	0.5	3.05	9.87	97.6	316
0.0125	154	F	0.5	1.32	2.3	106	184
0.0250	154	F	0.5	3.6	6.62	144	265
0.03125	154	F	0.5	3.43	12.9	110	413

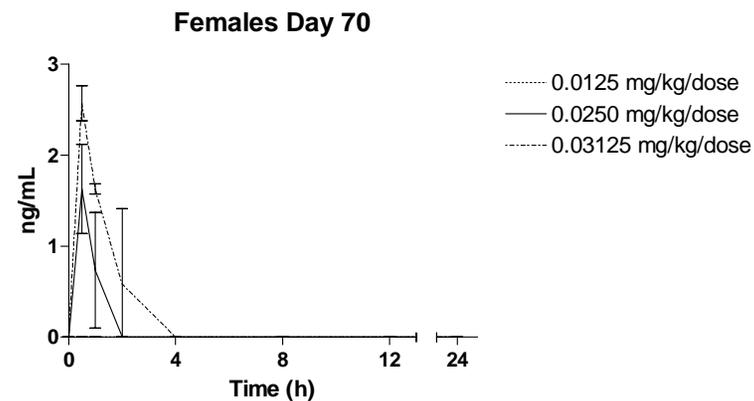
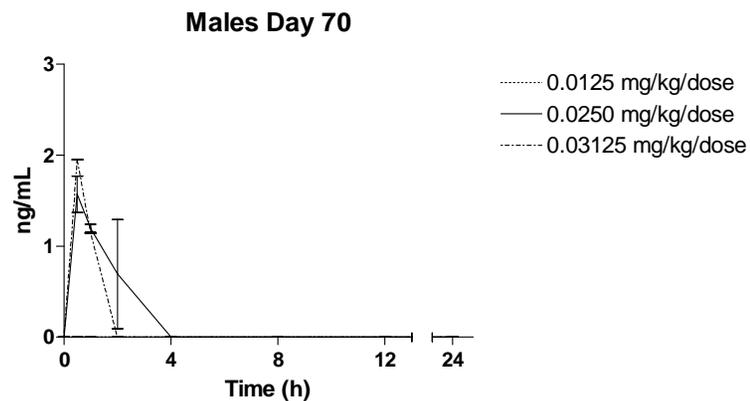
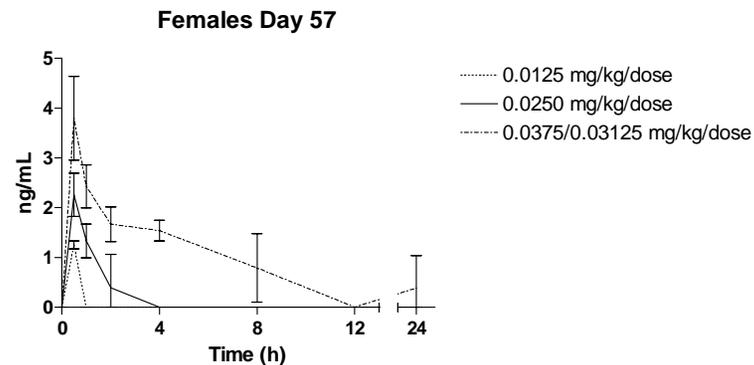
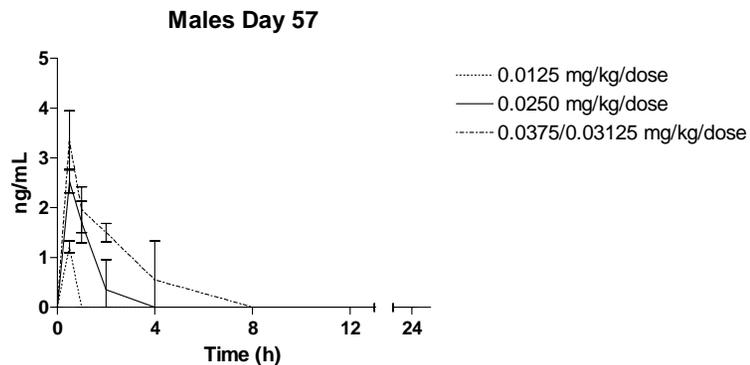
*no measurable plasma levels at any timepoint.

APPEARS THIS WAY ON ORIGINAL

HHT Plasma Levels

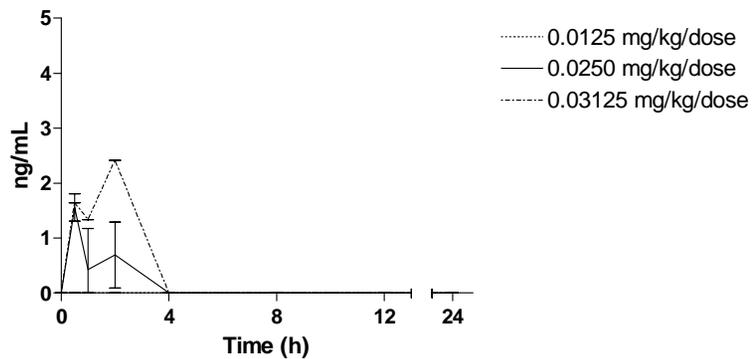


HHT Plasma Levels

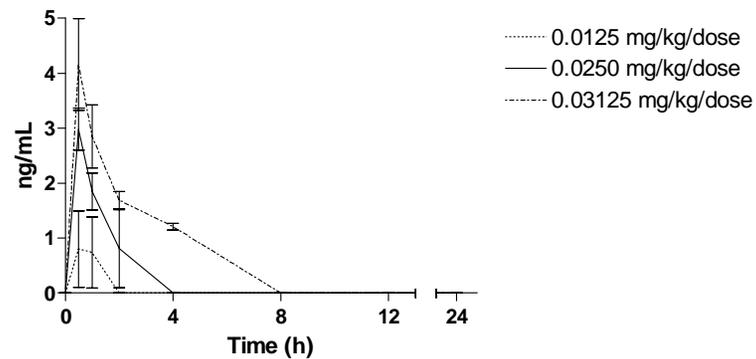


HHT Plasma Levels

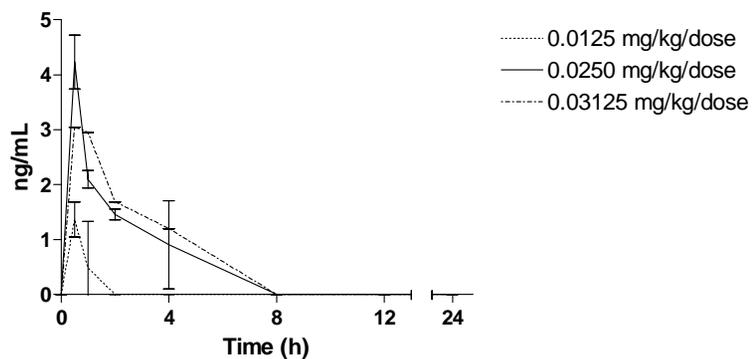
Males Day 141



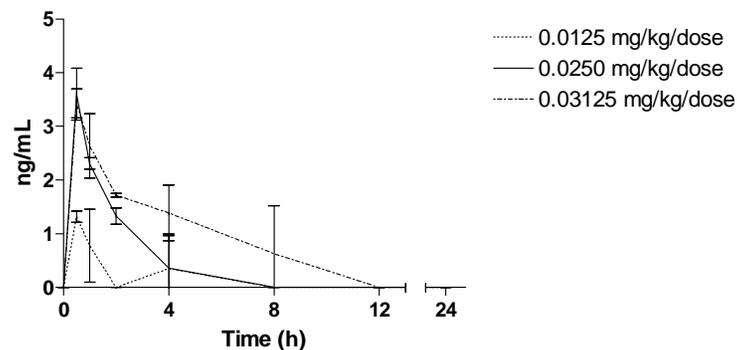
Females Day 141

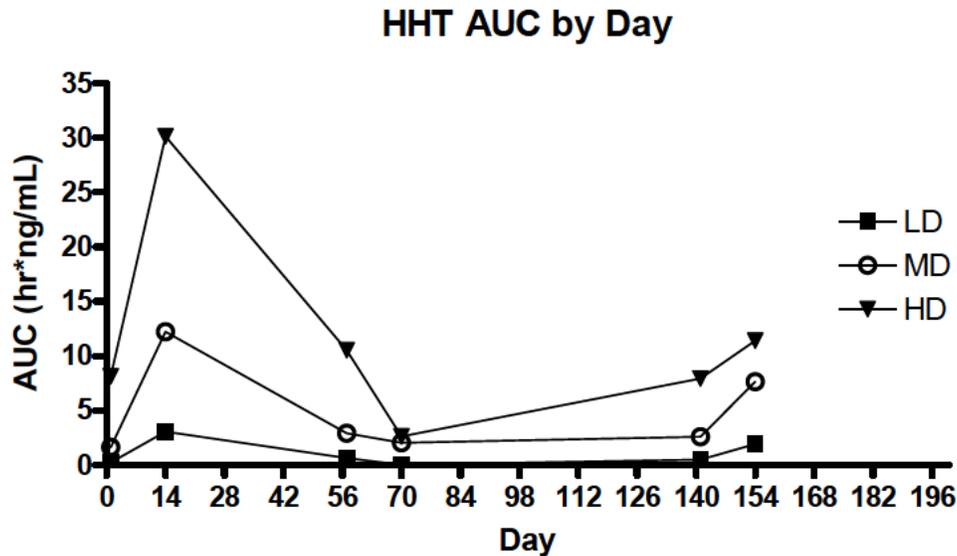


Males Day 154



Females Day 154





Conclusion:

Beagle dogs were treated for 6 cycles of twice daily subcutaneous treatment and 14 days treatment free with the last dosing period followed by 32 days of recovery. There were interim sacrifices on days 15 and 30 with final sacrifices on days 155/156 and 187. Overall there were early deaths for 2/10 MD males, 3/10 HD males, and 4/10 HD females.

The target organs of HHT toxic action were primarily the bone marrow, cardiovascular system (as related to widespread hemorrhage and increases in heart weight), and the lymphoid tissues. The dose-response relationship of HHT in dogs is relatively steep with large increases in deaths at or less than dose doubling (between LD and MD males and between MD and HD females). Findings in the lung may be due to a direct effect of HHT but may also be due to lymphoid and hematologic effects. The majority of the other findings appear to be secondary to the action of HHT on bone marrow and lymphoid tissues – possibly in conjunction with hemorrhage - or general ill health.

The hemorrhaging due to HHT-treatment is a significant finding in this study due to the widespread nature, seriousness of this endpoint, and the non-reversibility of this finding after a month of recovery. This is particularly striking in light of the fact that washout of HHT appears to occur by 12 h. The effect is at least partially due to drastically decreased platelets but does not appear to be wholly responsible as many animals such as many MD males, appeared to have similar decreases in platelets but did not have any hemorrhage findings in histopathology in either incidence or severity. It should be noted, however, that the two early death animals at the MD had gross pathology findings consistent with hemorrhage (red foci on organs) but no histopathological correlates were found. Indeed, there were many gross pathology

findings that had no histopathological correlates, such as gelatinous organs and tissues, pale organs, some GI contents, black organs, and increased heart weight (cardiac troponin I sampling was unremarkable as well).

Hematology findings were consistent with the bone marrow depletion found. Slight changes in MCHC suggest slight iron deficiency but this would not be responsible for the magnitude of the changes in HB or HCT. Reticulocytes appear to be initially decreased along with the RBCs, but later in the study it appears that induction of reticulocyte production is able to sufficiently replenish RBCs, generally by the end of study except for MD and HD males. The changes of hematology parameters for males and females appear to be slightly different but this is difficult to compare due to the variations in exposure and the multiple deaths at the MD and HD levels (the lowest levels are likely to be removed from the average as these animals die).

BUN levels suggest impairment of kidney or liver function, but there are no correlates for this finding, either in histopathology or other clinical chemistry findings. There were no findings of muscle injury to correlate the slight decreases in creatinine. Glycogen was severely depleted in the liver in many HD animals correlating with hyperglycemia in the males.

Females significantly recovered from most effects with some mild lingering lymphoid depletion, mild hemorrhage in the stomach, and evidence of mild inflammation in the brain. Males, however, did not recover from the majority of toxicities found on day 155. The one HD male surviving appears to have tolerated HHT very well, it is the remaining 2 MD males which have findings similar to, and in most cases worse than, MD animals on day 155. Overall, it does not appear that toxicities due to HHT are easily recovered from in Beagle dogs.

Toxicokinetic conclusion:

HHT is the primary moiety found in the serum in dogs after HHT administration. DMHHT is present at lower levels and appears to reach a steady state within the timeframe of the dosing cycle. No increasing accumulation of DMHHT was noted. CTXOH was not detected in this study.

At similar dose levels male exposure was generally slightly greater than female exposure for the first cycle and generally similar between sexes through out the rest of the study. HHT is absorbed quickly and cleared quickly with the peak concentration occurring between 0.5 and 1 h and returning to base line by 12 hr. HHT C_{max} was roughly dose-proportional on all days. HHT total exposure, as measured by AUC, was greater than dose-proportional and appeared to increase over the 1st and last dosing periods but not during the 3rd. The lack of increase during the second cycle does not appear to be due to the dose reduction during this cycle as the same pattern was found at the MD and LD. Exposure was lower at the beginning of 3rd cycle dosing compared to the end of the 1st; exposure at the beginning of the 6th cycle was, however, higher than for similar dosing at the end of the 3rd cycle dosing.

Accumulation did not appear to occur as no HHT was above the LLOQ by 12 hours or at the predose timepoints. Even taking into account the fact that the amount of HHT administered to the HD animals was reduced during this study twice, it appears that initially there may be some impairment of clearance after which there may be induction of a clearance mechanism. On day 57, however, HHT was found in one HD female at 1.14 ng/mL at the 24 hr timepoint, which is 12 hr past the second daily dose. This was a singular finding and only occurred on day 57 but on none of the subsequent sampling days.

Histopathology inventory

Study	PTX019/ PTX022	PTX018a/ PTX023a
Species	Mouse	Dog
Adrenals	x*	x*
Aorta	x	x
Bone Marrow smear		
Bone (femur)		
Brain	x	x*
Cecum	x	x
Cervix	x	x
Colon	x	x
Duodenum	x	x
Epididymis	x*	x*
Esophagus	x	x
Eye	x	x
Fallopian tube		
Gall bladder	x	x
Gross lesions	x	x
Harderian gland		
Heart	x*	x*
Ileum	x	x
Injection site	x	x
Jejunum	x	x
Kidneys	x*	x*
Lachrymal gland		x
Larynx		
Liver	x*	x*
Lungs	x	x
Lymph nodes, cervical	x	x
Lymph nodes mandibular		
Lymph nodes, mesenteric	x	x
Mammary Gland	x	x
Nasal cavity		
Optic nerves	x	x
Ovaries	x*	x*

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

X, histopathology performed

*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Bacterial Mutagenicity Test – Ames Assay

Key findings: This study was not valid to test the mutagenicity due to inappropriate doses tested.

Study no.: PTX004

Volume #, and page #: vol 16, pg 2

Conducting laboratory and location:

(b) (4)

Date of study initiation: 14 Nov 2007

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: homoharringtonine (HHT), Lot 07758, 99.1%

Methods: Plate incorporation assay

Strains/species/cell line: TA97a, TA98, TA100, TA1535, TA102

Concentrations used in definitive study: 100.14, 316.46, 1000.00 ng/plate with and without S9 activation.

Basis of concentration selection: No toxicity or precipitate was noted at any concentration in the concentration-range assay including the top dose of 1000.00 ng/plate.

Negative controls: 0.9% saline

Positive controls:

Strain	Positive Control without S9	Positive Control with S9
TA97a	1.0 µg/plate ICR-191 acridine	10 µg/plate 2-aminoanthracene
TA98	10 µg/plate 2-nitrofluorene	10 µg/plate 2-aminoanthracene
TA100	1.5µg/plate sodium azide	10 µg/plate 2-aminoanthracene
TA102	200-400 µg/plate cumene*	10 µg/plate 2-aminoanthracene*
TA1535	1.5µg/plate sodium azide	1.6 µg/plate 2-aminoanthracene

*The concentrations of positive controls used for TA102 are not present in the report. The concentrations listed above are from the associated protocol.

Incubation and sampling times: Incubated for 48-72 h @ 37±2°C

Results

Average revertants/plate – without activation

	TA97a	TA98	TA100	TA102	TA1535
100 ng/plate	180	49	145	258	14
316 ng/plate	174	63	123	254	8
1000 ng/plate	165	56	130	236	15
- control	201	58	128	266	11
+ control	1898	1502	791	793	322

Average revertants/plate – with activation

	TA97a	TA98	TA100	TA102	TA1535
100 ng/plate	196	33	140	333	16
316 ng/plate	214	38	125	301	14
1000 ng/plate	208	43	124	303	17
- control	204	33	138	327	16

+ control	1593	862	706	1728	143
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Study validity:

It is unclear as to why the top concentration of 1000.00 ng/plate was chosen for either the range-finding or the definitive tests; justification was given that the top concentration should “meet a 10-fold maximum concentration requirement for patient testing”. This requirement is not explained nor justified. Particularly because an Ames assay is a hazard determination and not a risk determination, the levels of patient exposure in a certain trial are not valid criteria for setting a top concentration. When not limited by solubility or cytotoxicity, 5000 µg/plate is currently recommended by ICH S2, a level 5000 times above that which was tested here. Furthermore, the assay performed did not comport to the protocol submitted which notes that the test article “will be observed for toxicity up to a concentration of 5 mg/plate” which was not done. Due to the lack of any evidence of cytotoxicity or precipitates this assay is not considered valid to assess the mutagenicity of HHT.

Study outcome: This study was not valid for determining mutagenicity of HHT.

Study title: *In Vitro* chromosome aberration analysis in Chinese hamster ovary (CHO) cells.

Key Study Findings: Under the conditions of this assay HHT is considered a clastogen.

Study no.: PTX008

Location: Vol 16, pg 23

Conducting laboratory and location: (b) (4)

Date of study initiation: 12 Nov 2007

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: homoharringtonine (HHT), Lot 07758, 99.1%

Method: *In Vitro* clastogenicity test

Strain: Chinese hamster ovary cells

Doses used: 10.03, 31.69, 100.14, 316.46, 1000 ng/mL

Basis of Dose Selection: The applicant chose the concentrations prior to study with the top concentration representing a 10-fold increase of the C_{max} dose in patients according to the report. Toxicity was seen at the highest doses as seen in the results below (e.g. no mitotic spreads were seen after 20 h treatment with HHT).

Negative Control: 0.9% saline

Positive Control: Cyclophosphamide (CP) at both 5 and 15 µg/mL for the S9 activated cultures, mitomycin C (MMC) at both 0.25 and 1.0 µg/mL for the non-activated cells.

Incubation and sampling time:

- The cells were incubated at 37.0°C, 5% CO₂
- Treatment time was 3 h and 20 h without activation and 3h with S9 activation.

Results:

- pH and osmolarity data were not reported. pH was not tested but no media color changes were reported indicating that pH was unacceptable. Clastogenicity was positively observed without S9 metabolic activation after 20 h treatment.
- The majority of aberrations with test article or positive control were chromatid exchanges but breaks were also seen.

3 h assay

Treatment (ng/mL)	S9	% cells with aberrations (excluding gaps)
Saline	-	5.5
10	-	4.0
31.7	-	5.0
100.1	-	4.0
316.5	-	6.0
1000	-	6.0
low MMC	-	32.0*
high MMC	-	51.0*
Saline	+	5.0
10	+	5.0
31.7	+	3.0
100.1	+	7.0
316.5	+	3.0
1000	+	6.0
low CP	+	30.5*
high CP	+	65.0*

*P<0.001

20 h Assay

Treatment (ng/mL)	S9	% cells with aberrations (excluding gaps)
Saline	-	4.5
10	-	7.0
31.7	-	2.0
100.1	-	11.0*
316.5	-	16.0*
1000	-	NA [†]
low MMC	-	39.0*
high MMC	-	NA [‡]

*P≤0.001

[†]No cells to score due to cytotoxicity

[‡]The high dose MMC control is listed in the report as 'NA' without explanation.

Mean Mitotic Index (%)

Treatment (ng/mL)	3 h	3 h + S9	20 h
Saline	17.4	15.0	16.2
10	10.2	15.4	16.7
31.7	13.8	13.8	13.3
100.1	11.6	15.9	9.5
316.5	8.8	5.8	0.4
1000	9.1	4.7	0
low MMC	7.0	-	8.7
high MMC	20.1	-	NA [‡]
low CP	-	8.0	-
high CP	-	12.4	-

[‡]The high dose MMC control is listed in the report as 'NA' without explanation.

QSAR Analysis for Genetic Toxicity Results

Computational Toxicology Software:

MC4PC 2.1.0.11, MDL-QSAR 2.2, Derek for Windows 11, and Leadscope Model Applier 1.3.2-7 software programs were used

Key findings: Based on the entire weight of evidence, HHT is predicted to be positive for genetic toxicity, however, HHT was predicted to be negative for rodent carcinogenicity.

Summary of model system:

The models used employ significantly different approaches to identify molecular attributes associated with biological activity and are intended as complementary

systems. Consequently, a positive prediction by one model system is not necessarily negated by a negative prediction from another. The molecular composition of HHT was checked for representation (coverage) among the molecular structural features found in the control database (training set).

Results:

No structural alerts were found using Derek. HHT was predicted to be positive in the mouse lymphoma mutagenicity assay and for the *in vivo* micronucleus assay but negative in the bacterial mutagenicity and *in vitro* chromosome aberration assays as well in rodent carcinogenicity studies. There is a conflict with the negative result in for the *in vitro* chromosome aberration assay prediction with the actual positive result in study PTX008 (*In Vitro* chromosome aberration analysis in Chinese hamster ovary (CHO) cells). It is important to note however, that positive predictions carry more weight from predictive models because a negative prediction is confirming only that nothing in the training set is positive.

2.6.6.6 Reproductive and developmental toxicology**Study title: Developmental Toxicity Study of Homoharringtonine (HHT) in Mice: Pilot Study.**

Key study findings: HHT was 100% embryo-fetolethal at 0.415 and 0.835 mg/kg/dose twice/day on days 6-15 of gestation. No malformations were noted. Embryo-fetal lethality was found in the absence of maternal toxicity.

Study no.: PTX011

Volume #, and page #: Vol 16 pg 48

Conducting laboratory and location:

(b) (4)

Date of study initiation: 10 Jan 2008

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Homoharringtonine, Lot 05D08, 99.9%

Methods

Doses: vehicle, 0.105, 0.205, 0.415, 0.835 HHT mg/kg/dose

Species/strain: albino mice/Swiss-Webster

Number/sex/group: 6 mated females/group

Route, formulation, volume: subcutaneous injection, 0.9% saline, 5 mL/kg/dose

Satellite groups used for toxicokinetics: none

Study design: twice daily administration at approximately 10 hours apart on days 6 to 15 of gestation (the morning a vaginal plug was observed was considered day 0 of gestation). Termination on gestation day 18.

Parameters and endpoints evaluated: Body weight, clinical signs, food consumption, maternal gross pathology, pregnancy rate, pre-implantation loss, post-implantation loss, fetal weight, fetal external morphology.

Results

Mortality (dams): none

Clinical signs (dams):

- bloody vaginal discharge
 - 2 at 0.205 mg/kg on gestation day 11
 - 2 at 0.415 mg/kg on gestation day 12, 1 continued gestation day 12.
 - 2 at 0.835 mg/kg on gestation days 10 and 12 (one each)

Gross Pathology (dams):

- Enlarged spleen in 2 dams at 0.415 mg/kg and 4 at 0.835 mg/kg.
- Enlarged adrenal gland in 2 at 0.835 mg/kg.
- Enlarged iliac lymph nodes in 1 at 0.835 mg/kg

Body weight (dams): corrected weights for dose groups were similar and unremarkable. See table under C-section data for gravid uterine weight.

Food consumption (dams): not measured

Terminal and necroscopic evaluations: C-section data:

	vehicle	0.105 mg/kg	0.205 mg/kg	0.415 mg/kg	0.835 mg/kg
Pregnant (early resorption or fetus)	4	5	5	4*†	2†
Live fetuses/animal	10.3	10.6	11	0	0
Dead fetuses	0	0	0	0	0
Total resorptions/animal	0.75	0.60	0.80	8.25	10
Early resorptions/animal	0.75	0.60	0.80	6.75	10
Mean fetal body weight (g male/female)	1.01/1.03	1.20/1.13	1.07/1.01	-	-
Gravid uterine weight (g – avg for gravid dams)	14.6	16.2	15.4	1.5	0.28

* 4 dams had evidence of resorptions but 2 additional had thickened endometrium which may indicate pregnancy.

†No fetuses present but pregnancy was confirmed by the evidence of resorptions.

Offspring: no malformations noted

Study title: Developmental Toxicity Study of Homoharringtonine (HHT) in Mice Following Twice Daily Subcutaneous Administration.

Key study findings: Post-implantation loss due to increased early resorptions increased with HHT exposure. HHT did not induce malformations but lack of ossification and decreased fetal weight was noted in the HD group. No significant maternal toxicity was noted at any dose.

Study no.: PTX012

Volume #, and page #: Vol 16 pg 84

Conducting laboratory and location:

(b) (4)

Date of study initiation: 6 Feb 2008

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Homoharringtonine, Lot 05D08, 99.9%

Methods

Doses: vehicle, 0.105, 0.205 HHT mg/kg/dose

Species/strain: albino mice/Swiss-Webster

Number/sex/group: 24 mated females/group

Route, formulation, volume: subcutaneous injection, 0.9% saline, 5 mL/kg/dose

Satellite groups used for toxicokinetics: none

Study design: twice daily administration at approximately 10 hours apart on days 6 to 15 of gestation (the morning a vaginal plug was observed was considered day 0 of gestation). Termination on gestation day 18.

Parameters and endpoints evaluated: Body weight, clinical signs, food consumption, maternal gross pathology, pregnancy rate, pre-implantation loss, post-implantation loss, fetal weight, fetal morphology. Visceral and skeletal fetal examinations at control and high-dose groups only unless abnormalities noted.

Results

Mortality (dams): none

Clinical signs (dams): unremarkable

Gross Pathology (dams): one HD dam had an enlarged spleen.

Body weight (dams): unremarkable

Food consumption (dams): unremarkable

Terminal and necroscopic evaluations:C-section data:

	vehicle	0.105 mg/kg	0.205 mg/kg
# Pregnant per group (%) - all had viable fetuses	13 (54%)	16 (67%)	15 (63%)
Corpora lutea/animal	12.5	11.4	12.5
Implantation sites/animal	11.6	10.4	11.5
Pre-implantation losses/animal (total)	0.8 (11)	0.9 (15)	1.1 (16)
Post-implantation losses/animal (total)	0.5 (7)	0.8 (12)	1.7 (25)
Live fetuses/animal (total)	11.1 (144)	9.7 (155)	9.5 (143)
Dead fetuses	0	1	0
Total resorptions/animal (total)	0.5 (7)	0.7 (11)	1.7 (25)
Early resorptions/animal (total)	0.5 (7)	0.6 (10)	1.6 (24)
Mean fetal body weight (g)	1.36	1.36	1.16
Gravid Uterine Weight (g – avg for gravid females)	17.8	16.6	14.6

Offspring:

Fetal observation incidence

	vehicle	0.205 mg/kg
Unossified hyoid body/arch	-	2
Unossified sternebra 5 or 6	-	3
Unossified carpal/metacarpal	-	5
Unossified tarsal/metatarsal	-	1

2.6.6.9 DISCUSSION AND CONCLUSIONS

II. Summary of nonclinical findings

Omacetaxine shows activity in various anti-leukemic cells lines and appears to be the active moiety but the mechanism of action of omacetaxine as related to anti-cancer activity has not been fully elucidated. Omacetaxine is an inhibitor of protein elongation by blocking tRNA binding to the 60S ribosome subunit. Apoptosis appears to be due to decreases in the anti-apoptotic protein, Mcl-1 (Mcl-1 is required for myeloma cell survival¹²). Because Mcl-1 has a high turnover rate, inhibition of protein synthesis may be behind the decreases of Mcl-1 and subsequently to apoptosis. Given the indiscriminate nature of protein elongation, however, it is likely that other mechanisms of action are involved in omacetaxine's activity. Although cancer cells have high rates of translation, translation is necessary for survival of all cells and increased translation is not necessarily related to the ability of cells to do without protein production. If cancerous cells are less likely to survive without short-lived proteins than normal cells, then a differentiation could be made, but this is not known.

In response to omacetaxine treatment, Mcl-1 and Bim levels were decreased dose-dependently, the level of Bax was increased dose-dependently, and Puma and Bcl-X_L levels were not changed by omacetaxine. It appears that omacetaxine and cycloheximide both increase Bax. It's unclear how the inhibitors of protein elongation cause an increase in Bax. An increase of Bax due to omacetaxine is confirmed in another study found in the literature¹³.

There is another publically available paper¹⁴ reporting that omacetaxine and other protein elongation inhibitors have chemosensitizing effects for doxorubicin, and also reported cancellation of omacetaxine effect by the proteasome inhibitor MG132 similar to the applicant submitted study. Mcl-1, cyclin D1 and c-Myc were decreased. The authors also acknowledge the paradox that this MoA would not thought to be selective for cancer cells but is. It is important to note that a pro-apoptotic action decreasing Mcl-1, cyclin D1, and c-Myc by means other than inhibition of protein elongation would also be reversed by MG132 since the inhibition of proteasomes would keep the level of Mcl-1 and similar proteins up regardless of the cause.

The dose-toxic response curve is steep, which would be consistent with a drug that stopped protein synthesis. It could be, though, that the toxic mechanism of action and the anti-cancer mechanism of action are not the same. Anthracyclines also decrease proteins by way of decreasing transcription so anti-cancer action by inhibition of protein

¹² Derenne S et al. 2002. Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x(L) is an essential survival protein of human myeloma cells. *Blood*. 100: 194-9

¹³ Yinjun et al. 2004. Homoharringtonine Mediates Hyeloid Cell Apoptosis uia upregulation of pro-apoptotic bax and inducing caspase-3-mediated cleavage of poly(adp-ribose) polymerase (PARP). *Am J Hem* 76: 199-204.

¹⁴ Robert et al. 2009. Altering chemosensitivity by modulating translation elongation. *PLoS ONE*. 4: e5428

elongation may be plausible with cancer cells being more sensitive simply because they are producing (and supposedly requiring) a substantial amount transcription/translation. omacetaxine and anthracyclines also share some primary targets of toxicity: bone marrow, lymphoid tissue, and the heart. However, anthracyclines also have other mechanisms of anti-cancer action as well and a relationship between increased transcription/translation and sensitivity to anthracyclines is poorly understood.

While omacetaxine does seem to inhibit protein elongation, there is little information that this inhibition is related to the anti-cancer activity of omacetaxine and, therefore, the mechanism of omacetaxine anti-cancer mechanism should be considered unknown.

There is no evidence that hematological malignancies that contain the T315I mutation of the Bcr-Abl fusion gene are more sensitive to omacetaxine than wild type Bcr-Abl; in fact, pharmacology studies submitted by the applicant demonstrate that omacetaxine activity is similar in cells with either oncogene polymorphism.

In both species omacetaxine was the primary moiety found and was absorbed quickly and cleared quickly with peak concentration occurring at 0.5 h and washing out by approximately 12 hours. Dose proportionality varied over the course of the study for mice but for dogs exposure was generally greater than dose proportional. In both species, induction of metabolism or clearance appears to occur over the course of the study but this finding was not consistent. omacetaxine did not appear to be accumulative over the course of the 6-month studies; however, for certain cycles accumulation did appear to minimally occur.

The order of stability in plasma of omacetaxine was dog < monkey < human < mouse plasma. Omacetaxine was either completely unstable in rat plasma or the assay was invalid. The amount of HHT that was protein bound was 2-50%, with binding in dog plasma < monkey plasma < mouse plasma < human plasma.

Omacetaxine appears to have low permeability and is subject to P-gp efflux. This correlates with evidence that omacetaxine is approximately 15 times less effective as an anticancer agent in cell lines with multidrug resistance (MDR) than those without¹⁵. The susceptibility to P-gp efflux is notable as MDR is a characteristic of refractory and relapsed acute leukemia and blast phase CML.

Omacetaxine appears to preferentially distribute to the bone marrow (in rats), liver, and kidneys in rodents. By 24 h post dose, HHT was only detected in the liver and that was eliminated by 72 h post dose.

Omacetaxine does not appear to be metabolized significantly by CYP-mediated oxidative metabolism or by esterases in human liver microsomes but there is some

¹⁵ Russo D, Michelutti A, Melli C, Damiani D, Michieli MG, Candoni A, Zhou DC, Marie JP, Zittoun R, Baccarani M. 1995. MDR-related P170-glycoprotein modulates cytotoxic activity of homoharringtonine. *Leukemia*. 9(3): 513-516.

evidence that a CYP-inducible enzyme is involved in omacetaxine metabolism in rat and rabbit liver microsomes¹⁶. In human liver microsomes, omacetaxine showed weak inhibition of 1A and 2D6 isozymes and strong inhibition of the 3A4 isozyme in the irreversible inhibition assay; DMHHT showed weak inhibition of 1A, 2C19, and 2D6 isozymes. Omacetaxine showed possible weak induction of the 1A isozyme. DMHHT also appeared to weakly inhibit 1A and weakly induce 3A4 in the induction assay, consistent with the irreversible inhibition assay results.

Omacetaxine and metabolites appear to be excreted by both the urine and bile with the bile becoming a greater amount as time approaches 48 h. The amounts found in the intestines and in the bile at early timepoints suggest that excretion by way of the liver and into the bile is a rapid process in the rat. The majority of omacetaxine appears to be metabolized before excretion.

In general toxicology studies, omacetaxine showed a steep dose-response relationship (see tabulated summary table below). This may be due to omacetaxine's effect on protein elongation. Of particular note are the effects on bone marrow depletion. At 0.835 mg/kg/dose, 16 of 17 male dogs had decreased cellularity in the bone marrow (sternum) with severity between moderately severe while at 0.585 mg/kg dose, no dogs had any bone marrow findings of any sort. Bone marrow depletion appears to be the primary toxicity associated with omacetaxine, with platelets particularly affected. Other targets of omacetaxine toxicity included the heart, skin, and possibly the kidney and liver.

Serious hemorrhage and lymphoid depletion were also related to omacetaxine but it is unclear if the finding of hemorrhage is solely due to the severe drop in platelets as more hemorrhage in both incidence and severity was seen in HD male dogs despite the levels of platelets being similar to those in the MD (-96% and -93%, respectively). The hemorrhaging due to omacetaxine-treatment is significant due to the widespread nature, seriousness of this endpoint, and the non-reversibility of this finding after a month of recovery in dogs. This is particularly striking in light of the fact that washout of omacetaxine appears to occur by 12 hours.

There was little histopathological correlation for many findings in the general toxicology studies. Most strikingly, the pale organs such as the brain and adrenal glands, the increased heart weights, and the black contents of the stomach and intestines of early death animals had no histopathological correlates. Red foci, striations, and general color were also noted and may be related to hemorrhage although, these gross pathology findings effected more organs than the histopathology findings can confirm.

The heart is clearly a target of omacetaxine toxicity as demonstrated by not just the significant increases in heart weight at every dose level in both mice and dogs but also by the other findings in dogs: 3-4 cm adherent thrombus in right ventricle, mottled red heart, red foci in/on all chambers, red striations in the ventricles, thickened heart walls in gross pathology and epicardial, endocardial, and myocardial hemorrhage and

¹⁶ Cui Y, Wang, M. 1991. The metabolism of homoharringtonine by liver microsomes of rats and rabbits. *Yao Xue Xue Bao*. 26(4): 274-279.

fibrosis, chronic myocardial inflammation, and pericardial fat necrosis. *Ex vivo* findings in the safety pharmacology study (study ZNA19021.002), suggested that QT may be decreased *in vivo*. This was confirmed in the 6-month, repeat dose dog study. It may be that contractility and conduction velocity are decreased as well, but no functional deficits were noted in dogs.

There are significant incidences of ‘gelatinous’ contents, organs, or tissues seen in mainly in dogs but also in a early death mouse. Gelatinous contents in the GI tract do not have other correlates in the study; indeed, most animals with this finding did not have evidence of any effect on the GI tract by omacetaxine. Gelatinous thymus, injection site, and jugular groove (also a red and thick left jugular groove) similarly did not have other correlates. These findings may suggest a vascular issue that may or may not be related to platelets but this can not be known without further investigation.

Clinical chemistry in mice and dogs suggests slight impairment of glomerular filtration but these can not be further characterized with the information from the studies performed. Hyperglycemia was significant in mice but the cause is unclear and was not significant in dogs. Thymus and spleen were also affected by omacetaxine with changes in lymphoid depletion. Thymus atrophy/involution was also increased and while it is a natural process, it appears that omacetaxine is accelerating it. Minimal to slight findings of necrosis/degeneration of hepatocytes were found in many mice and a few dogs in response to omacetaxine; other findings in the liver, such as hypertrophy, appear to solely adaptive in nature. Muscosal ulceration of the stomach in mice or urinary bladder in dogs was also noted in a few high dose animals in dogs and mice. One high dose male dog had serious ulceration in the oral cavity and tongue which was the cause of early death. The skin, independent from injection sites, also was affected with acanthosis and atrophy of sebaceous glands in mice and one serious hemorrhage in a 0.025 mg/kg/dose dog. Injection sites in mice and dogs were associated with hemorrhage, fibrosis and inflammation in addition to the findings in general skin sections.

Other findings in the general toxicology studies are likely secondary to other effects noted above.

All mice and female dogs significantly recovered from most effects with some mild lingering lymphoid depletion, minimal liver necrosis/degeneration in one mouse, mild hemorrhage in the stomach, and evidence of mild inflammation in the brain. Males, however, did not recover by day 187 from the majority of toxicities found on day 155. While the one high dose male surviving appears to have tolerated omacetaxine very well throughout the study, the remaining 2 mid-dose male dogs for recovery had findings similar to, and in most cases worse than, mid-dose animals at the terminal sacrifice. As noted above, this is particularly striking due to the rapid clearance of omacetaxine and the nature of many of the toxicities. Significantly, hemorrhages are found in the heart and brain; inflammation is active in the heart, and bone marrow depletion is at a moderately severe level in both surviving dogs. This is in contrast to the mid-dose dogs at the terminal sacrifice 32 days earlier whom had no findings in the heart and bone marrow depletion between mild and moderate.

In a small pilot embryofetal developmental study in mice, omacetaxine appeared to be 100% embryofetoletal at 0.415 mg/kg/dose and higher given twice daily on days 6-15 of gestation. 0.415 mg/kg/dose is approximately the proposed human dose on a mg/m² basis (toxicokinetic analysis was not performed) Some minimal signs of maternal toxicity were noted at 0.415 mg/kg/dose and above such as enlarged spleen, adrenal gland and iliac lymph nodes. No malformations were noted at any level.

In the definitive embryofetal developmental study in mice (PTX012), dosing twice daily over gestation days 6-15, omacetaxine exposure lead to post-implantation loss due mainly to an increase in early resorptions, decreased mean fetal body weight and lack of ossification in the hyoid body/arch, sternebra 5/6, carpal/metacarpal and tarsal/metatarsal bones in 2, 3, 5, and 1 high dose (0.205 mg/kg/dose) fetuses, respectively, out of 143 high dose fetuses with no similar findings in control animals and with no apparent maternal toxicity. No malformations were noted in fetuses. No maternal toxicity was noted other than one high dose dam with an enlarged spleen in the definitive study. The decrease in fetal weights suggest that development may be delayed due to omacetaxine exposure. If ossification continues to be absent in the tissues noted, malformations will occur as fetal development continues. The high dose used in the definitive study was half the proposed human dose of 1.25 mg/m².

Omacetaxine was positive for clastogenicity in Chinese hamster ovary (CHO) cells in the only adequately performed genetic toxicology study submitted with this NDA (PTX008). An Ames assay was submitted but was invalid for determining mutagenicity because the top concentration was only 5 µg/plate and did not cause cytotoxicity nor precipitated in the culture medium. Adequate justification for the low value for the maximum concentration was not given.

A published paper was submitted (Huang et al. 1983) that investigated sister chromatid exchange in CHO cells. The study was not reviewed as it was not GLP, the source or purity of the semi-synthetic HHT was not given, and many study details are not reported. Furthermore, the study PTX008, is more pertinent an rigorous study to investigate the propensity of HHT to cause chromosomal rearrangement.

Another paper (Singh and D'Ambrosio, 1984¹⁷) was submitted that assessed sister chromatid exchange (SCE) in human lymphocytes 2 h post-HHT dose in three patients in a phase 1 trial after 2-5 days of HHT (2 at 3.8 mg/day for 2 days and 1 patient for 5 days at 8 mg/day). No increase in sister chromatid exchange was seen in the lymphocytes of the three patients compared to non-treated volunteers. Cell cycle was only inhibited in one patient, indicating possible lack of HHT activity so the relevance of the findings is questionable particularly since the paper also finds that SCE and inhibition of mitosis appear to be dependent on both dose and duration of exposure. Due to these issues this study is not sufficient to determine SCE due to HHT administration. It is noteworthy that

¹⁷ Singh NP and D'Ambrosio SM. 1984. Sister chromatid exchange frequency and cell cycle kinetics in cancer patients treated with cytostatic drugs. *Basic Life Sci.* 29 Pt B: 885-93.

breaks were the main finding in the CHO assay which while not completely comparable would be expected to be similar.

No carcinogenicity study was submitted. The proposed patient population is refractory to imatinib - and if they truly harbor the T15I mutation, are unlikely to respond to other approved lines of therapy for CML. However, the life expectancy of a significant proportion of the imatinib resistant chronic phase CML patient population is greater than 2-3 years¹⁸. The 95% CI for *median* overall survival is 44.4-59.5 months for tyrosine kinase inhibitor (TKI) – refractory chronic phase CML patients and 18.2-48.5 months from the time of T315I mutation detection. These estimates do not take into account any positive effect on survival that omacetaxine may have. Genotoxicity results available suggest also raise questions about the possibility of secondary tumors over the lifetime of the treated patients but are equivocal overall.

TABLE OF EXPOSURE LEVELS WITH ASSOCIATED TOXICITIES (sorted by AUC from pharmacokinetic results)

Species	Sex	Dose mg/m ²	AUC (h·ng/mL)	Primary Effects
Dog	F	0.25	0.633	sporadic effect on WBC parameters, thrombocytopenia, lymphoid depletion
Dog	F	0.5	5.11	decreased WBC parameters, thrombocytopenia, lymphoid depletion
Dog	M	0.25	5.48	decreased WBC parameters, lymphoid depletion, thrombocytopenia
Dog	M	0.5	19.3	Death; bone marrow depletion w/ correlating decreases in WBC and RBC parameter; widespread hemorrhaging; thrombocytopenia; lymphoid depletion; heart inflammation /fibrosis; hyperglycemia
Dog	F	1	22.5	Death; bone marrow depletion w/ correlating decreases in WBC and RBC parameter; widespread hemorrhaging; thrombocytopenia; lymphoid depletion; increased heart weight and fibrosis
Dog	M	1	37.7	Death; bone marrow depletion w/ correlating decreases in WBC and RBC parameters; widespread hemorrhaging; lymphoid depletion; heart inflammation/necrosis/fibrosis; hyperglycemia; thrombocytopenia
Mouse	M	1.755	93.4	Death; moderate ↓ in WBC, RBC and related parameters; ↓ BUN; ↑ bilirubin
Mouse	F	0.87	99.3	moderate ↓ in WBC, RBC and related parameters; ↑ glucose

¹⁸ Nicolini EF et al. 2009. Epidemiological study on survival of chronic myeloid leukemia (CML) and Ph+ acute lymphoblastic leukemia (ALL) patients with BCR-ABL T315I mutation. *Blood*. 114: 5271-5278.

Mouse	M	0.87	100	moderate ↓ in WBC, RBC and related parameters; ↓ BUN; ↑ bilirubin
Mouse	F	1.755	110	moderate ↓ in WBC, RBC and related parameters; ↑ glucose
Human proposed dose	M&F	1.25	188	Thrombocytopenia
Mouse	M	3.495	209	Death, severe bone marrow depletion characterized by ↓ in WBC and related parameters, moderately severe ↓ RBC and related parameters; hemorrhage; necrosis/ulceration of epithelial tissues; increased heart weight
Mouse	F	3.495	230	Death, severe bone marrow depletion characterized by ↓ in WBC, RBC and related parameters; hemorrhage; necrosis/ulceration of epithelial tissues; hyperglycemia; increased heart weight;

AUC levels are for day 14 for mice and dogs and day 11 for humans.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

We recommend approval of omacetaxine from a pharmacology/toxicology standpoint for the proposed indication.

Additional studies needed

Based on the life expectancy of a significant proportion of the imatinib-resistant chronic phase CML patient population, a full battery of genotoxicity studies should be performed per ICH S2. These studies are necessary to adequately inform patients of the possible risks of omacetaxine and to determine the possible need for carcinogenicity studies.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22374	ORIG-1	CHEMGENEX PHARMACEUTICA LS	Omapro

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/s/

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03/05/2010

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