CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-991

PHARMACOLOGY REVIEW(S)

MEMORANDUM
Oct. 5, 2006

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

SUBJECT: NDA 21-991

I concur with the recommendation by the pharmacology/toxicology reviewer, Dr. S. Leigh Verbois, that the marketing application for vorinostat (ZOLINZA®) may be approved based on review of submitted data. The proposed product label is acceptable.

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

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

/s/

Kenneth Hastings
10/5/2006 12:16:29 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-991**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**04/05/06**
PRODUCT:	**ZOLINZA® (vorinostat)**
INTENDED CLINICAL POPULATION:	**Refractory Cutaneous T-Cell Lymphoma**
SPONSOR:	**Merck**
DOCUMENTS REVIEWED:	**Vol. 4**
REVIEW DIVISION:	**Division of Drug Oncology Products**
PHARM/TOX REVIEWER:	**S. Leigh Verbois, Ph.D**
PHARM/TOX SUPERVISOR:	**David Morse, Ph.D.**
DIVISION DIRECTOR:	**Robert Justice, M.D.**
PROJECT MANAGER:	**Paul Zimmerman**

Date of review submission to Division File System (DFS): September 13, 2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
The nonclinical studies submitted to this NDA provide sufficient information to support the use of vorinostat (ZOLINZA®) for [redacted]

B. Recommendation for nonclinical studies
None

C. Recommendations on labeling
See separate labeling review

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings
The pivotal nonclinical toxicity studies of vorinostat (AKA: SAHA, AP390, L-00107938, MK0683 and RG10) in support of this NDA were conducted, mimicking the clinical route of administration, in rats and dogs. Nonclinical studies included single- and repeat-dose toxicity studies, reproductive toxicity studies, genotoxicity studies and local tolerance studies. These studies were conducted in compliance with Good Laboratory Practice regulations. Nonclinical pharmacokinetics and toxicokinetics studies of vorinostat were also evaluated in rats and dogs.

B. Pharmacologic activity
Submitted data suggest vorinostat is acting as an inhibitor of histone deacetylase 1, 2, 3 (Class I) and 6 (Class 2) with nanomolar affinity. The activity of vorinotsat and its major metabolites were tested *in vitro* (murine erythroleukemia cells-MEL) for proliferation and HDAC1 inhibition. None of the major human metabolites inhibited HDAC1 activity at concentrations up to 10 μM, or inhibited MEL growth

Vorinostat, *in vivo* and *in vitro,* results in hyperacetylation of histones. Studies demonstrated a dose and time dependant accumulation of acetylated histones, cell cycle arrest (G1, G2/M phase) and apoptosis in some transformed cell linesand xenograft models. Inhibition of proliferation was evident i*n vitro* in the NCI cell line screen (GI50=38.6 nM -6.2 μM) and *in vivo* in various xenograft models. Induction of apoptosis and inhibition of proliferation has been shown to occur at concentrations which induce accumulation of hyperacetylated histones (≥600 nM). In addition, *in vitro,* vorinostat has been show to result in acetylation of HSP90, and tubulin when incubated with transformed cell lines. The acetylation

of HSP90 and tubulin is associated with reductions of pro-survival proteins HER2, BCL-X_L, BCL2, XIAP and Survivin.

The sponsor has asserted that the antineoplastic effects of vorinostat are associated with hyperacetylation of histones resulting from antagonism of HDACs. However, hyperacetylation of histones could also be the result of agonism of histone acetyltransferase. The activity of vorinostat on histone acetyltransferase has not been investigated, therefore the mechanism of the antineoplastic activity of vorinostat has not been fully characterized.

C. Nonclinical safety issues relevant to clinical use

Safety Pharmacology

The safety profile of orally administered and locally applied vorinostat was evaluated *in vitro* and *in vivo* in rats and dogs. Evaluation of CNS and pulmonary effects did not identify vorinostat-induced CNS or pulmonary toxicity at the highest doses evaluated (900 mg/m2) when administered to rats. Evaluation of cardiovascular toxicity was performed *in vitro* in transgenic CHO-K1 cells and *in vivo* in dogs. Evidence of potential cardiotoxicity was limited to 7-8% increase in hERG currents at 300μM and a ~25% increase in maximal heart rate following administration of ≥1800 mg/m2 [versus a clinical dose of 400 mg (222 mg/m2)].

Specificity as defined by off target inhibition of receptors by vorinostat was not evaluated.

Toxicology

Toxicities in rats and dogs after oral administration of vorinostat were predictive of adverse effects in humans (anorexia, weight loss, fatigue, hematologic suppression, and gastrointestinal [GI] effects).

In the 26-week GLP repeat-dose oral toxicity study of vorinostat in Sprague-Dawley rats, a significant, dose-dependent reduction in food consumption and body weight gain was observed in both females and males at doses of 50 mg/kg/day (300 mg/m2/day) or 150 mg/kg/day (900 mg/m2/day). Lower white blood cell counts (WBC; ↑20-70%) (primarily due to the lower lymphocyte counts, but also including monocytes, eosinophils, and neutrophils), decreased globulin (up to 40%) and increased absolute reticulocyte counts (↑ 162%) were observed at all doses in at least one sex at more than one interval. The magnitude of changes were does dependent. Decreased thymus weight, and splenic and thymic lymphoid depletion along with bone marrow erythroid hyperplasia/myeloid hypoplasia were treatment-related findings. These treatment-related findings are likely related to the mechanisms by which vorinostat induces cell differentiation and cell death. All these effects were partially or completely reversible by 4 weeks of recovery. Based on the findings at the lowest dose, a no-observable-adverse-effect level (NOAEL) could not be established in this study.

In the 26–week GLP repeat-dose oral toxicity study in beagle dogs with a 4-week recovery, no adverse effects were found at doses of 20 mg/kg/day (400 mg/m2/day) and 60 mg/kg/day (1200 mg/m2/day). The doses administered via capsule were 20, 60, or 80/100/125/160 mg/kg/day. Due to a lack of findings throughout the study in the high-dose group, dosing began at 80 mg/kg/day (1600 mg/m2/day) and was escalated in succession to 100 mg/kg/day (2000 mg/m2/day), 125 mg/kg/day (2500 mg/m2/day), and 160 mg/kg/day (3200 mg/m2/day) on Drug Days 16, 30, and 97, respectively. Reversible GI toxicity, characterized by non-formed or liquid feces, macroscopic (red foci) or microscopic (villous blunting with crypt epithelium regeneration, inflammation and necrosis in the large and small intestine) findings, caused by vorinostat was associated with the high-dose regimen (at the 160-mg/kg/day [3200 mg/m2/day]). Histologically, there was no evidence of serious, irreversible damage to any organ observed. No treatment-related findings at any dose were noted for the endpoints of mean body weight, mean food consumption, ophthalmologic abnormalities, electrocardiographic parameters, or blood pressure. The NOAEL of vorinostat was 60 mg/kg/day (1200 mg/m2/day) in this study.

Genetic Toxicology
Positive genotoxic effects were obtained for vorinostat *in vitro* assays (AMES Assay) for mutagenicity in the presence and absence of metabolic activation. vorinostat was positive for clastogenicity in an *in vitro* CHO cell assay in the presence and absence of metabolic activation and an *in vivo* mouse micronucleus assay. Additional testing was conducted in human peripheral lymphocytes. vorinostat was not found to be clastogenic in this assay, however, a confirmatory assay was not conducted.

Reproductive and Developmental Toxicology
Assessment of reproductive toxicity of vorinostat in showed early embryonic development (Segment I) impairments in female rats treated with 300 mg/m2. This was manifested by treatment-related increases in peri-implantation loss, a treatment-related increase in postimplantation loss secondary to increases in the percentage of resorptions and dead fetuses per implants. Decreases in the mean number of live fetuses/pregnant female (900 mg/m2/day) were also observed. The NOEL for embryonic development is 90 mg/m2. Based on a dose dependent increase in corpora lutea at 90 mg/m2, the lowest dose administered in the study, a NOEL for female fertility was not identified.

In male rats, there were no treatment related effects of vorinostat on reproductive parameters at doses up to 900 mg/m2 (the highest dose administered in this study).

In the absence of maternal toxicity, developmental toxicity was observed with vorinostat, 50 mg/kg/day, when administered rats (GD6-20) and rabbits (GD 7-20) at 300 mg/m2 and 240 mg/m2, respectively. In rats, findings consisted of marked decreases in weight and increases in fetuses with skeletal variations

(cervical ribs, supernumerary ribs and vertebral count, and sacral arch variations) and sites of incomplete ossifications (skull, thoracic vertebra and sternebra). In the rabbit, findings consisted of decreased fetal weight, incomplete ossification of the metacacarpals, increased incidence in 13th rib, and gall bladder malformations).

TK was not conducted concurrent with fertility and reproductive toxicology studies. Additional studies in the rat and rabbit to assess placental transfer of vorinostat conducted. Vorinostat, Vorinostat Glucoronide and N-phenyl-succinamic acid were found to cross the placenta and the fetus in both rats and rabbits. Fetal serum concentrations were found to be ≤50% that of maternal serum concentrations and to have a delay in the tmax of metabolites, indicative of slow transplacental transfer of vorinostat metabolites. In rats, following 300 and 900 mg/m2, the mean $AUC_{Vorinostat}$ was 0.13 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{Vorinostat\ glucuronide}$ was 0.04 and 0.61x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic.acid}$ was 0.62 and1.92x the observed clinical concentration. In rabbits, following 600 and 1800 mg/m2, respectively, the mean $AUC_{Vorinostat}$ was 0.12 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{Vorinostat\ glucuronide}$ was 0.19 and 0.52x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic\ acid}$ was 0.94 and4.5x the observed clinical AUC. TK was not determined for the lowest dose utilized in the rabbit study (240 mg/m2).

Carcinogenicity Carcinogenicity studies were not conducted and are generally not required to support the safety of the product for the proposed cancer indication.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

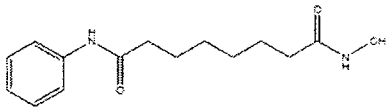
NDA number: 21991
Review number: 1
Sequence number/date/type of submission: 000/12-06-2005/RRP
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Merck
Manufacturer for drug substance: Merck

Reviewer name: S. Leigh Verbois, Ph.D.
Division name: Drug Oncology Products

Review completion date: September 13, 2006

Drug:

Trade name:	ZOLINZA®
Generic name:	Vorinostat
Code name:	SAHA, L-001079038, MK0683, RG-10, AP390
Chemical name:	suberoylanilide hydroxamic acid or N-hydroxy-N'-phenyl-octane-1, 8-diotic acid diamide,
CAS registry #:	149647-78-9
Molecular formula/MW:	$C_{14}H_{20}N_2O_3$; MW=264.32
Structure:	



Relevant INDs/NDAs/DMFs: 58915

Drug class: Histone deactylase inhibitor

Intended clinical population: Refractory Cutaneous T-Cell Lymphoma

Clinical formulation:

Ingredient	Reference	Function	mg/capsule
Vorinostat[1]	---	Active	100.0
Microcrystalline Cellulose	NF, Ph. Eur.		
Croscarmellose Sodium	NF, Ph. Eur.		
Magnesium Stearate	NF, Ph. Eur.		
Capsule Fill Weight	---	---	150.0
Size #3 Opaque White Hard Gelatin Capsules		Dosage Vehicle	approx. 48[2]
Black Printing Ink[3]		Product Identification	---

[1] Composed of
[2] The average empty capsule weight will be determined prior to manufacturing.
[3] The printing ink is applied to the hard gelatin capsule by the capsule manufacturer.

Route of administration: Oral

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

Studies reviewed within this submission:

Pharmacology		
Pharmacodynamics		
270-001	*In vitro* and *in vivo* pharmacological studies of vorinostat	4.2.1.1:PD001
Report 8	*In vivo* evaluation of SAHA inhibitors vs. mda-231 breast 8 HCT-116 colon tumor xenograft models	4.2.1.1:PD008
Report 5	Tolerability of Intraperitoneal SAHA (RG10) on a T.i.d. x 21 Schedule, and Comparison of Tumor Growth Inhibition on Qd x 21 and TID. x 21 Dosing Schedules, in Nude Mice Bearing the Human HCT-116 Colon Carcinoma	4.2.1.1:PD005
Report 6	Evaluation of Vorinostat (SAHA) Administered by Intravenous Infusion or Intraperitoneally in Nude Rats Bearing the Human HCT 116 Colon Carcinoma	4.2.1.1:PD003
Report 7	Evaluation of 24 hour and 8 hour CIV infusions of vorinostat (SAHA, RG10) in nude rats bearing the human HCT-116 colon carcinoma	4.2.1.1:PD006
Safety Pharmacology		
TT #04-4720	L-001079038: Cellular Electrophysiological Evaluation of a Histone Deacetylase Inhibitor on HERG.	4.2.1.3:TT044720
TT #05-5654	Oral Respiratory Study in Rats	4.2.1.3:TT055654
TT #05-5655	Oral FOB Study in Rats	4.2.1.3:TT055655
TT #05-5656	Oral CV Telemetry Study in Dogs	4.2.1.3:TT055656
Pharmacokinetics		
TT #03-5518	Analytical Report for the Determination of SAHA, SAHA Glucuronide and N-Phenyl-Succinamic Acid in Rat Serum (26 wk)	4.2.3.2:TT035518

TT #03-5519	Analytical Report for the Determination of SAHA, SAHA Glucuronide and N-Phenyl-Succinamic Acid in Dog Serum	4.2.3.2:TT035519
TT #035502	26-Week Repeat Dose Toxicity and Toxicokinetic Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 13-Week Interim Sacrifice and a 4-Week Recovery	4.2.3.2:TT035502
TT #04-5504	26-Week Repeat Dose Toxicity and Toxicokinetic Study in Beagle Dogs Administered AP390 Capsules with a 4-Week Recovery	4.2.3.2:TT045504
TT #04-7450	Oral TK Study in Rats with Evaluation of Placental Transfer	4.2.3.5.2:TT047450
TT #04-7480	Oral TK Study in Rabbits with Evaluation of Placental Transfer	4.2.3.5.2:TT047480
Absorption		
PK001	High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of SAHA and its Metabolites, SAHA Glucuronide and N-Phenyl-Succinamic acid in Rat Serum	4.2.2.2:PK001
PK002	High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of SAHA and its Metabolites, SAHA Glucuronide and N-Phenyl-Succinamic acid in Dog Serum	4.2.2.2:PK002
PK003	PK of L-001079038 in Male SD Rats Male Beagle Dogs following IV and Oral Administration	4.2.2.2:PK003
PK004	Analysis of Caco-2 Permeability of SAHA	4.2.2.2:PK004
Distribution		
TT #04-5506	In-vitro Evaluation of SAHA Binding to Plasma Proteins by Ultrafiltration	4.2.2.3:TT045506
Metabolism/Excretion		
PK005	*In vitro* and Cytochrome P450 Gene Expression studies with L-001079038	4.2.2.3:PK005
PK006	Excretion and Metabolism of [14C]L-001079038 in Rats and Dogs	4.2.2.3:PK006
TT #04-5507	*In vitro* Metabolic Profiling of SAHA in S9 Fractions from Dog, Rat, Monkey, and Human Livers	4.2.2.4:TT045507
PK007	*In vitro* Evaluation of SAHA as an inducer of CYP450 Expression	4.2.2.4:PK007
Toxicology		
Single Dose		
TT #04-2511	L-001079038: Exploratory Acute Oral Range-Finding Study in Mice	4.2.3.1:TT042511
TT #92-5513	Exploratory Acute Tolerance Study for WIN 50829 and WIN 64652 adminsitered IV to Mice	4.2.3.1:TT925513
TT #94-5531	WIN 64652: An Acute IV Infusion Dose-Ranging Study in Mice	4.2.3.1:TT945531

TT #94-5532	WIN 64652: An Acute Intravenous Infusion Tolerance Study in Mice	4.2.3.1:TT945532
TT #93-5503	Exploratory Acute Tolerance Study for WIN 50829, WIN 64652, WIN 65397, WIN 65593, WIN 65594, and WIN 65595 Administered IV to Rats	4.2.3.1:TT935503
TT #94-5530	WIN 64652 A Single Dose Four-Hour Intravenous Infusion Toxicity and Dose Range-Finding Study with Plasma Concentration Analysis in Dogs (Protocol 00416)	4.2.3.1:TT945530
Repeat Dose		
TT #03-5511	Four-Week Repeat Dose Toxicology Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 2-Week Recovery	4.2.3.2:TT035511
TT #035502	26-Week Repeat Dose Toxicity and Toxicokinetic Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 13-Week Interim Sacrifice and a 4-Week Recovery	4.2.3.2:TT035502
TT #035512	Four-Week Repeat Dose Toxicology Study in Beagle Dogs Administered AP390 Capsules with a Recovery Period	4.2.3.2:TT035512
TT #04-5504	26-Week Repeat Dose Toxicity and Toxicokinetic Study in Beagle Dogs Administered AP390 Capsules with a 4-Week Recovery	4.2.3.2:TT045504
	Genotoxicity	
TT #93-5506	WIN 64652: *Salmonella*/Mammalian Microsome Preincubation Mutagenicity Assay (AMES Test) and *Escherichia coli* WP2*uvr*A Reverse Mutation Assay with ConfirmatoryAssay	4.2.3.3.1LTT935506
TT #04-8026	Microbial Mutagenisis Assay with Preincubation	4.2.3.3.1:TT048026
TT #93-5505	WIN 64652: *In vitro* Chromosome Aberration Analysis in CHO Cells	4.2.3.3.1:TT935505
TT #04-8633 TT #04-8639 TT #04-8651	Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells TT #04-8633, TT 004-8639, and TT #04-8651	4.2.3.3.1:TT048633
TT #04-5501	Chromosomal Aberrations in Cultured Purified Human Blood Lymphocytes with AP390	4.2.3.3.1:TT045501
TT#03-5510	Screening Assay for Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes	4.2.3.3.1:TT035510
TT#05-8662 TT#05-8818	L-001079038: Exploratory Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells (TT #05-8662) and Exploratory Flow Cytometric Assay for Measuring DNA Synthesis Inhibition and Cell Cycle Kinetics (TT #05-8818) in Chinese Hamster Ovary Cells.	4.2.3.3.1:TT058662
TT #04-8626	L-001079038 Assay for Micronucleus Induction in Mouse Bone Marrow	4.2.3.3.2:TT048626

Reproductive and Developmental Toxicity		
TT #04-7360	L-001079038 Oral Fertility in Female Rats	4.2.3.5.1:TT047360
TT #04-7440	L-001079038 Oral Fertility in Male Rats	4.2.3.5.1:TT047440
TT #04-7295	L-001079038 Oral Range-Finding Reproduction Study in Female Rats	4.2.3.5.1:TT047295
TT# 04-7290	L-001079038 Oral Developmental Toxicity Study in Rats With Prenatal Evaluation	4.2.3.5.1:TT047290
TT #04-7450	L-001079038 Oral Toxicokinetic Study in Rats With Evaluation of Placental Transfer	4.2.3.5.1:TT047450
TT #04-7305	L-001079038 Oral Range-Finding Study in Pregnant Rabbits	4.2.3.5.2:TT047305
TT #04-7300	L-001079038 Oral Developmental Toxicity Study in Rabbits	4.2.3.5.2:TT047300
TT #04-7380	L-001079038 Oral Toxicokinetic Study in Rabbits With Evaluation of Placental Transfer	4.2.3.5.2:TT047380
Local Tolerance		
TT #04-5518	L-001079038-000K: Local Lymph Node Assay (LLNA) in Mice	4.2.3.6:TT045518
TT #04-5517	L-001079038-000K: Acute Dermal Irritation Study in Rabbits	4.2.3.6:TT045517
TT #04-5503	Bovine Corneal Opacity and Permeability Assay	4.2.3.6:TT045503
TT #04-5504	Skin Irritation Test Using the — Skin Model with Optional IL-1α	4.2.3.6:TT045504

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

SAHA was evaluated in numerous in vivo and in vitro models of cancer therapy. SAHA is a histone deacetylase inhibitor with affinity for numerous subtypes as described below.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

***In vitro* and *in vivo* Pharmacological Studies of Vorinostat (PD001, Volume 4.2.1.1, non-GLP)**

Inhibition of HDAC activity by vorinostat

- SAHA inhibits of HDAC1, 2, 3 (Class I) and 6 (Class II) with approximately equal nanomolar affinity and has approximately 10-fold less inhibitory activity (as determined with the — Flourescence Microscopy Assay*) against HDAC8 (Class I).

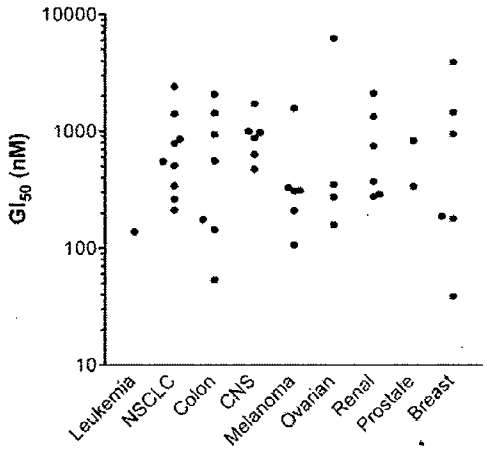
Enzyme	IC50 ± SEM (nM)	N	Expression Construct	Purification Method
HDAC1	30 ± 9	5		
HDAC2	49	1		
HDAC3	86 ± 7	4		
HDAC4-FL	>10,000	3		
HDAC4-CD	>10,000	3		
HDAC4-GOF	408	4		
HDAC5	58% at 5000	1		
HDAC6	37± 5	4		
HDAC7	>5000	1		
HDAC8	779 ± 183	5		
SIRT1	>100,000	1		Purchased Purified

Lots utilized- 0010709038-000K020, L-00107903800K001, L-001079038-000K005

[redacted]™ utilized for the SIRT1 Assay.

Inhibition of proliferation in transformed cell lines

The ability of SAHA to inhibit proliferation in transformed cell lines was determined using the NCI *in vitro* cell line screen. Growth inhibitory activity was equivalent throughout the panel (38.6 nM-6.2 μM), with no one tumor type more sensitive than another.



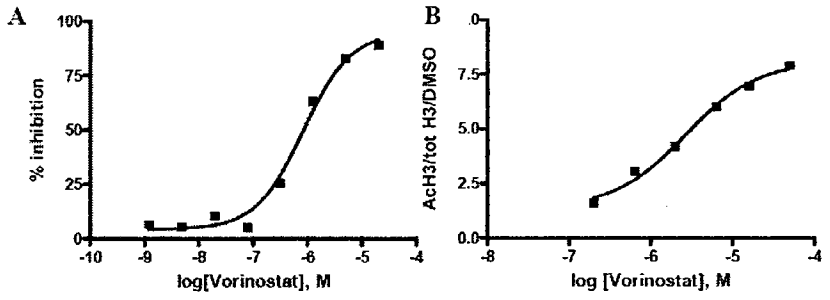
†Growth was determined by SRB assay on day 2 of treatment.
†Points on graph represent individual GI_{50} results for each cell line.

Excerpted from the sponsor's submission

Inhibition of proliferation and induction of histone hyperacetylation in human HCT-116 colon carcinoma cells:Vorinostat induces cell cycle arrest or apoptosis of transformed cells at concentrations which induce accumulation of acetylated histones.

- Using bioluminescence to determine proliferation and ELISA to measure histone acetylation levels, SAHA inhibits proliferation of HCT-116 cells with an IC50 of approximately 800 nM. A 3 fold increase in acetylated histone H3 was detectable following a 6 hour incubation with 600 nM SAHA.

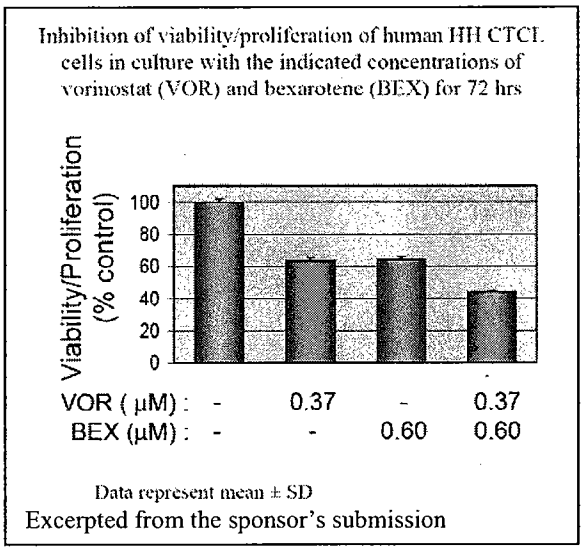
Vorinostat (**A**) inhibits proliferation (48 hr culture) and (**B**) induces histone H3 hyperacetylation (6 hr culture) in HCT-116 colon carcinoma cells



Excerpted from the Sponsor's submission

SAHA Inhibition of proliferation in human HH cutaneous T cell Lymphoma cells in combination with bexarotene

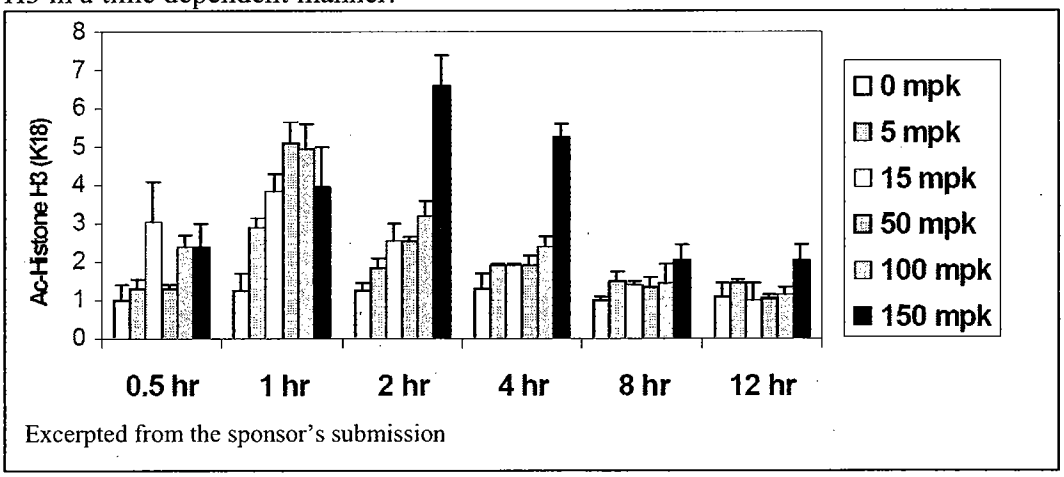
- The effect of SAHA (0-30 μM) and bexarotene (0.01, 0.06, 0.1, 0.6, 3 and 6μM) combinations on the proliferation of HH cells was evaluated following 72 hr culture using bioluminescence.
- A less than additive effect on viability/proliferation was noted with combination treatment.



Excerpted from the sponsor's submission

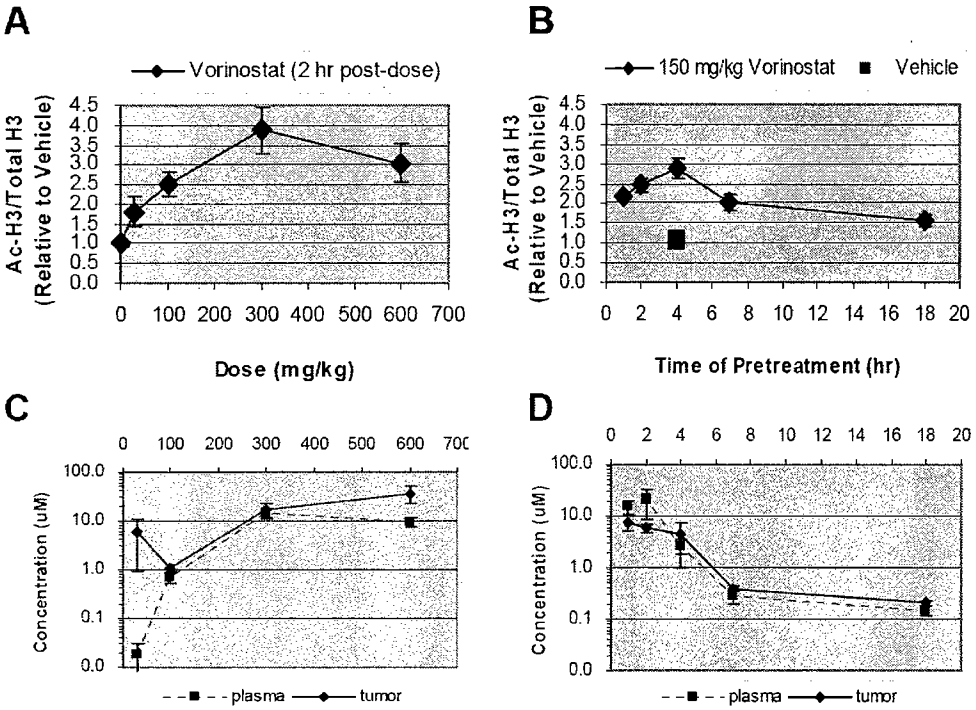
Histone hyperacetylation and SAHA plasma and tumor levels in HCT-116 xenograft models following a single dose in mice

- SAHA (5, 15, 50, 100, or 150 mg/kg) administered mice bearing HCT-116 xenografts. Tumors were collected at 0.5, 1, 2, 4, 8, and 12 hours post-dose. Histone acetylation determined with ELISA.
- SAHA administration resulted in a dose dependent accumulation of acetylated histone H3 in a time dependent manner.



Excerpted from the sponsor's submission

- SAHA (30, 100, 300, or 600 mg/kg single dose IP) was administered to mice once tumor xenografts (450-550 mm3). PK and tumor samples obtained at 2 hours post dose. SAHA (150 mg/kg, single dose IP) was also administered and tumor and PK samples were obtained at 1, 2, 4, 7 and 18 hours post-dose)
- Accumulation of acetylated histone H3 in tumors and plasma is dose (Figures A and C) and time dependent (Figures and D)

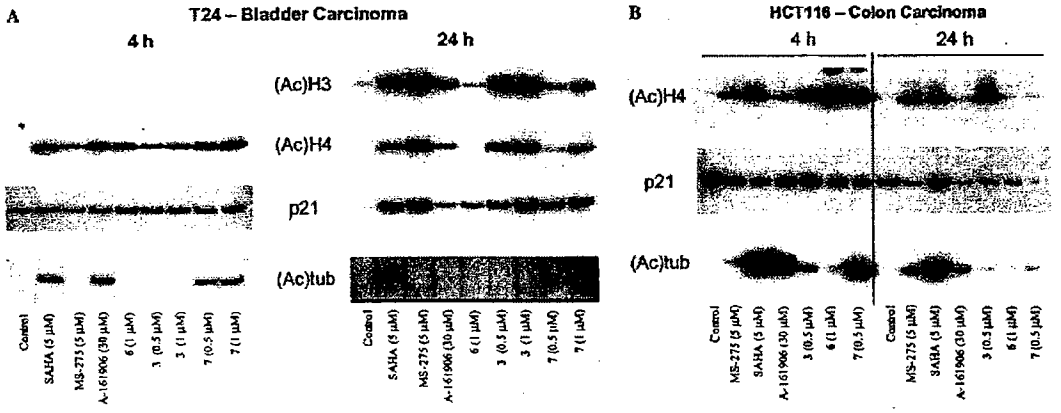


Tubilin and HSP90 (non-histone proteins) are acetylated on lysine residues and undergo hyperacetylation following exposure to SAHA[1,2]

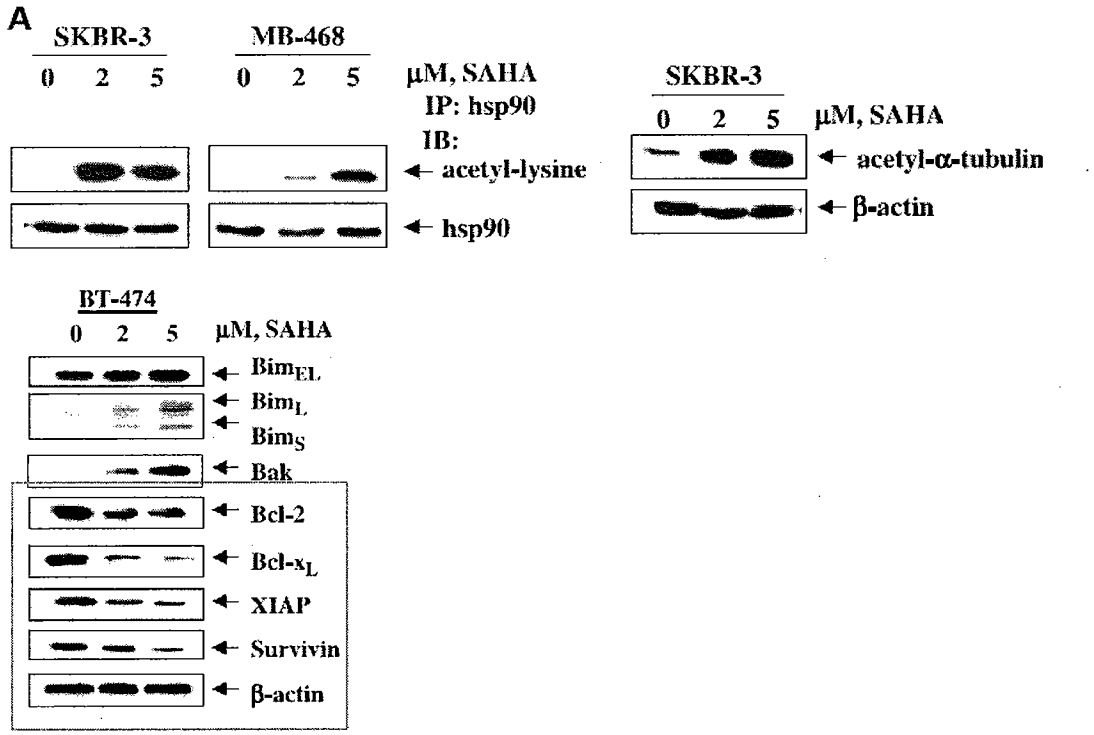
- SAHA (5μM) incubation of T24 bladder carcinoma cells and HCT116 colon carcinoma cells for 4 and 24 hours caused a hyperacetylation of tubulin (see figure excerpted from Glaser et al. 2004.)

APPEARS THIS WAY ON ORIGINAL

[1] Glaser KB, Li J, Pease LJ, Staver MJ, Marcotte PA, Guo J, et al. Differential protein acetylation induced by novel histone deacety-lase inhibitors. Biochem Biophys Res Commun 2004;325:683-90.
[2] Bali P, Pranpat M, Swaby R, Fiskus W, Yamaguchi H, Balasis M, et al. Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of Her-2. Clin Cancer Res 2005;11(17):6382-9.



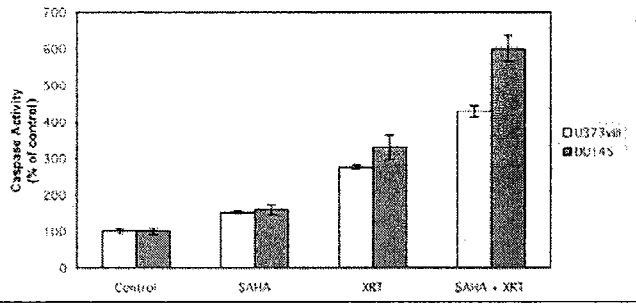
- SAHA (2 and 5 μM) incubation for 48 hours resulted in a hyper acetylation of HSP90 and tubulin in SKBR3 and MB468 cells (Figure A excerpted from Bali et al, 2005), which leads to a decrease in pro survival proteins Her 2, Bcl-X_L, Bcl2, XIAP and Survivin (Figures B excerpted from Bali et al, 2005)



In some but not all cultured human transformed cell lines, vorinostat has additive activity in combination with radiation, tyrosine kinase inhibitors, cytotoxic agents and differentiating agents.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
See table	50 nM to 5μM (96 hrs)	none	**Table 1.** Inhibition of proliferation (ED50) of lymphoid cell lines by VPA and SAHA	3

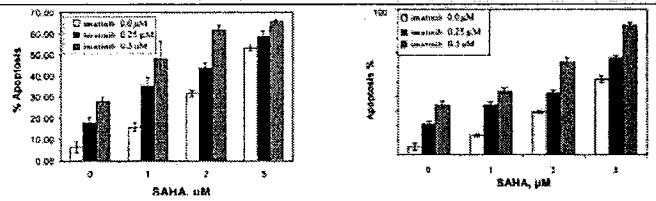
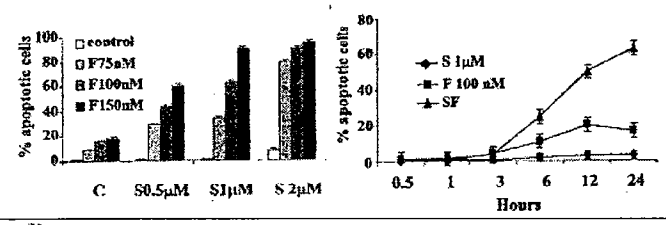
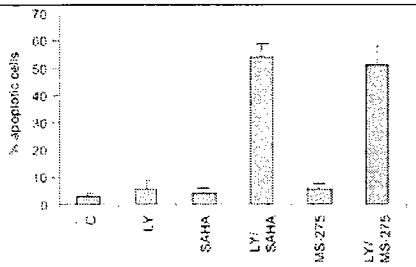
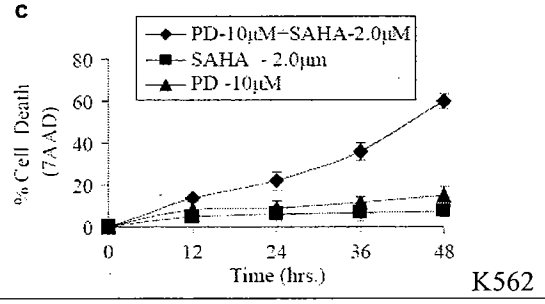
		VPA	SAHA
(A) Burkitt's lymphoma	Ramos	1.7×10^{-3} M	2.2×10^{-6} M
	Raji	9.3×10^{-4} M	2.0×10^{-6} M
	Daudi	2.2×10^{-3} M	2.5×10^{-6} M
(B) ALL (B-cell)	Blin1	7.3×10^{-4} M	1.4×10^{-6} M
	Nalm6	9.0×10^{-4} M	1.2×10^{-6} M
	Reh	1.0×10^{-3} M	1.6×10^{-6} M
(C) MCL	Jeko1	1.0×10^{-3} M	1.3×10^{-6} M
	NCEB1	1.3×10^{-3} M	9.6×10^{-7} M
	Granta	N.D.	9.6×10^{-7} M
(D) DLBCL	SUDHL6	1.3×10^{-3} M	8.3×10^{-7} M
	SUDHL16	9.3×10^{-4} M	1.9×10^{-6} M
(E) ATL	SLB-1	1.3×10^{-3} M	2.1×10^{-6} M
(F) ALL (T-cell)	Jurkat	N.D.	6.5×10^{-7} M
	RPMI8402	N.D.	7.3×10^{-7} M

MJ Hut78 HH (CTCL lines) PBL from patients with atypical	2.5 and 5 μM (48 hr)	none	• Dose dependent apoptosis (annexin binding) MJ: 9 and 24% Hut 78: 40 and 56% HH: 61 and 75%	4
DU145 (Prostate Carcinoma)	1μM for (4 hours)	6 Gy XRT		5

[3] Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP. Histone deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell lines. Exp Hematol 2005;33:53-61.

[4] Zhang C, Ni X, Talpur R, Richon V, Duvic M. SAHA, a histone deacetylase inhibitor, induces apoptosis in cutaneous T cell lymphoma cells and is synergistic with 5-Aza-2 deoxycytidine [abstract]. 0683_0700, 2003.

[5] Chinnaiyan P, Vallabhaneni G, Armstrong E, Huang S-M, Harari PM. Modulation of radiation response by histone deacetylase inhibition. Int J Radiat Oncol Biol Phys 2005;62(1):223-9.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
K562 (CML) and LAMA-84 (Leukocytic)	0-5 μM (48 hr)	0-0.5μM Gleevec		6
U937 (lymphoma)	0-2 μM (varied)	0-150 nM Flavopiridol SAHA + Floavopiridol (SF)		7 8
U937	1μM (24 hr)	30 μM LY294002 (Akt inhitior) 1 μM MS275 (HDACi)		9
K562 LAMA84	2.0 μM (48 hr)	10 μM PD184352 (MEK1/2 Inhib)		10

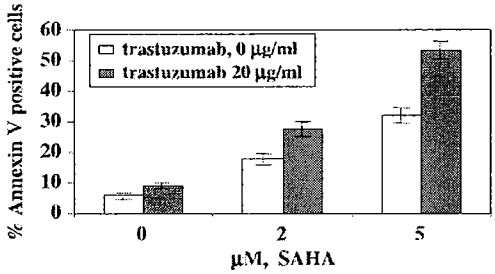
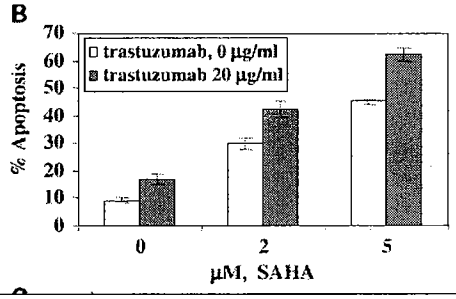
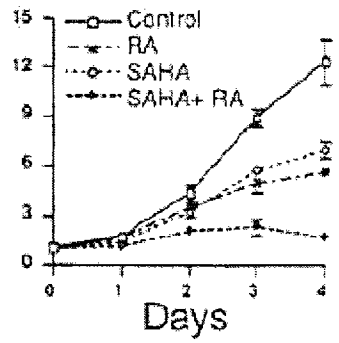
[6] Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. Blood 2003;101(8):3236-9.

[7] Almenara J, Rosato R, Grant S. Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). Leukemia 2002;16:1331-43.

[8] Gao N, Dai Y, Rahmani M, Dent P, Grant S. Contribution of disruption of the nuclear factor-KB pathway to induction of apoptosis in human leukemia cells by histone deacetylase inhibitors and flavopiridol. Mol Pharmacol 2004;66(4):956-63.

[9] Rahmani M, Yu C, Reese E, Ahmed W, Hirsch K, Dent P, et al. Inhibition of PI-3 kinase sensitizes human leukemic cells to histone deacetylase inhibitor-mediated apoptosis through p44/42 MAP kinase inactivation and abrogation of p21 CIP1/WAF1 induction rather than AKT inhibition. Oncogene 2003;22:6231-42.

[10] Yu C, Dasmahapatra G, Dent P, Grant S. Synergistic interactions between MEK1/2 and histone deacetylase inhibitors in BCR/ABL + human leukemia cells. Leukemia 2005;19:1579-89.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
BT474 (Mammary) SKBR3 (breast)	0-5μM (48 hr)	20μM Trastuzumab (48 hr)	A  B  	[11]
NB4 cells (Promyelocytic)	0.6μM	1 Retinoic Acid μM	 	[12]

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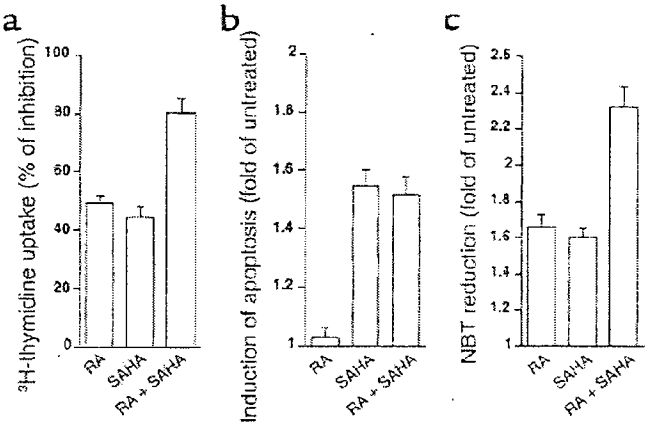
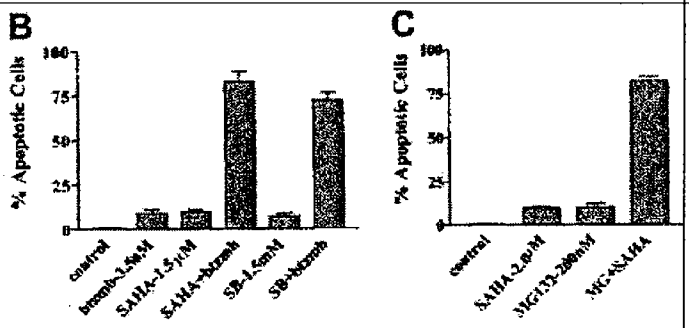
[11] Bali P, Pranpat M, Swaby R, Fiskus W, Yamaguchi H, Balasis M, et al. Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of Her-2. Clin Cancer Res 2005;11(17):6382-9.

[12] He L-Z, Tolentino T, Grayson P, Zhong S, Warrell RP, Jr., Rifkind RA, et al. Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leuke-mia. J Clin Invest 2001;108(9):1321-30.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
PLZF-RARα/RARαPLZF double TM	0.6 μM (44 hours)	1μM Retinoic Acid		9
K562 LAMA 84	1.5 μM (48 hrs) 2.0μM (48 hr)	4.5 nM Bortezomib 200 nM MG132 (proteosome inhibitor) 1.5 mM sodium butyrate (SB)		13

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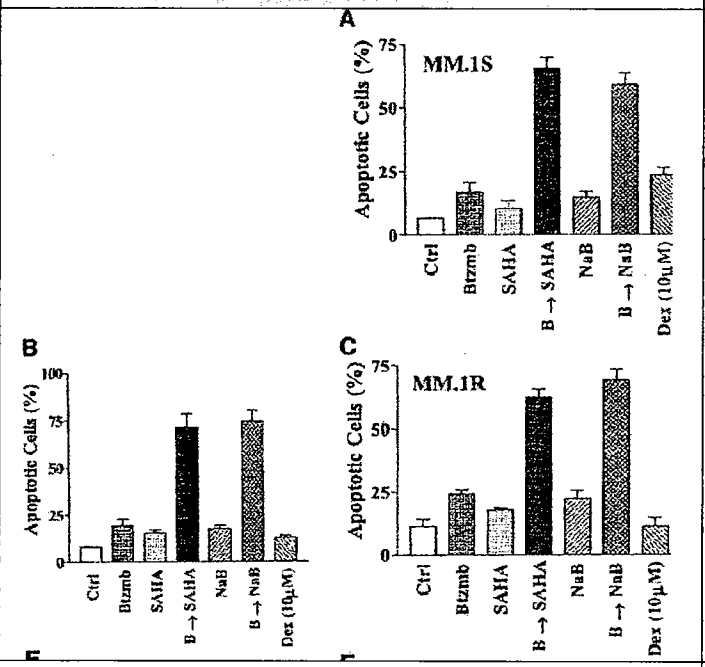
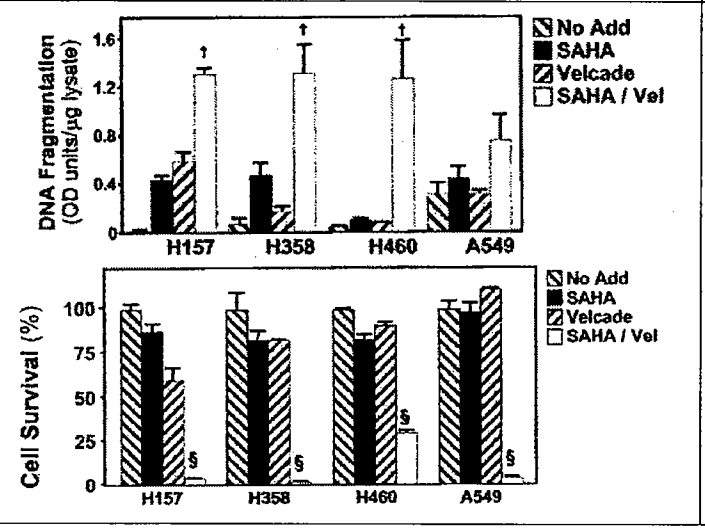
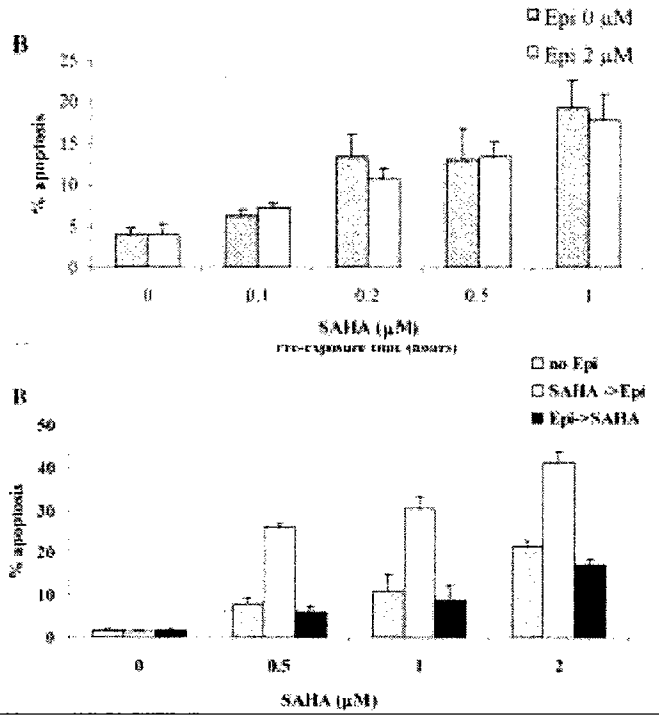
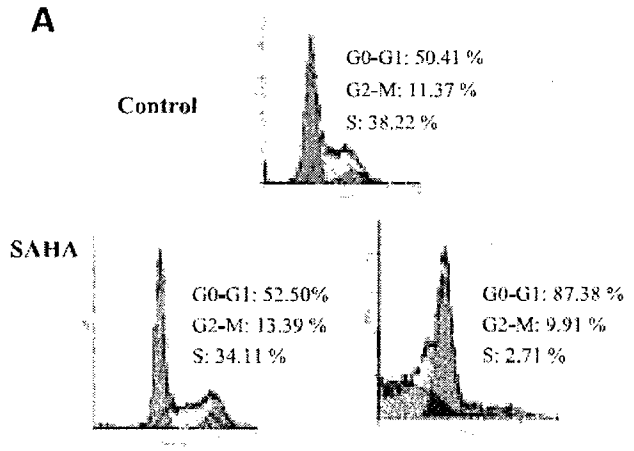
[13] Yu C, Rahmani M, Conrad D, Subler M, Dent P, Grant S. The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571. Blood 2003;102(10):3765-74.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
U266 MM.1S MM.1R (myeloma)	1μM (20 hours) Initiated 6 hours after BTZ	2.4 nM Bortezomib (BTZ) 1mM sodium butyrate (SB) Dexamethasone (Dex) → indicated of serial treatment		14
H157 H358 H460 A549 (NSCLC)	5 μM (24, 48, 72 hr)	50 nM Velcade(24 hr)		15

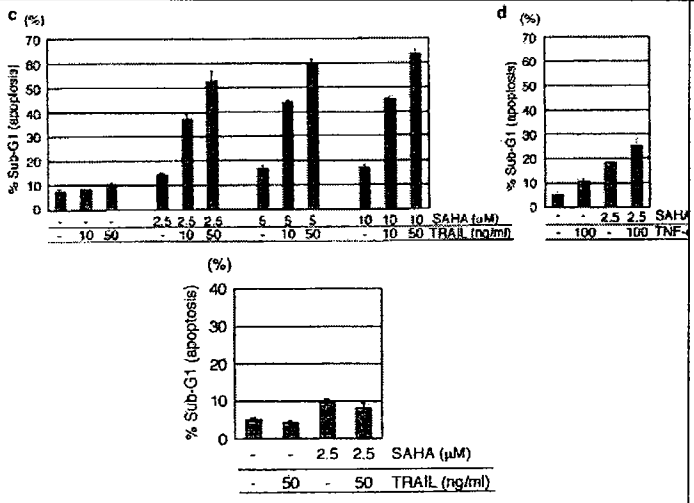
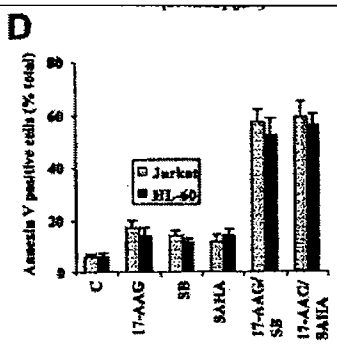
[14] Pei X-Y, Dai Y, Grant S. Synergistic induction of oxidative injury and apoptosis in human multiple myeloma cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitors. Clin Cancer Res 2004;10:3839-52.

[15] Denlinger CE, Rundall BK, Jones DR. Proteasome inhibition sensitizes non-small cell lung cancer to histone deacetylase inhibitor-induced apoptosis through the generation of reactive oxygen species. J Thorac Cardiovasc Surg 2004;128(5):740-8.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
MCF7 (Breast)	0-2 μM (48 hours)	0.5 μM Epirubicin (4 hours)	Potentiation of epirubicin toxicity is schedule dependent. 	16
U937	2μM (24 and 48 hours)	none	 Cell cycle arrest is observed with 2μM SAHA following 24 and 48 hours (left and right figure, respecitively).	17

[16] Marchion DC, Bicaku E, Daud AI, Richon V, Sullivan DM, Mun-ster PN. Sequence-specific potentiation of topoisomerase II inhibitors by the histone deacetylase inhibitor suberoylanilide hydroxamic acid. J Cell Biochem 2004;92:223-37.

[17] Rosato RR, Almenara JA, Dai Y, Grant S. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells. Mol Cancer Ther 2003;2(12):1273-84.

Cell Line	[Vorinostat] (time)	[Additional treatment	Activity of SAHA (excerpted from literature)	
Jurkat (T cell Leukemia)	2.5-10 μM (24 h)	10 -50 ng/mL TNF related apoptosis inducing ligand		18
HL60 (promyelocytic) Jurkat (lymphblastic)	0.75 μM (48 hr)	500 nM 17AAG for HL60 2000 nM 17AAG fro Jurkat		19

Drug activity related to proposed indication:

Vorinostat demonstrates anti-tumor activity in a variety of rodent xenograft tumor models including prostate, breast, and colon.

Reports reviewed within the section include and various literature references:

- **Report 5: Tolerability of Intraperitoneal SAHA (RG10) on a T.i.d. x 21 Schedule, and Comparison of Tumor Growth Inhibition on Qd x 21 and TID. x 21 Dosing Schedules, in Nude Mice Bearing the Human HCT-116 Colon Carcinoma (PD005, Volume 4.2.1.1, non-GLP)**
- **Report 6: Evaluation of Vorinostat (SAHA) Administered by Intravenous Infusion or Intraperitoneally in Nude Rats Bearing the Human HCT 116 Colon Carcinoma (PD003, Volume 4.2.1.1, non-GLP)**

[18] Nakata S, Yoshida T, Horinaka M, Shiraishi T, Wakada M, Sakai T. Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells. Oncogene 2004;23:6261-71.

[19] Rahmani M, Yu C, Dai Y, Reese E, Ahmed W, Dent P, et al. Coadministration of the heat shock protein 90 antagonist 17- allylamino-17-demethoxygeldanamycin with suberoylanilide hy-droxamic acid or sodium butyrate synergistically induces apopto-sis in human leukemia cells. Cancer Res 2003;63:8420-7.

- **Report 7: Evaluation of 24 hour and 8 hour CIV infusions of vorinostat (SAHA, RG10) in nude rats bearing the human HCT-116 colon carcinoma (PD006, Volume 4.2.1.1, non-GLP)**
- **Report 8: *In vivo* evaluation of SAHA inhibitors vs. mda-231 breast 8 HCT-116 colon tumor xenograft models (PD008, Volume 4.2.1.1, non-GLP)**

Study Report	Model		N/ group	Route	Dose/day (mg/kg)	Duration	Major Findings
PD003	HCT116 (colon)	Rats	6-8 ♀	CIV	25	14 days	5% Growth Inhibition (GI)
					50		77% GI
				IP	150/QD	12 days	27% GI
PD005	HCT116	Mice	10 ♀	IP	100/QD	21 days	16% GI
					150/QD		57% GI
					5/TID		NS
					15/TID		NS
					50/TID		NS
					100/TID		53% GI
					150/TID	10 days*	Intolerable
PD006	HCT116	Rats	5 ♀	CIV	50	7 days	59% GI
				8 hr inf	50	14 days	64% GI
					100	14 days	Intolerable
PD008	HCT116	Mice	8-9 ♀	IP	25/QD	23 days	NS
					100/QD	23 days	49% GI
	MDA231 (breast)	Mice	8-9♀	IP	25/QD	23 days	34% GI
Butler et al[20]	CWR22 (prostate)	Mice	5-9♂	IP	25/QD	21 days	78% GI; regression in 1/8
					50/QD	21 days	97% GI; regression in 5/9
					100/QD	21 days	97% GI; regression in 3/5
He et al[21]	T(11;17 APL)	trans mice	11	IP	50/QD	28 days	22 day ↑ in survival; 43 day compared with 21 day placebo
[22]Desai et al.	NNK induced Lung tumors	Mice	10♀	PO-food	450 ppm	18 weeks; 1 week before NNK	80% inhibition of tumor multiplicity
	MDA-	Mice	5-7♀	PO	86 QD	18 days	NS

[20] Butler LM, Agus DB, Scher HI, Higgins B, Rose A, Cordon-Cardo C, et al. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in Vitro and in Vivo. Cancer Res 2000;60:5165-70.

[21] He L-Z, Tolentino T, Grayson P, Zhong S, Warrell RP, Jr., Rifkind RA, et al. Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. J Clin Invest 2001;108(9):1321-30

[22] Desai D, Das A, Cohen L, El-Bayoumy K, Amin S. Chemopre-ventive efficacy of suberoylanilide hydroxamic acid (SAHA) against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)- induced lung tumorigenesis in female A/J mice. Anticancer Res 2003;23:499-503.

[23] — Chemotherapy evaluation for SAHA (Aton), Jan- 2004.

Study Report	Model		N/ group	Route	Dose/day (mg/kg)	Duration	Major Findings
	MB-231 (breast) A549 (lung)				144 QD	18 days	NS
					240 QD	18 days	NS
					400 QD	18 days	NS
	A549-(lung)	Mice	20♀	PO	120 QD	21 days	77% GI; max on day 11
	HCT15 (colon)	Mice	10♀	PO	120 QD	21 days	45% GI; max on day 11

***reduced schedule due to toxicity**
NS-not significant; APL-Acute Promyeolocytic Leukemia

[24] Profiling of HDAC inhibitors in vivo, 31-Jul-2003.

2.6.2.3 Secondary pharmacodynamics

***In vitro* and *in vivo* Pharmacological Studies of Vorinostat (PD001, Volume 4.2.1.1, non-GLP)**

- Specificity of SAHA inhibition (10μM was assessed in 25 enzyme assays at — — (only summary submitted). Vorinostat exhibited significant inhibitory activity of CYP450 2C19 (80%) and 2D6 (64%). No other significant (= 50%) inhibitory activity was observed in this panel of enzyme assays at the tested concentration of 10 μM. No other information regarding other enzymes assayed were presented.
- The activity of SAHA and its major metabolites were tested *in vitro* (murine erythroleukemia cells-MEL) for proliferation and HDAC1 inhibition. None of the major human metabolites inhibited HDAC1 activity at concentrations up to 10 μM nor inhibited MEL growth (See table excerpted from the sponsor's submission).

Analyte	HDAC1 IC_{50} (μM)[a]	MEL IC_{50} (μM)[b]
Vorinostat	0.07	0.66
Vorinostat glucuronide	>10	>20
Vorinostat succinate	>10	>20
Vorinostat adipate	>10	>20
Vorinostat primary amide	>10	>20
Vorinostat acid	>10	>20
Acetanilide	>10	>20

[a] IC_{50} for inhibition of affinity purified HDAC1 [(Aton) Notebook/page: AP47/23-24]
[b] IC_{50} for inhibition of MEL cell growth [(Aton) Notebook/page: AP50/3. 4. and 11]

2.6.2.4 Safety pharmacology

Neurological effects:

L-001079038: Oral Functional Observational Battery Study in Rats (TT#05-5655, Volume 4.2.1.3, GLP)

The CNS pharmacology of L-001079038 following a single oral dose of L-001079038 was administered as a single oral dose to male rats to determine its effects on central nervous system function.

Assessments consisted of home cage, hand-held, and open-field observations, stimulus activity responses, and grip strength, foot splay, and body temperature measurements were performed approximately 30 minutes after administration of vehicle or 20, 50, or 150 mg/kg of L-001079038 or vehicle (1.0% (w/v) Carboxymethylcellulose Sodium (CMC; Medium Viscosity)/0.5% (v/v) Polysorbate 80 in Deionized Water). There were no treatment-related neurobehavioral effects observed. Oral administration of L-

001079038 did not induce any modification of behavior or body temperature with administration of up to 150 mg/kg at 30 minutes post-dose (~Cmax).

The dose 150 mg/kg is determined to be the NOEL for behavioral effects in male rats.

Cardiovascular effects:

L-001079038: Cellular Electrophysiological Evaluation of a Histone Deacetylase Inhibitor on HERG. (TT #04-4720; Volume 4.2.1.3, non-GLP)

The effect of L001079038 (100 and 300 μM, solubility issues at concentration higher than 300 μM) on *in vitro* hERG current was evaluated to assess the potential for delayed repolarization and prolongation of the QT interval. HERG (human-Ether-a-go-go Related Gene) is a gene encoding the pore forming subunit of a human delayed rectifying potassium channel, and blockade of hERG current has been associated clinically with delayed repolarization and proarrythmic responses in humans. Current was elicited with a voltage step from -80 mV to a test potential of +20 mV or when by a voltage ramp designed to roughly mimic a cardiac action potential. These conditions elicited a ≤8% change and ≤7% change, respectively; therefore the highest concentration resulted in minimal effect of hERG current.

L-001079038 Oral Cardiovascular Telemetry Study in Dogs (TT #05-5656, Volume 4.2. 1.3, GLP*)

*Metabolite characterized with non-GLP metabolite standards.

The effect of oral (capsule) L-001079038 on cardiovascular parameters and body temperature was assessed in telemetered female dogs (n=4/sex). L-001079038 was administered at successive doses of 30, 90 and 240 mg/kg producing plasma concentrations of 0.24 ± 0.07, 0.35 ± 0.18, and 0.96 ± 0.35 μM, respectively, at 1 hours post-dose. L-000341257 (4-anilino-4-oxobutanoic acid) concentrations were 1.81 ± 0.52, 2.28 ± 1.25, and 3.99 μM, and L-001302381 (SAHA-O-Glucuronide) were 0.19 ± 0.05, 0.25 ± 0.13, and 0.67 ± 0.12 at 1 hour post-dose.

- No effects on systolic, diastolic or mean arterial blood pressure, QRS interval or QTcf were observed. Drug related findings are summarized in the table below.

	90 mg/kg	240 mg/kg
HR	↑24% from baseline of 95 ± 6 bpm up to 5 hrs post-dose; peak at 3.75 hrs.	↑25% from baseline of 95 ± 3 bpm up to 20 hours, peak at 9.25 hrs
PR	↓9% from baseline of 0.091± .003 sec up to 5 hrs post-dose; peak at 3.75 hrs.	↓ 9% from baseline of 0.087±0.003 sec up to 20 hours, peak at 9.25 hrs
QT	↓5% from baseline of 0.219 ±0.011 sec up to 5 hours, peak at 4.5 hrs	↓12% from baseline of 0.233 ± 0.010 sec up to 5 hours, peak 3.75 hrs

- Given PK was not conducted it is not possible to relate peak QT changes with peak plasma concentration for this study, however in other PK and toxicology studies, Tmax of L-001079038 and it's primary metabolites occurred at < 1.0 hours post-dose.

Pulmonary effects:

L-001079038 Oral Respiratory Study in Rats (TT #05-5654; Volume 4.2.1.3; GLP)

The effect of oral L-001079038 (20, 50, and 150 mg/kg) on pulmonary function was evaluated in conscious male Sprague Dawley rats (n=6/dose). Indices of pulmonary function [respiratory rate, tidal volume, minute ventilation, and PenH (i.e. airway resistance)] were measured approximately 120 minutes prior to dosing and continued 6 hour post dose every 10 minutes.

All parameters measured varied in all groups immediately following dosing. These changes were due to the excitatory reaction to handling during dosing and were not considered treatment related. Based on these findings it appears that the NOAEL for L-001079038 on respiratory function is 150 mg/kg.

2.6.2.5 Pharmacodynamic drug interactions

No data submitted

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Summary of Safety Pharmacology Studies

Study #/ Organ System	Method of Administration	Species	Doses	Gender/N	Findings
TT#05-5655/ CNS	Oral	Rat	20, 50, or 150 mg/kg	Male/6	No significant CNS or body temp effect up to 150 mg/kg **NOEL= 150 mg/kg**
TT #04-4720 hERG current	*In vitro*	NA; transgenic CHO-K1 cells	0, 100, 300 µM	NA; N=7-9	300 µM resulted in a 7-8% inhibition when analyzed with the voltage step and voltage ramp protocols.
TT #05-5656 Cardiovascular Conscious telemetered	Oral	Dogs	30, 90, 240 mg/kg	Female; N=4 sex	90mg/kg HR max ↑24% at 3.75 PR interval max ↓9% at 3.75 hr QT interval max↓5% at 4.5 hr 240 mg/kg: HR max ↑25% at 9.25 hr PR interval max ↓9% at 9.25 hr QT interval max ↓12% at 3.75 hr **$NOEL_{CV}$=30 mg/kg**
TT#05-5654 Pulmonary	Oral	Rat	20, 150, 150 mg/kg	Male; N=6	**$NOEL_{Pulmonary}$=150 mg/kg**

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

PK studies were conducted in rat, rabbit, guinea pig, monkey and dog. The rat and dog are the pivotal species reviewed herein.

2.6.4.2 Methods of Analysis

Study Title: High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of SAHA and its Metabolites, SAHA Glucuronide and N-Phenyl-Succinamic acid in Rat Serum (PK001, Volume 4.2.2.1, GLP)

Study Title: High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of SAHA and its Metabolites, SAHA Glucuronide and N-Phenyl-Succinamic acid in Dog Serum (PK002, Volume 4.2.2.1, GLP)

Rat and Dog serum concentrations of vorinostat, L-001302381 (*O*-glucorinide of vorinostat), and L-000341257 (β-oxidation product, 4-anilino, 4-anilino-4oxobutanic acid) were determined by a validated LC-MS/MS method. The validated assay was linear for vorinstat and the two metabolites in the concentration range of 5 to 5000 ng/mL. The lower limit of quantitation for each of the analytes was 5 ng/mL. The inter-batch accuracy for quality control samples was within the range of 98.6 to 103.6% and 88.0 to 97.9% for rat and dog serum samples, respectively. The validated assay demonstrated good reproducibility for vorinostat and the two metabolites (precision [%CV] =10.1%). The rat and dog serum samples were stable at -80C for up to 234 and 241 days, respectively.

2.6.4.3 Absorption

Study Title: PK of L-001079038 in male SD rats and male beagle dogs following IV and oral administration (PK003, Volume 4.2.2.2, non-GLP)

The pharmacokinetics of L-001079038 were evaluated in rats and dogs following 2 mg/kg intravenous (IV) and 20 mg/kg oral (P.O.) administration. In both rats and dogs, L-001079038 possessed very high serum clearance properties (129.5 and 54.7 mL/min/kg, respectively). In rats, the Vdss exceeded total body water (1.6 L/kg) whereas in dogs, the Vdss was 0.6 L/kg. The half-life of the compound was ~ 12 minutes in both rats and dogs (12 min), bioavailability was low (11 and 1.8% for rat and dog, respectively), and absorption was rapid (Tmax <1 hr).

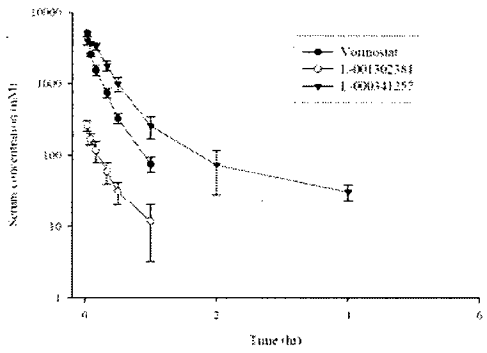
Metabolites L-001302381 and L-000341257 were also measured. Following oral dosing, the relative exposure (AUC) to L-001302381, was 0.95- and 2.31-fold that of parent compound in rats and dogs, respectively. For L-000341257, the relative exposure after oral dosing was 10- and 23.2-fold higher than that of parent compound in rats and dogs, respectively. See tables and graphs excerpted from the sponsor's submission.

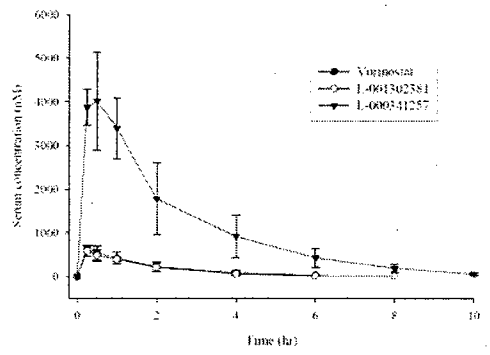
Pharmacokinetics of Vorinostat in the Rat Following IV and P.O. Administration

IV Parameters	Values[a]
Dose (mg/kg)	2
$AUC_{(0-t)}$ (μM·hr) vorinostat	0.96 ± 0.09
$AUC_{(0-inf)}$ (μM·hr) vorinostat	0.98 ± 0.10
% AUC extrapolated	2.3 ± 0.4
$Cl_{(s)}$ (mL/min/kg)	129.5 ± 14.1
Vd_{ss} (L/kg)	1.6 ± 0.1
$t_{1/2}$ (min)	12 ± 1
$AUC_{(0-t)}$ (μM·hr) L-001302381	0.05 ± 0.02
$AUC_{(0-t)}$ Ratio L-001302381 : vorinostat	0.05
$AUC_{(0-t)}$ (μM·hr) L-000341257	1.77 ± 0.26
$AUC_{(0-t)}$ Ratio L-000341257 : vorinostat	1.84
P.O. Parameters	
Dose (mg/kg)	20
$AUC_{(0-t)}$ (μM·hr) vorinostat	1.05 ± 0.46
C_{max} (μM)	0.66 ± 0.07
T_{max} (hr)	0.3 ± 0.1
Bioavailability (%)	11[b]
$AUC_{(0-t)}$ (μM·hr) L-001302381	1.0 ± 0.33
$AUC_{(0-t)}$ Ratio L-001302381 : vorinostat	0.95
$AUC_{(0-t)}$ (μM·hr) L-000341257	10.5 ± 3.53
$AUC_{(0-t)}$ Ratio L-000341257 : vorinostat	10

[a]The values are the mean ± SD of n=4 rats.
Serum levels of L-001302381 (*O*-glucuronide of vorinostat) and L-000341257 (β-oxidation product) also were measured.
[b]Based on AUC_{0-t} (dose normalized) values after IV and P.O. dosing (non-crossover study design).

Serum Concentration-Time Profiles of Vorinostat, L-001302381, and L-000341257 Following Administration of a 2 mg/kg IV (top panel) or 20 mg/kg P.O. Dose (bottom panel) of Vorinostat to Male Sprague-Dawley Rats (Mean ± SD; n=4)



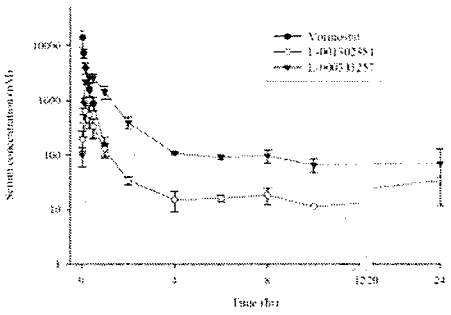


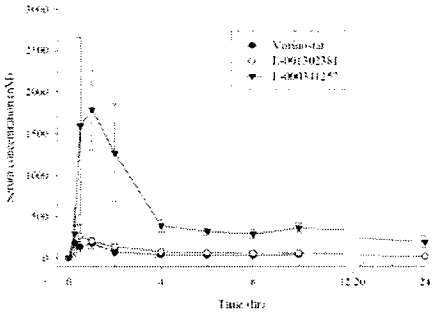
Pharmacokinetics of Vorinostat in the Dog Following IV and P.O. Administration

IV Parameters	Values[a]
Dose (mg/kg)	2
$AUC_{(0-t)}$ (μM·hr) vorinostat	2.38 ± 0.62
$AUC_{(0-inf)}$ (μM·hr) vorinostat	2.42 ± 0.64
% AUC extrapolated	1.8 ± 0.5
$CL_{(s)}$ (mL/min/kg)	54.7 ± 12.9
Vd_{ss} (L/kg)	0.6 ± 0.2
$t_{1/2}$ (min)	12 ± 1
$AUC_{(0-t)}$ (μM·hr) L-001302381	0.58 ± 0.30
$AUC_{(0-t)}$ Ratio L-001302381 : vorinostat	0.24
$AUC_{(0-t)}$ (μM·hr) L-000341257	4.21 ± 0.87
$AUC_{(0-t)}$ Ratio L-000341257 : vorinostat	1.77
P.O. Parameters	
Dose (mg/kg)	20
$AUC_{(0-t)}$ (μM·hr) vorinostat	0.42 ± 0.22
C_{max} (μM)	0.26 ± 0.11
T_{max} (hr)	0.7 ± 0.4
Bioavailability (%)	1.8 ± 0.9[b]
$AUC_{(0-t)}$ (μM·hr) L-001302381	0.97 ± 0.20
$AUC_{(0-t)}$ Ratio L-001302381 : vorinostat	2.31
$AUC_{(0-t)}$ (μM·hr) L-000341257	9.75 ± 0.18
$AUC_{(0-t)}$ Ratio L-000341257 : vorinostat	23.2

[a]The values are the mean ± SD of n=4 dogs.
Serum levels of L-001302381 (*O*-glucuronide of vorinostat) and L-000341257 (β-oxidation product) also were measured.
[b]Based on AUC_{0-t} (dose normalized) values after IV and P.O. dosing (crossover study design).

Serum Concentration-Time Profiles of Vorinostat, L-001302381, and L-000341257 Following Administration of a 2 mg/kg IV (top panel) or 20 mg/kg P.O. Dose (bottom panel) of Vorinostat to Male Beagle Dogs (Mean + SD; n=4)





Study Title: Analysis of Caco-2 Permeability of SAHA (PK004, Volume 4.2.2.2, non-GLP)

This study evaluated the ability of SAHA to be transported from the apical to the basolateral surface of intact Caco-2 cell monolayers in vitro. SAHA was found to cross the intact intestinal cell monolayer with an apparent permeability coefficient (P_{app}) of 1.70×10^{-6} cm/sec. P_{app} for propranolol (1.57×10^{-5}, highly permeable) and atenolol (1.89×10^{-7}, poorly permeable) were also determined. Based on SAHA's P_{app} score, It is considered to be moderately permeable in this system; less permeable than propranolol which is ~90% bioavailable in humans but more permeable than atenolol which is ~50% bioavailable in humans. The potential for of other HSA bound drugs by SAHA or the potential for other HSA drugs to displace SAHA was not addressed in this study.

2.6.4.4 Distribution

Study Title: *In vitro* Evaluation of SAHA Binding to Plasma Proteins by Ultrafiltration (#04-5506; Volume 4.2.2.3, non-GLP)

The extent of binding of SAHA to mouse, rat, rabbit, dog, and human plasma proteins and major binding proteins in human plasma was evaluated by comparing specific binding of SAHA to protein solutions [human serum albumin, (HSA), and α_1 acid glycoprotein (AAG)] via ultrafiltration and liquid chromatography mass spectrometry. Human plasma showed the greatest binding of SAHA followed by rabbit plasma, dog plasma, rat plasma and mouse plasma. Mean percentages of bound SAHA within the concentration range of 0.5 µg/mL to 50.0 µg/mL were 71.3%, 62.5%, 43.6%, 32.4% and 31.1%, respectively for these species. The major binding protein in human plasma was determined to be HSA (mean binding percentage = 60.6%) although some binding of SAHA to AAG was also observed (mean binding percentage = 15.9%) with nonspecific binding (< 5%).

2.6.4.5 Metabolism

Study Title: In vitro and Cytochrome P450 gene expression studies with L-001079038 (PK005; Volume 4.2.2.3, non-GLP)

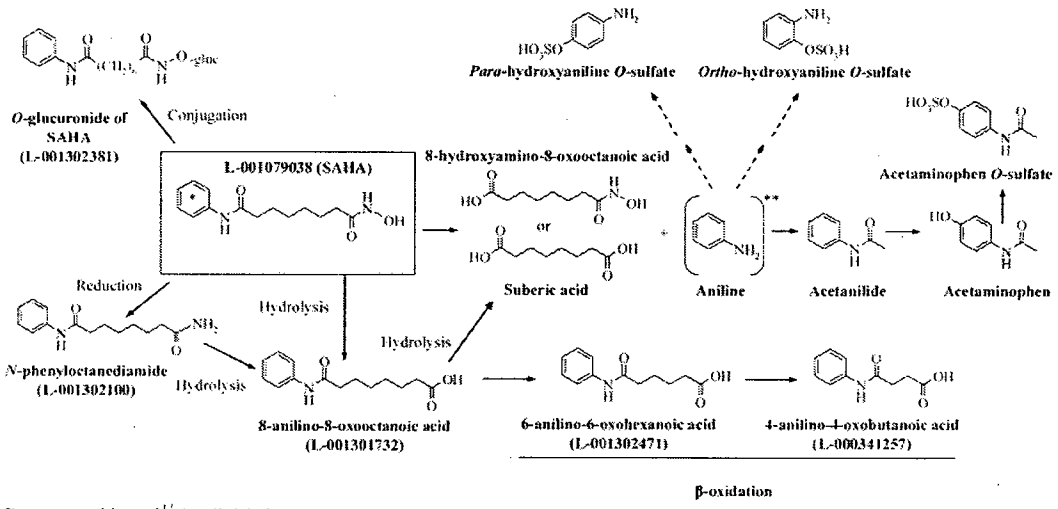
The in vitro metabolism of [14C]SAHA (L-001079038; 1, 10 and 100 µM, 60 minutes) was examined in liver microsomes and S9 fractions from rat, dog and humans. Direct glucuronidation of the parent compound to form the O-glucuronide (L-001302381) was the major metabolic pathway of L-001079038 in liver microsomes and S9 fractions in all three species studied. A minor pathway was hydrolysis of the parent compound to the carboxylic acid moiety (L-001301732, 8-anilino-8-oxooctanoic acid). The in vitro metabolism of [14C]SAHA (1, 10 and 100 µM) was also examined utilizing human cDNA-expressed UDP-Glucuronosyltransferases (UGTs). SAHA (10 and 100 µM) was glucuronidated by several human cDNA-expressed UGTs including UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, UGT2B7, and UGT2B17.

The ß-oxidation pathways of [14C]SAHA were evaluated in the presence of rat and guinea pig liver S9 fractions. Incubations with 100 µM SAHA (120 minutes) led to the formation of two products, 6-anilino-6-oxohexanoic acid (L-001302471) and 4-anilino-4-oxobutanoic acid (L-000341257). R,S-flurbiprofen (100 and 250 µM) completely

inhibited ß-oxidation of SAHA in guinea pig liver S1 fractions, while valproic acid (100 µM) and amiodarone (250 µM) inhibited ß-oxidation by 51 and 33%, respectively.

Following incubation of [14C]SAHA (1, 10, 100 µM, 1 or 4 hours) with rat, dog and human hepatocytes, several metabolites were formed. The major metabolites identified in all three species were the ß-oxidation product L-000341257 and the carboxylic acid moiety L-001301732. Additionally, the O-glucuronide L-001302381 was a major metabolite in dog hepatocytes. Minor metabolites that were present in all three species included another ß-oxidation product, L-001302471. Dog hepatocytes also generated para- and ortho-hydroxyaniline O-sulfate, while rat hepatocytes showed the presence of acetanilide. Human hepatocytes contained small amounts of the O-glucuronide and the reduction product N-phenyloctanediamide (L-001302100).

Overall, the major metabolites formed in vitro were largely attributed to glucuronidation and hydrolysis of the parent compound followed by ß-oxidation pathways (See figure excerpted from the sponsor's submission).



*Denotes position of ^{14}C radiolabel
** Aniline was not detected in samples but is a proposed intermediate

SAHA was not a reversible inhibitor of cytochromes P450 (CYP) CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 or a time-dependent inhibitor of CYP1A2, CYP2C9, CYP2C19 or CYP3A4 in human liver microsomes when incubated for 10-30 minutes. Additionally, SAHA (up to 10 µM, 24 and 48 hour incubations) did not alter mRNA expression or activity levels in human and rat hepatocytes, with the exception of increase in CYP1A1 mRNA in rat hepatocytes.

The mean blood-to-plasma partition ratio of [14C]SAHA was 1.2, 0.7 and 2.0 in rat, dog and human, respectively and was independent of concentration (1 and 10 µM).

Study Title: In Vitro Evaluation of SAHA as an Inducer of CYP450 Expression (PK007; Volume 4.2.2.4, non-GLP)

The purpose of this study was to determine the cytochrome P450 (CYP450) induction potential of SAHA in freshly cultured human hepatocytes (n=1). Induction was measured by catalytic activity assays selective for CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4.

SAHA caused a statistically significant induction (up to 2.3-fold but within historical values compared to 59 fold induction by Phenacetin) of CYP1A2 catalytic activity at 2 μM, 10 μM, and 50 μM in cultured human hepatocytes. The lowest concentration tested (2 μM) approximates the maximum SAHA plasma concentration (528 ng/mL; uncorrected for plasma protein binding) observed following oral administration of 400 mg SAHA in a Phase 1 clinical trial.

In contrast to CYP1A2, SAHA caused a statistically significant, concentration-dependent suppression of CYP2B6, CYP2C9, and CYP2C19 catalytic activities in cultured human hepatocytes (10 μM to 50 μM) and suppression of CYP3A4 catalytic activity in the hepatocytes (2 μM to 50 μM). (See table excerpted from the sponsor's submission)

Table 3. Effect of SAHA on CYP450 catalytic activities in cultured human hepatocytes (mean ± SD, n = 3; expressed as pmol/mg protein†)

Treatment (Probe Substrate)	CYP1A2 (Phenacetin)	CYP2B6 (S-Mephenytoin)	CYP2C9 (Diclofenac)	CYP2C19 (S-Mephenytoin)	CYP3A4 (Testosterone)
0 μM (DMSO vehicle)	184 ± 6.7	263 ± 88	2750 ± 269	562 ± 200	1403 ± 35
2 μM SAHA	310 ± 28*	161 ± 60	2117 ± 507	503 ± 10	740 ± 150*
10 μM SAHA	430 ± 57*	106 ± 14*	1362 ± 54*	256 ± 16*	388 ± 60*
50 μM SAHA	234 ± 2.9*	23 ± 3.4*	354 ± 5*	148 ± 41*	208 ± 14*
Positive Controls	**ß-naphthoflavone**	**Phenobarbital**	**Rifampicin**	**Rifampicin**	**Rifampicin**
Solvent only	184 ± 6.7	161 ± 76	2750 ± 269	562 ± 200	1403 ± 35
Inducing chemical	10770 ± 1428	5138 ± 455	13383 ± 852	3401 ± 173	48724 ± 1003
Inducing chemical plus 50 μM SAHA‡	8237 ± 244	4978 ± 141	14150 ± 702	3407 ± 244	54386 ± 12810
Percent inhibition of activity by SAHA‡	24	3	-6	0	-12

† – Determined over 30 minutes for testosterone, 60 min for phenacetin and diclofenac, and 240 minutes for S-mephenytoin.

‡ – Cells were treated with 50 μM SAHA for 30 minutes prior to enzyme assay.

* – Significantly different from vehicle control ($p \leq 0.05$).

Study Title: In-vitro Metabolite Profiling of SAHA in S9 Fractions from Dog, Rat, Monkey, and Human Livers (#TT04-5507 Volume 4.2.2.4, non-GLP)

SAHA was incubated in vitro with pooled liver S9 fractions isolated from beagle dogs, Sprague-Dawley rats, cynomologus monkeys, and human donors. The disappearance of the parent compound and formation of metabolites was monitored by LC-MS and subsequent metabolite identification was putatively assigned on the basis of molecular weight, retention time, and m/z ratios.

SAHA was extensively metabolized (primarily via glucuronidation) by the S9 liver fractions across all species studied with 47%, 0%, 44%, and 80% of the parent compound remaining after 60 minutes in the rat, dog, monkey, and human incubations, respectively. SAHA glucuronide was a major metabolite in dog, rat, monkey and human S9 fractions where it accounted for 95%, 58%, 29%, and 29% of the total chromatographic area, respectively (See table excerpted from the sponsor's submission).

Table 3. Putative Metabolite Summary from Tandem (Sequential) Phase I and II Metabolism of SAHA by Rat, Dog, Monkey, and Human Liver S9 Incubations (% Total Chromatographic Area)

Name	% Total Chromatographic Area											
	Human (0 min)	Human (30 min)	Human (60 min)	Dog (0 min)	Dog (30 min)	Dog (60 min)	Rat (0 min)	Rat (30 min)	Rat (60 min)	Monkey (0 min)	Monkey (30 min)	Monkey (60 min)
SAHA primary amide					0.441			3.13	3.49		8.53	11.2
SAHA acid		1.68	2.60		0.353	0.295		3.73	4.38		15.2	18.6
SAHA	97.7	61.2	41.4	100	0.364		100	33.6	17.8	100	44.9	24.2
Methylated-SAHA			5.02					6.18	9.77			
Hydroxylated SAHA		4.42	8.89		1.28	1.70			2.76			8.75
Dihydroxylated SAHA		2.91	4.60			0.729		2.27	4.13		4.82	8.72
SAHA adipic acid glucuronide		1.44				0.799						
SAHA acid glucuronide	2.30	6.69	8.13			1.04						
SAHA glucuronide		21.6	29.3		97.6	95.4		51.1	57.7		21.8	28.5

SAHA acid glucuronide was also detected in dogs and human incubations (1.0% and 8.1%, respectively. Trace amounts of SAHA adipic acid glucuronide were observed in dog and human incubations (<1%). SAHA was reduced to a primary amide in rat and monkey S9 incubations (3.5% and 11.2%, respectively). Putative oxidation products were also detected. Peaks with an m/z ratios consistent with hydroxylation and dihydroxylation were seen in all species (approximately 1% to 9% of the chromatographic area). Methylated SAHA products were detected in human and rat S9 liver incubations (5% and 10%, respectively). Hydrolysis also resulted in SAHA acid (L-000341257, 8-anilino-8-oxooctanoic acid, N-phenyl succinamic acid) in rat, dog, monkey, and human incubations (4.4%, 0.3%, 18.6, and 2.7%, respectively). (See table below for m/z ration and retention times)

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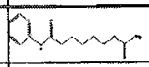
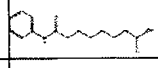
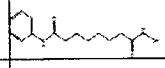
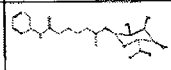
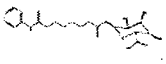
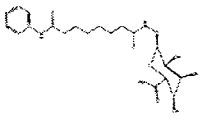
Table 5. Metabolite Structure, M/Z ratios, and Retention Time (minutes) of Putative SAHA Metabolites Observed *In Vitro*

AP#		NAME	FORMULA	m/z Ratio	MW	RT (min)
595		SAHA primary amide	C14H20N2O2	249.32	248.32	8.8
193		SAHA acid	C14H19NO3	250.31	249.31	11.14
390		SAHA	C14H20N2O3	265.32	264.32	8.2
NA	unknown	Methylated-SAHA[a]		279.00	278.00	14.3, 15.8, 17.4
NA	unknown	Hydroxylated SAHA[a]	C14H20N2O4	281.32	280.32	15.2, 16.7, 17.1, 19.3
NA	unknown	Dihydroxylated SAHA[a]		297.00	296.00	14.3, 15.8
NA		SAHA adipic acid glucuronide	C18H23NO9	398.38	397.38	7.6
NA		SAHA acid Glucuronide	C20H27NO9	426.43	425.43	9.6
970		SAHA Glucuronide	C20H28N2O9	441.45	440.45	8.8

[a] Not confirmed by coelution; appears as multiple peaks

Study Title: Excretion and metabolism of [14C-L-001079038] in rats and dogs (PK006; Volume 4.2.2.4, non-GLP)

The in vivo excretion of SAHA was studied in intact rats and dogs over a 96-hr collection period following a single intravenous (IV) or oral (P.O.) dose of [14C]SAHA. The primary route of excretion is via urine.

	% of recovered radioactivity			
	Rat		Dog	
	IV	PO	IV	PO
Urine	90.9	88.5	81.4	67.5
Feces	1.8	1.2	7.8	9.1
Total	92.7	89.7	89.2	76.6

The in vivo metabolism of SAHA was studied in intact rats and dogs following a single IV or P.O. dose of [14C]SAHA. Radiochromatograms generated from urine samples suggested that the compound was eliminated almost exclusively as metabolites in both rats and dogs. The major metabolites in rat urine were acetaminophen O-sulfate and the ß-oxidation products L-000341257 (4-anilino-4- oxobutanoic acid) and L-001302471 (6-anilino-6-oxohexanoic acid). L-001302381 (O-glucuronide of SAHA) was also detected in trace amounts. The major metabolites in dog urine were L-000341257 and ortho-hydroxyaniline O-sulfate. L-001302381 and carnitine esters of L-001302471 and L-001301732 (8-anilino-8-oxooctanoic acid, a carboxylic acid moiety) were also found in minor amounts in dog urine. Circulating metabolites detected in rat serum included L-

000341257, acetaminophen O-sulfate, L-001301732, L-001302471, and acetanilide. Circulating metabolites detected in dog serum included L-000341257, L-001301732 and both para- and ortho-hydroxyaniline O-sulfate. Overall the major metabolites formed in vivo were attributed to hydrolysis of the parent molecule at the hydroxamic acid functional group followed by ß-oxidation pathways. (see table below for

	Levels of [14]-SAHA and metabolites as % of Dose in Urine (0-24 hours)			
	Rat		Dog	
	2 mg/kg	20 mg/kg	2 mg/kg	20 mg/kg
Acetaminophen O-sulfate	15.9 ± 1.6	18.5 ± 0.9		
L-000341257	47.2 ± 3.5	47.9 ± 1.3	33.5 ± 4.7	31.1 ± 3.5
SAHA	5.0 ± 0.9	0.7 ± 0.1	ND	ND
L-001302471	14.4 ± 3.2	9.5 ± 2.5		
L-001302381	0.7 ± 0.3	1.2 ± 0.3	1.0 ± NA	ND
Ortho-hydroxyaniline O-sulfate			20.6 ± 3.2	17.2 ± 1.2
Carnitine ester of L-001302471			2.1 ±1.8	0.4 ± NA
Carnitine ester of L001301732			8.0 ± 2.9	6.8 ± 0.8
% dose excreted in urine (0-24 hr)*	88.2	85.3	66	56.7
Total	**83.2 ± 4.8**	**77.8 ± 2.0**	**65.2 ± 5.9**	**55.4 ± 3.7**

*excluding cage wash

2.6.4.6 Excretion
See Section 2.6.4.5

2.6.4.7 Pharmacokinetic drug interactions
None Submitted
2.6.4.8 Other Pharmacokinetic Studies
None Submitted

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2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

	Rat	Dog	Human
	26 week TK	**26 week TK**	**QD x22 days (P008)**
Dose (mg/kg/day)	20, 50, 150	20, 60, 80/100/125/160	400 mg
Dose (mg/m2/day)	120, 300, 900	400, 1200, 1600/2000/2500/3200	
SAHA			
Dose Proportionality	Dose proportional exposure excepting: Day 7 females and week 26 males and females between 50 and 150 mg/kg for AUC. Day 7 and week 26 males and females between 50 and 150 for Cmax. These findings were slightly less than dose proportional.	Less than dose proportional increases in AUC and Cmax were noted following 60 and 80/100/125/160 mg/kg.	AUC_{0-24}=6.46 μM hr (1707 ng hr/mL) Cmax=0.91 μM (240 ng/mL) Tmax=4.2 hr $T_{1/2}$=1.34 hr
Gender Differences	Clear gender differences were not observed.	Clear gender differences were not observed	Not apparent (n=14)
Accumulation	Minimal accumulation was observed	Minimal accumulation (<2) was observed days 56, 116, and 175 at all dose levels.	Minimal accumulation (1.21) was observed
SAHA Glucuronide (L-001302381)			
Dose Proportionality	AUC and Cmax increased in a less than dose proportional manner between 50 and 150 mg/kg day 7 and week 26.	Less than dose proportional increases in AUC and Cmax were noted following 60 and 80/100/125/160 mg/kg.	Serum exposures 3-4 fold greater than SAHA (fasted) AUC_{0-24}=15.78 μM hr (6950 ng hr/mL) Cmax=3.07 μM (1350 ng/mL) Tmax=2.7 hr $T_{1/2}$=2.03 hr
Gender Differences	Clear gender differences were not observed	Clear gender differences were not observed	Not apparent (n=14)
Accumulation	Minimal accumulation at 50 mg/kg	Accumulation was not apparent at any dose	Accumulation was not apparent.
N-Phenyl-Succinamic acid (L-000341257)			
Dose Proportionality	Dose proportional increases from 20-50 mg/kg but less than dose proportional increases from 50-150 mg/kg on day 7 and week 26	Less than dose proportional increases in AUC and Cmax were noted following 60 and 80/100/125/160 mg/kg.	Serum exposures 10-13 fold greater than SAHA AUC_{0-24}=43.11 μM hr (8540 ng hr/mL) Cmax=4.78 μM (947 ng/mL) Tmax=3.92 hr $T_{1/2}$=8.9 hr (fasted)
Gender Differences	Clear gender differences were not observed	Clear gender differences were not observed	Not apparent (n=14)
Accumulation	Minimal accumulation at 50 and 150 mg/kg on week 26	Accumulation was not apparent at any dose level.	Minimal accumulation was observed (1.44)

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

26-Week Repeat Dose Toxicity and Toxicokinetic Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 13-Week Interim Sacrifice and a 4-Week Recovery (TT#04-5502, Volume 4.2.3.2)

Table B: Summary of Toxicokinetic Parameters of SAHA in Serum of Male and Female Rats – Dose Levels 20, 50 and 150 mg/kg/day– Study Day 7 and Week 26

Group Number	Dose (mg/kg/day)	Period	Gender	AUC_{0-t} (ng•h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)	Accumulation Ratio*	AUC_{0-t}/Dose	Cmax/Dose
5	20	Day 7	Female	196	97.6	0.50	1.30	NA	9.81	4.88
		Day 7	Male	177	96.5	0.50	0.970	NA	8.86	4.82
		Week 26	Female	265	260	0.25	0.830	1.4	13.3	13.0
		Week 26	Male	234	165	0.25	1.26	1.3	11.7	8.25
6	50	Day 7	Female	499	195	0.25	2.01	NA	10.0	3.90
		Day 7	Male	476	172	0.75	2.18	NA	9.52	3.43
		Week 26	Female	571	313	0.25	1.08	1.1	11.4	6.25
		Week 26	Male	671	244	0.25	1.40	1.4	13.4	4.87
7	150	Day 7	Female	1042	355	0.75	2.55	NA	6.95	2.36
		Day 7	Male	1644	372	0.50	1.36	NA	11.0	2.48
		Week 26	Female	1164	437	0.75	1.39	1.1	7.76	2.91
		Week 26	Male	1229	460	0.51	1.58	0.7	8.19	3.07

*Calculated as AUC_{0-t} Week 26/ AUC_{0-t} Day 7
NA: Not applicable

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Table C: Summary of Toxicokinetic Parameters of SAHA Glucuronide in Serum of Male and Female Rats – Dose Levels 20, 50 and 150 mg/kg/day– Study Day 7 and Week 26

Group Number	Dose (mg/kg/day)	Period	Gender	AUC_{0-t} (ng•h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)	Accumulation Ratio*	AUC_{0-t}/Dose	Cmax/Dose
5	20	Day 7	Female	430	223	0.50	1.44	NA	21.5	11.1
		Day 7	Male	412	228	0.50	1.05	NA	20.6	11.4
		Week 26	Female	513	502	0.25	0.914	1.2	25.6	25.1
		Week 26	Male	462	316	0.48	3.07	1.1	23.1	15.8
6	50	Day 7	Female	961	384	0.50	1.66	NA	19.2	7.69
		Day 7	Male	854	342	0.75	1.58	NA	17.1	6.84
		Week 26	Female	1090	613	0.25	1.04	1.1	21.8	12.3
		Week 26	Male	1203	615	0.49	1.52	1.4	24.1	12.3
7	150	Day 7	Female	1761	624	0.75	2.20	NA	11.7	4.16
		Day 7	Male	2403	622	0.75	1.72	NA	16.0	4.15
		Week 26	Female	2225	1236	0.75	1.37	1.3	14.8	8.24
		Week 26	Male	2222	1013	0.51	1.40	0.9	14.8	6.75

*Calculated as AUC_{0-t} Week 26/ AUC_{0-t} Day 7

Table D: Summary of Toxicokinetic Parameters of N-Phenyl-Succinamic Acid in Serum of Male and Female Rats – Dose Levels 20, 50 and 150 mg/kg/day– Study Day 7 and Week 26

Group Number	Dose (mg/kg/day)	Period	Gender	AUC_{0-t} (ng•h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)	Accumulation Ratio*	AUC_{0-t}/Dose	Cmax/Dose
5	20	Day 7	Female	3565	1680	0.50	2.07	NA	178	84.0
		Day 7	Male	3933	1667	0.50	2.11	NA	197	83.3
		Week 26	Female	4697	2993	0.25	2.13	1.3	235	150
		Week 26	Male	5273	2473	0.25	3.68	1.3	264	124
6	50	Day 7	Female	9848	2650	0.50	1.81	NA	197	53.0
		Day 7	Male	10390	2553	0.50	1.50	NA	208	51.1
		Week 26	Female	9553	3907	0.25	1.76	1.0	191	78.1
		Week 26	Male	14836	3660	0.49	3.16	1.4	297	73.2
7	150	Day 7	Female	19994	3943	0.75	2.17	NA	133	26.3
		Day 7	Male	23224	4270	0.50	2.08	NA	155	28.5
		Week 26	Female	22561	4515	0.75	1.77	1.1	150	30.1
		Week 26	Male	32163	5633	0.51	2.44	1.4	214	37.6

*Calculated as AUC_{0-t} Week 26/ AUC_{0-t} Day 7
NA: Not applicable

26-Week Repeat Dose Toxicity and Toxicokinetic Study in Beagle Dogs Rats Administered AP390 by Oral Gavage with a 13-Week Interim Sacrifice and a 4-Week Recovery (TT#04-5504, Volume 4.2.3.2)

Dogs TK Parameters for SAHA in Serum of Male and Female Dogs

Dose (mg/kg/d)	Dose mg/m2/d	Dose ratio	Sex	Day	AUC (ng h/mL)	±SD	Dose Ratio	F:M Ratio	Accumulation ratio	Cmax (ng/mL)	±SD	Dose Ratio	M:F Ratio	Accumulation Ratio
20	400	1	F	7	76	54.1	1			40	48	1		
			F	175	145	12.4	1		2	72	36	1		2
		1	M	7	92	99	1	1		42	76	1	1	
			M	175	249	124	1	2	3	84	87	1	1	2
60	1200	3	F	7	237	36	3			65	33	2		
			F	175	384	67	3		2	98	51	1		2
		3	M	7	250	27	3	1		73	16	2	1	
			M	175	346	117	1	1	1	131	93	2	1	2
80/10	1600/	>4	F	7	298	49	4			94	48	2		
0/125	2000/		F	175	398	53	3		1	126	25	2		2
/160	2500/	>4	M	7	221	41	2	1		67	65	2	2	
	3200		M	175	325	28	1	1	1	134	36	2	1	2

Dogs TK Parameters for SAHA Glucuronide in Serum of Male and Female Dogs

Dose (mg/kg/d)	Dose mg/m2/d	Dose ratio	Sex	Day	AUC (ng h/mL)	±SD	Dose Ratio	F:M Ratio	Accumulation ratio	Cmax (ng/mL)	±SD	Dose Ratio	M:F Ratio	Accumulation Ratio
20	400	1	F	7	537	55	1			156	50	1		
			F	175	935	50	1		2	307	31	1		2
		1	M	7	458	39	1	1		128	42	1	1	
			M	175	560	25	1	1	1	144	33	1	0.5	1
60	1200	3	F	7	1110	62	2			227	61	1		
			F	175	1164	45	1		1	267	37	1		1
		3	M	7	1160	39	3	1		242	40	2	1	
			M	175	1437	70	3	1	1	450	82	3	2	2
80/10	1600/	>4	F	7	937	23	2			196	48	1		
0/125	2000/		F	175	2507	137	3		3	405	90	1		2
/160	2500/	>4	M	7	927	46	2	1		213	64	2	1	
	3200		M	175	1167	35	2	0.5	1	356	55	2	1	2

Dogs TK Parameters for n-Phenyl-Succinamic Acid in Serum of Male and Female Dogs

Dose (mg/kg/d)	Dose mg/m2/d	Dose ratio	Sex	Day	AUC (ng h/mL)	±SD	Dose Ratio	F:M Ratio	Accumulation ratio	Cmax (ng/mL)	±SD	Dose Ratio	M:F Ratio	Accumulation Ratio
20	400	1	F	7	2278	47	1			356	30	1		
			F	175	3402	39	1		1	775	52	1		2
		1	M	7	1912	54	1	1		389	47	1	1	
			M	175	2272	17	1	1	1	451	41	1	2	1
60	1200	3	F	7	3942	44	2			658	40	2		
			F	175	4698	55	1		1	932	33	1		1
		3	M	7	3534	49	2	1		519	33	1	1	
			M	175	3855	48	2	1	1	681	63	2	1	1
80/10	1600/	>4	F	7	4190	29	2			770	51	2		
0/125	2000/		F	175	6386	73	2		2	1211	26	2		2
/160	2500/	>4	M	7	3577	20	2	1		629	345	2	1	
	3200		M	175	5436	63	2	1	2	1150	39	2	1	2

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

In the 26-week GLP repeat-dose oral toxicity study of vorinostat in Sprague-Dawley rats, a significant, dose-dependent reduction in food consumption and body weight gain was observed in both females and males at doses of 50 mg/kg/day (300 mg/m2/day) or 150 mg/kg/day (900 mg/m2/day). Lower white blood cell counts (WBC; ↑20-70%) (primarily due to the lower lymphocyte counts, but also including monocytes, eosinophils, and neutrophils), decreased globulin (up to 40%) and increased absolute reticulocyte counts (↑ 162%) were observed at all doses in at least one sex at more than one interval. The magnitude of changes were dose dependent. Decreased thymus weight, and splenic and thymic lymphoid depletion along with bone marrow erythroid hyperplasia/myeloid hypoplasia were treatment-related findings. These treatment-related findings are likely related to the mechanisms by which vorinostat induces cell differentiation and cell death. All these effects were partially or completely reversible by 4 weeks of recovery. Based on the findings at the lowest dose, a no-observable-adverse-effect level (NOAEL) could not be established in this study.

In the 26–week GLP repeat-dose oral toxicity study in beagle dogs with a 4-week recovery, no adverse effects were found at doses of 20 mg/kg/day (400 mg/m2/day) and 60 mg/kg/day (1200 mg/m2/day). The doses administered via capsule were 20, 60, or 80/100/125/160 mg/kg/day. Due to a lack of findings throughout the study in the high-dose group, dosing began at 80 mg/kg/day (1600 mg/m2/day) and was escalated in succession to 100 mg/kg/day (2000 mg/m2/day), 125 mg/kg/day (2500 mg/m2/day), and 160 mg/kg/day (3200 mg/m2/day) on Drug Days 16, 30, and 97, respectively. Reversible GI toxicity, characterized by non-formed or liquid feces, macroscopic (red foci) or microscopic(villous blunting with crypt epithelium regeneration, inflammation and necrosis in the large and small intestine) findings, caused by vorinostat was associated with the high-dose regimen (at the 160-mg/kg/day [3200 mg/m2/day]). Histologically, there was no evidence of serious, irreversible damage to any organ was observed. No treatment-related findings at any dose were noted for the endpoints of mean body weight, mean food consumption, ophthalmologic abnormalities, electrocardiographic parameters, or blood pressure. The NOAEL of vorinostat was 60 mg/kg/day (1200 mg/m2/day) in this study.

Genetic toxicology:

Positive genotoxic effects were obtained for SAHA in the *in vitro* AMES Assay for mutagenicity in the presence and absence of metabolic activation. SAHA was positive for clastogenicity in an *in vitro* CHO cell assay in the presence and absence of metabolic activation and an *in vivo* mouse micronucleus assay. Additional testing was conducted in human peripheral lymphocytes. SAHA was not found to be clastogenic in this assay however a confirmatory assay was not conducted.

Carcinogenicity:

Carcinogenicity studies were not conducted and are generally not required to support the safety of the product for the proposed cancer indication.

Reproductive toxicology:
Assessment of reproductive toxicity of SAHA showed early embryonic development (Segment I) impairments in female rats treated with 300 mg/m2. This was manifested by treatment-related increases in the peri-implantation loss, a treatment-related increase in postimplantation loss secondary to increases in the percentage of resorptions and dead fetuses per implants. Decreases in the mean number of live fetuses/pregnant female (900 mg/m2/day) were also observed. The NOEL for embryonic development is 90 mg/m2. Based on a dose dependent increase in corpora lutea at 90 mg/m2, the lowest dose administered in the study, a NOEL for female fertility was not identified.

In male rats, there were no treatment related effects of SAHA on reproductive parameters at doses up to 900 mg/m2 (the highest dose administered in this study).

In the absence of maternal toxicity, developmental toxicity was observed with SAHA when administered GD6-20 rats and rabbits at 300 mg/m2 and 600 mg/m2, respectively. In rats, findings consisted of marked decreases in weight and increases in fetuses with skeletal variations (cervical ribs, supernumerary ribs and vertebral count, and sacral arch variations) and sites of incomplete ossifications (the skull, thoracic vertebra and sternebra). In the rabbit, findings consisted of decreased fetal weight, incomplete ossification of the metacacarpals, and increased incidence in 13^{th} rib)

TK was not conducted concurrent with fertility and reproductive toxicology studies. Additional studies in the rat and rabbit to assess placental transfer of SAHA was conducted. SAHA, SAHA Glucoronide and N-phenyl-succinamic acid were found to cross the placenta and the fetus in both rats and rabbits. Fetal serum concentrations were found to be $\leq$50% that of maternal serum concentrations and to have a delay in the tmax of metabolites, indicative of slow transplacental transfer of SAHA metabolites. In rats, the mean AUC_{SAHA} was 0.13 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{SAHA\ glucuronide}$ was 0.04 and 0.61x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic\ acid}$ was 0.62 and1.92x the observed clinical concentration. In rabbits, the mean AUC_{SAHA} was 0.12 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{SAHA\ glucuronide}$ was 0.19 and 0.52x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic\ acid}$ was 0.94 and4.5x the observed clinical concentration.

Local Tolerance:
Vorinostat did not cause sensitization in female CBA/JHsd mice, nor did it produce dermal irritation of New Zealand White rabbits. Vorinostat was not an irritant in the bovine corneal opacity and permeability assay. Evaluation of the irritant potential indicated that Vorinostat was a non-irritant in the in vitro skin irritation test. Although the mean viability of the three treated tissues was >50% (54%), which would result in a non-irritant prediction, the individual viability values of 2 of 3 tissues were less than 50%, which is suggestive of an equivocal prediction.

2.6.6.2 Single-dose toxicity

Study #	Species	Method of Admin	Doses (mg/kg)	Gender (N)	Treatment Related Findings	NOAEL (mg/kg)
TT #04-2511 (non-GLP)	Mouse-ICR	Oral	2000	3F/3M	None	2000
TT #92-5513 (non-GLP)	Mouse TAC:SWBR	IV-Bolus	0, 0.1, 1.0, 10, 20	3F	≥1 mg/kg- dyspnea, ↓motor activity ≥10 mg/kg ↓ respiratory rate, ataxia, ptosis, dark red lungs and renal cortex 20 mg/kg- 1 mouse found dead	0.1
TT#94-5531 (non-CLP)	Mouse: ICR-	IV 4 hr infusion	0, 250, 400, 600, 900, 1250	2F	0.5 hours post-dose: ≥900 mg/kg- ptosis, lacrimation, labored breathing 2.hours post dose: 1250 mg/kg: ruffled fur, lacrimation	600
TT #94-5532 (non-GLP)	Mouse: ICR	IV 4 hr infusion	0, 1250, 1500, 1750, 2000	5M/5F	1250 mg/kg: Labored breathing, lacrimation, touch, bloated. 1500 mg/kg: Day 1: cool to touch, dyspnea, labored breathing, lacrimation, bloated, and motor activity; 1 female found dead 3 hours after dosing. 1750 mg/kg: Day 1: clonic convulsions, motor activity, ataxia, prostration, loss of righting reflex, tremors, labored breathing, lacrimation, bloated, and cool to touch. 3 females died (2 during infusion, one approximately 1.5 hours after infusion); 2 males died (one 0.5 hour after infusion, one on Day 2). Macroscopic observations: cool viscera, glossy subcutis, clear fluid in abdominal cavity, gelatinous pancreas, diffuse dark red discoloration of lung, bilateral, and mild enlarged kidney 2000 mg/kg: 100% mortality within 3.5 hours. Macroscopic observations: cool viscera, glossy subcutis, clear fluid in abdominal cavity, gelatinous pancreas, mottled liver, bilateral, and mild enlarged kidney.	Not identified
TT#93-5503 (non-GLP)	Rat: SD	IV-Push	0, 1, 10, 50	3F	1 mg/kg: None 10 mg/kg: None 50 mg/kg: Dyspnea (3 of 3) and labored respiration (2 of 3) 1 minute after dosing and gone by 2.5 hours related to 10% hydroxypropyl-ß-cyclodextrin vehicle.	10
TT#94-5530	Dog: Beagle	IV 4 hour infusion	Phase 1: 0, 48, 68, 96 mg/kg	1 F	48 mg/kg: No clinical signs. 68 mg/kg: Day 3: 27% ↓WBCs, 24%↓ neutrophils, and 73% ↓monocytes, 45% eosinophils 96 mg/kg: Day 3: 33% ↓WBCs, 27%↓	48

Study #	Species	Method of Admin	Doses (mg/kg)	Gender (N)	Treatment Related Findings	NOAEL (mg/kg)
					neutrophils, and 100% ↓monocytes and eosinophils. Reversible: Day 7. 150 mg/kg: Emesis in both dogs. ↓body weight in male; ↓WBC and neutrophils in female; ↑WBC and neutrophils in male; ↑ALT in males ↑ bilirubin in both sexes. C(ss): 7.6 µg/mL female; 4.4 µg/mL male.	
			Phase 2: 0, 150, 200 mg/kg	1M/1F	200 mg/kg: Emesis in both dogs, biting, aggression, salivation, vocalization, ↓body weight, Day 3: female: ↓WBC, ↓neutrophil, ↓Hg Male: ↑WBC, ↑neutrophil, ↓Hg Both: ↑ALT, ↑Bili Css: 10.9 µg/mL female, 8.8 µg/mL male	150
TT#95-5555	Dog: Beagle	IV 120 hr infusion	0 (Saline and HBCD), 72 (0.02 mg/min/kg), 144 (0.04 mg/min/kg),	1-3	HBCD: reddened ears and gums, bloodshot eyes, diarrhea and vomiting. 72 mg/kg/day: ataxia, muscle rigidity, recumbent @52 hrs with tremors and rapid respiration. Animals moribund and euthanized at ~72 hrs. As early as 48 hrs post dose findings included: ↓Ca (32%), ↓K (24%), ↓ Cl (12%), ↓WBC (68%), ↓Lymh (96%), ↓mono/eosinophils (100%), ↓ Ret (67%), $Neutro_{seg}$, ↑ALP/AST/ALT (>175%), ↑CPK (448%), ↑BUN (88%), ↑LDH (3x), ↑chol (70%), ↑Prothrombin time. Similar findings were observed in the high dose with euthanasia occurring as early as 48 hrs.	Not identified

2.6.6.3 Repeat-dose toxicity

Study title: Four-Week Repeat Dose Toxicology Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 2-Week Recovery (TT#03-5511, Volume 4.2.3.2)

This study was not reviewed since studies of longer duration, utilizing similar doses were conducted and reviewed herein

Study title: Four-Week Repeat Dose Toxicology Study in Beagle Dogs Administered AP390 by Oral Gavage with a 2-Week Recovery (TT#03-5512, Volume 4.2.3.2)

Beagle dogs were treated with 0, 15 mg/kg/day (300 mg/m2/day), 40 mg/kg/day (800 mg/m2/day), or 100 mg/kg/day (2000 mg/m2/day) vorinostat daily for 28 days.

Clinical signs [thin appearance, dehydration, hypoactive behavior, abnormal fecal excretion (discolored, liquid, mucoid, non-formed), pale gums and the presence of vomitus] resulted the sacrifice of 3 dogs/sex in the high dose group on Day 17. Dosing ceased in the remainder of HD animals, the animals were designated for recovery and

sacrificed day 29/30. Mortality was not observed below 100 mg/kg. Clinical signs in the low and mid dose were similar but lower in magnitude.
At the end of the treatment period, body weight gain was 3-4% in the control, low and mid dose groups, however, there was an approximate 20% decrease in body weight in the HD group on day 17. Full recovery was not observed during the recovery period although supplemental food was initiated on day 16, given a virtual complete cessation of feeding during the six hour feeding period.

No treatment-related findings occurred in the ophthalmology, electrocardiology, or indirect blood pressure examinations. There were multiple abnormalities observed in the clinical pathology from the 100 mg/kg/day dose group (2000 mg/m2/day). Although none were statistically significant, they may be clinically relevant.

Clinical pathology alterations considered treatment-related on Day 17 in both females and males when compared to Week 2 or Week 4 control data included the following: hemoglobin and hematocrit levels and erythrocyte counts increased in 2 female dogs and decreased in 2 other female dogs. WBCs, neutrophils and monocytes increased (2-4 fold) in both sexes while lymphocytes decreased to <1000/mm3 in one female and male dog each. APTT (activated partial thromboplastin time) was >12 sec in most females and all males. Total protein, albumin increased slightly (<2 fold), while A/G ratios and/or calcium decreased (10-30%) in 1 female and 2 males, possibly due to dehydration and decreased food consumption. Serum creatinine (~2 fold), and urea nitrogen (~3 fold) increased while inorganic phosphorus, sodium, potassium and chloride decreased (10-25%) in females and may be the result of dehydration. Increased serum glucose levels (40%) and platelet counts (2 fold) were considered to be secondary effects of treatment with vorinostat. The urinary changes at this dose included decreased specific gravity (5%), and increased urine volume (10 fold before sacrifice). The consequential decreased urinary chemistry parameters reflect the dilute nature of the urine. The positive occult blood was considered an indirect effect of treatment with vorinostat.

At recovery, the 3 hematologic values that remained decreased compared with the vehicle group control levels were hemoglobin and erythrocyte counts and hematocrit (<25%) in the 2 female dogs.

No macroscopic findings indicative of a treatment-related effect were observed in dogs sacrificed on Days 29 and 30. However, treatment-related microscopic gastro-enteropathy, increased splenic pigment and hypocellularity of the bone marrow and testicular degeneration was observed in several males in the 100 mg/kg/day dogs sacrificed in a moribund condition on Day 17. Lymphoid depletion was also observed in several lymphoid tissues but the relationship to treatment is uncertain, since these animals were stressed and moribund. Gastroenteropathy, splenic pigment, bone marrow hypocellularity, and testicular degeneration persisted in some early recovery female and/or male dogs sacrificed on Day 29. Although still present, lymphoid depletion was abated in the early recovery animals. No treatment-related changes, including GI, were present at the terminal sacrifice in the 15 or 40 mg/kg/day dose groups.

Serum samples were obtained day 28 in the 15-mg/kg/day and 40-mg/kg/day groups Day 17 in the 100-mg/kg/day group on Day 17. Overall, Cmax and AUC) increased in both sexes with dose, but in a less than dose-proportional manner and were higher on Day 17/28 than on Day 1, indicating probable accumulation. Given the low number of animals comparisons are difficult.

In light of the results of the 4-week repeat-dose oral toxicology study in beagle dogs, an exploratory repeat-dose oral toxicity study was performed to determine whether a dose level of 60 mg/kg/day (1200 mg/m2/day) or 80 mg/kg/day (1600 mg/m2/day) of vorinostat would be tolerated in a 26-week study, and to determine the dose level at which adverse effects were noted during escalating-dose levels of 80 mg/kg/day (1600 mg/m2/day), 120 mg/kg/day (2400 mg/m2/day), 160 mg/kg/day (3200 mg/m2/day), and 200 mg/kg/day (4000 mg/m2/day) (Phase 1) (TT #04-5503) [Sec. 2.6.7.6]. During Phase 1 of the study, vorinostat was administered once daily for 63 days. Only male dogs were used for this portion of the study. Phase 2 of this study was designed to determine whether adverse effects were noted following repeated exposure to vorinostat in previously untreated female and male dogs at a dose of 160 mg/kg/day (3200 mg/m2/day) for 13 days.

TK Parameters in males and females following SAHA Administration to Dogs (Female/male))

Day	Dose		
	15 mg/kg	40 mg/kg	100 mg/kg
SAHA			
AUC (ng hr/mL)	220 ± 8.8/230 ± 60.5	381±159/512±120	522±211/571±304
Cmax (ng/mL)	87.4±6/161±16	258±181/287±91	188±88/204±110
SAHA Glucoronide			
AUC (ng hr/mL)	916±342/1309±166	2129±1062/2953±298	3598±3143/4459±2602
Cmax (ng/mL)	393±78/869±294	1223±853/1780±853	1187±981/1490±964
N-Phenyl-Succinamic Acid			
AUC (ng hr/mL)	1714±689/1668±324	3120±1612/5906±3886	7546±2285/7780±4385
Cmax (ng/mL)	601±126/742±175	1246±788/1915±347	2005±516/2114±1172

Given the findings of this study, 80 mg/kg/day was chosen for the six month study. Additional changes to the study protocol included additional feeding window which appears to have abrogated dose limiting GI toxicity.

Study title: 26-Week Repeat Dose Toxicity and Toxicokinetic Study in Sprague-Dawley Rats Administered AP390 by Oral Gavage with a 13-Week Interim Sacrifice and a 4-Week Recovery (TT#04-5502, Volume 4.2.3.2)

Key study findings:

Based on the mortality, clinical pathology and anatomic pathology findings observed in all dose groups, a NOAEL was not established in this study and is therefore estimated to be< 20mg/kg/day. Treatment-related changes included reduced body weight and food consumption; lower globulin and white blood cell counts (primarily lymphocytes, including all B- and T-cell subtypes from immunophenotyping); higher absolute reticulocyte counts; decreased thymus weight; and histopathology findings in the thymus,

bone marrow, and spleen. All of these effects were reversible or partially reversible following 4 weeks of recovery.

Conducting laboratory and location: —
Date of study initiation: January 19th, 2004
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: SAHA, 1004D, —

Methods

Doses: 0, 20, 50, or 150 mg/kg per day

Species/strain: Sprague Dawley

Number/sex/group or time point (main study): 10/sex at interim sacrifice; 20/sex after 26 weeks

Route, formulation, volume, and infusion rate: oral gavage, 1% (w/v) carboxymethylcellulose, 0.5% (v/VTween® 80 in DI water, 10 mL/kg

Satellite groups used for toxicokinetics or recovery: 12/sex for TK, 10/sex for recovery

Age: 8 weeks

Weight: males:206-273 g; females: 146-210 g

Sampling times: pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 3, 6, 12, 18, and 24 hours post on day 7 and week 26 and predose and 0.5 hours during week 13.

Observations and times:

Mortality: twice daily
Clinical signs: twice daily, detailed weekly
Body weights: prior to treatment, day 1 and weekly thereafter
Food consumption: prior to treatment, day 1 and weekly thereafter
Ophthalmoscopy: prior to randomization, weeks 13, 26, and 30.
Hematology: week 14, week 27, and week 31
Clinical chemistry: week 14, week 27, and week 31
Urinalysis: week 14, week 27, and week 31
Gross pathology: all animals found dead or sacrificed in extremis were necropsied.
Organ weights: see histopath table
Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( )

Results

Mortality:

Dose Group	Animal #	Timing	Findings
0 mg/kg male	C61793	Day 188	• No observations
20 mg/kg male	C61830	Day 17	• Thin, few feces, rough and red haircoat. • ↑urea nitrogen/creatinine • Large renal pelvis, with tan discoloration with evidence of pyelonephritis **Renal Failure**

Dose Group	Animal #	Timing	Findings
20 mg/kg male	C61823	Day 84	• Limited use of hindlimbs, thin • ↑WBC, abs neutrophil, abs reticulocyte, globulin and ↓ RBC, Hb, HCT, albumin • Enlarged bilateral tarsal and metatarsal joints and protruding penis **Joint Infection and Inflammation, Blood Loss**
50 mg/kg male	C61846	Day 101	• Limited use of hindlimb, sensitive to touch • ↓abs lymphocyte count, albumin and A/G ratio, ↑globulin • Enlarged left tarsal joint that was swollen, red and gelatinous, enlarged mandibular LN **Joint infection and inflammation**
50 mg/kg female	C62107	Day 119	• Malocclusion, bloody nasal discharge inhibiting respiration
50 mg/kg female	C62034	Day 132	• Found dead, slight spinal cord hemorrhage and loss of distinct zones in the adrenal cortex. **Unknown cause**
150 mg/kg female	C62053	Day 42	• Cold to touch, tremors, labored respiration • Clear fluid in thoracic cavity • Necrosis and hypocellularity of femur and sternum bone marrow. Lymphoid necrosis of the spleen, lymph nodes and thymus; pleural effusion **Infection/inflammation**
150 mg/kg female	C62102	Day 86	**Bleeding Procedure-TK**

Clinical signs:

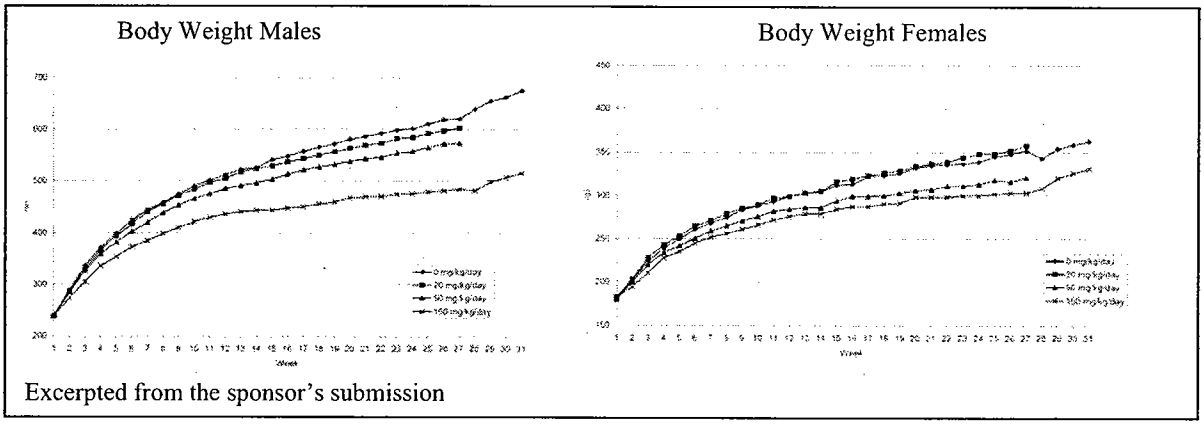
Observation	0 mg/kg	20 mg/kg	50 mg/kg	150 mg/kg
Limited use of hind limb left (infection)			1M	
Limited use of hind limb right (infection)	1M			
Limited use of hind limbs (infection, joints)		1M		
Malocclusion	3M	2F	3M/2F	7M
Missing Teeth	1M		1M	3M/2F
Masses (head/maxillary) week 17, 20, or 25-transient				1M/1F
Masses (mammary) weeks 24-transient		1F		
Masses (mammary) weeks 27*				1M

*Upon necropsy- Mass, single, 15x15x12 mm, red, tan, and firm on cut surface exudes a red semifluid.

Body weights:

Changes in Body Weight	20 mg/kg	50 mg/kg	150 mg/kg
Initiation of ↓	Males: week 15 Females: No dif	Males: week 6 Females: week 14 (intermittent ↓)	Males: week 2 Females: week 2
Maximal ↓ (end of treatment period)	Males: ↓3% Females: ↑1%	Males: ↓8% Females: ↓9%	Males: ↓22% Females: ↓14%

- Recovery: Weight gain significantly higher than controls but still body weight still significantly lower at end of recovery↓24% compared to control males and ↓9% compared to control females.



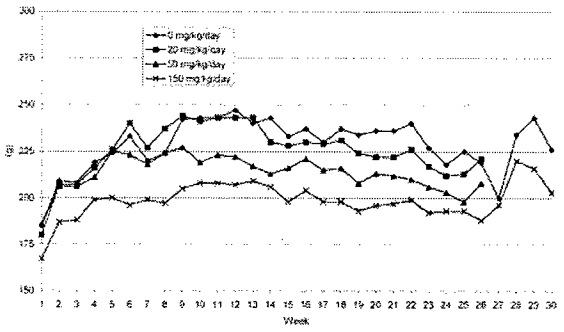
Excerpted from the sponsor's submission

Food consumption:

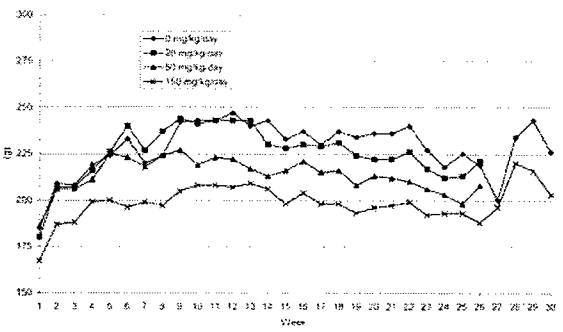
Changes in Food Consumption	20 mg/kg	50 mg/kg	150 mg/kg
Initiation of ↓	Males: week 8, 20-22 Females: No dif	Males: week 9- Females: week- 6-	Males: week 1- Females: week 1-
Maximal Difference compared to control	Males: ↓6%	Males: ↓14% Females: ↓15%	Males: ↓17% Females: ↓14%

- Decreases in food consumption were reversible in females and males during the first two weeks of recovery and partially reversible in males during the second two weeks of recovery.

Male Food Consumption



Female Food Consumption



Excerpted from the sponsor's submission

Ophthalmoscopy: none

Hematology:

% change in hematology parameters		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
RBC, HGB, HCT, MCV, MCH, MCHS	Alterations of less than 20% were noted throughout treatment and recovered in full								
Reticulocyte Count ($10^3/\mu L$)	Week 4	192	160	↑32	↑28	↑55	↑63	↑176	↑162
	Week 14	160	183	↑45		↑48		↑179	↑113
	Week 27	191	179	↑14		↑40	↑17	↑153	↑132
Platelets	Week 4, 14, & 27	Minimal reductions of ≤30%, complete recovery except high dose males where ↓17% was still noted							
WBC ($10^3/\mu L$)	Week 4	8.9	9.2		↓23	↓40	↓35	↓70	↓69
	Week 14	8.25	5.4	↓31		↓50		↓75	↓60
	Week 27	7.29	4.8	↓23	↓22	↓30	↓25	↓66	↓54
	Recovery	5.19	3.1					↓20	
Neutrophil ($10^3/\mu L$)	Week 14	2.09		↓60		↓70		↓79	
	Week 27	1.46	0.6	↓42		↓54		↓74	↓50
Lymphocytes ($10^3/\mu L$)	Week 4	7.32	7.8			↓45	↓35	↓76	↓70
	Week 14	5.77	4.4			↓43		↓73	↓60
	Week 27	5.44	3.8	↓17	↓22	↓17	↓27	↓69	↓55
	Recovery	3.91	2.3					↓25	
Monocyte ($10^3/\mu L$)	Week 4		0.21						↓50
	Week 14	0.20	0.13					↓55	↓50
	Week 27	0.18	0.11					↓34	
Eosin ($10^3/\mu L$)	Week 4		0.11						↓50
	Week 14	0.11	0.09					↓64	↓66
	Week 27		0.12		↓25	↓32	↓25	↓62	↓50

Clinical chemistry:

% Change in Clinical Chemistry Parameters		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
Total Protein	5-15% reduction including during recovery period in males								
Globulin (g/dL)	Week 14	3.1	2.7	↓5		↓24		↓39	↓23
	Week 27	3.1	2.7	↓13		↓13	↓15	↓36	↓23
	Week 31	3.1	2.8					↓17	
Cholesterol (mg/dL)	Week 14	100	94					↓57	↓31

% Change in Clinical Chemistry Parameters		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
	Week 27	87	103			↓30	↓17	↓48	↓36
CK (IU/L)	Week 14		736						↑93
K (mM)	Week 14	6.3	5.5			↓13		↓16	↓19
	Week 27	6.3	5.3	↓6		↓12		↓15	↓8

Urinalysis:

% Change in Urinalysis Parameters		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
pH	Week 14	7.1				↓9		↓7	
	Week 27	7.1	6.7			↓9		↓12	↓6
	Week 31	6.9						↓4	
Urinary CA	Week 14	4.6						↑215	
	Week 27	4.7		↑53		↑110		↑297	
CA Ex (mg)	Week 14	0.61						↑3 fold	
	Week 27	0.65	1.28					↑4 fold	↑59

Peripheral Blood Immunophenotyping:

- All changes were reversible during the recovery period

% Change in Peripheral Blood Immunophenotyping Data-Week 4	0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
	M	F	M	F	M	F	M	F
Lymphocyte Recovery and Purity (#/μL)	4867	4582	↓22	↓25	↓59	↓44	↓84	↓77
Total T-Lymphocytes (#/μL)	2190	2363		↓31	↓52	↓52	↓80	↓72
Helper T-Lymphocytes (#/μL)	1616	1676		↓31	↓56	↓53	↓82	↓73
Suppressor Cytotoxic T Lymphocytes(#/μL)	702	845		↓30	↓44	↓52	↓87	↓70
Total B Lymphocytes (#/μL)	2050	1921	↓44	↓37	↓69	↓49	↓92	↓88
Natural Killer Cells (#/μL)	101	109	↑62		↑32		↑43	

% Change in Peripheral Blood Immunophenotyping Data-Week 26	0 mg/kg		150 mg/kg	
	M	F	M	F
Lymphocyte Recovery and Purity (#/μL)	3831	1903	↓67	↓37
Total T-Lymphocytes (#/μL)	1290	657	↓48	↓6
Helper T-Lymphocytes (#/μL)	863	422	↓35	↑7
Suppressor Cytotoxic T Lymphocytes(#/μL)	509	261	↓66	↓37
Total B Lymphocytes (#/μL)	1750	916	↓82	↓57
Natural Killer Cells (#/μL)	249	88	↑88	↑69

Gross pathology:

Organ	Observation	Macroscopic Observations							
		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
		n=*10*/20/ **10**	n=*10*/20/ **10**	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20 /**10**	n=*10*/20/ **10**
Stomach	Dark Focus/Areas				1			*1*/2	*3*/2
	Red Focus							1	1
	Raised area					1			
Kidney	Large Pelvis		1			1			
	Granular Material					1		1	
	Semifluid material/pelvis					1			
LN Mesenteric	Mottled							2	
	Large								**1**

Organ	Observation	Macroscopic Observations							
		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
		n=*10*/20/**10**	n=*10*/20/**10**	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20/**10**	n=*10*/20/**10**
	Diffusely Red								**1**
Seminal Vesicle	Small							1	
Mammary	Mass							1	

Interim Sacrifice/Terminal Sacrifice/**Recovery Sacrifice**

Organ weights:

Organ	Percent change in absolute Organ Weight							
	0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
	M	F	M	F	M	F	M	F
Liver (g)	13.4	7.9					↓*23*/↓27/**↓28**	↓*15*/↓24
Kidney (g)	3.8	2.1					↓*29*/↓22/**↓19**	↓*8*/↓9
Thymus (g)	0.31	0.33					↓*49*/↓35	↓*50*/↓35
Seminal Vesicle(g)	2.03						↓37	

Histopathology:

Organ	Observation	Microscopic Observations							
		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
		n=*10*/20/**10**	n=*10*/20/**10**	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/20/**10**	n=*10*/20/**10**
Marrow, Femur	Erythroid hyperplasia/myeloid hypoplasia		*1*	*2*/2	*3*/3	*4*/7	*3*/12	*9*/12	*8*/5
Marrow, Sternum	Erythroid hyperplasia/myeloid hypoplasia		*1*	2	*1*/4	*3*/12	*4*/12	*8*/8	*5*/5
Bone, femur	Degeneration, cartilage	**1**	**2**					**2**	**3**
Eye	Inflammation, chronic							**1**	**1**
Kidney	Pyelonephritis	1	1					2	
	Hyperplasia	1	3					7	2
	Mineralization increased	1	2/**1**					5/**1**	5/**2**
	Cyst	1							1
Liver	Lipidosis								**1**
Spleen	Depletion, lymphocytic					*1*		*7*/13	*2*/11
	Pigment, increased	**1**	**2**	*1*/9	*4*/6	*9*/19	*7*/17	*10*/19/**10**	*9*/20/**9**
	Inflammation, capsule					*1*		*2*	
Thymus	Depletion, lymphocytic	*1*/**1**				*2*		*7*/**5**	*3*
LN, mesenteric	Infiltrate, macrophage, pigmented↑			*1*		*1*		*6*	*1*
Adrenal Cortex	Loss of distinct zona fasiculata and reticularis	**1**		*3*		*8*	*4*	*9*/**8**	*4*/**3**
	Degeneration, cystic/hemorrhagic	**1**	**1**					**4**	**4**
Pancreas	Infiltrate, lymphohistiocytic	**1**	**1**					**3**	**3**
	Zymogen Granule decreased								**2**
Stomach	Erosion			*1*		*1*		*1*	2

Organ	Observation	Microscopic Observations							
		0 mg/kg		20 mg/kg		50 mg/kg		150 mg/kg	
		M	F	M	F	M	F	M	F
		n=*10*/20/ 10	n=*10*/20/ 10	n=*10*/20	n=*10*/20	n=*10*/20	n=*10*/2 0	n=*10*/20 /10	n=*10*/20/ 10
	Ectasia, glandular							*1*	
	Ulcer							*1*	
Testis	Syncytial cells					*1*		*1*	
Ovary	Corpora Lutea decreased		2		4		9		6

Interim Sacrifice/Terminal Sacrifice/**Recovery Sacrifice**

Toxicokinetics:

- Serum samples were assayed for the parent (SAHA) and its metabolites (SAHA glucuronide and N phenyl-succinamic acid) using a validated HPLC with mass spectrometric detection method.
- Toxicokinetic parameters (Cmax, tmax, AUC and t1/2 for the parent and its metabolites in serun were determined by noncompartmental methods. (See tables excerpted from the sponsor's submission)
- Toxicokinetic results showed that SAHA is rapidly and extensively metabolized after oral administration to SAHA glucuronide and N-phenyl-succinamic acid, the major rnetabolite.
- For the parent, systemic exposure appeared to be similar between males and females over the dose range of 20 to 150 mg/kg on Day 7 and Week 26 and appeared to be dose proportional across the range 20-150 mg/kg Day 7 except between 50 and 150 mg/kg for females. During week 26 AUC values increased in a dose proportional fashion over the dose range of 20-50 mg/kg for both sexes and in a less than dose proportional fashion between 50 and 150 mg/kg. Cmax values increased in a less than dose proportional manner over the dose range of 50-150 mg/kg.
- For the metabolite SAHA glucuronide, systemic exposure appeared to be similar between male, and females on Day 7 and Week 26 at all doses. Systemic exposure to N-phenyl-succinamic acid was similar between males and females on Day 7 and Week 26 over the dose range of 20-150 mg/kg except at a dose level of 50 and 150 mg/kg on Week 26.
- There was an observed minimal accumulation (<2 fold) of SAHA at 20 mg/kg for females and at 50 mg/kg for males on Week 26. In addition, minimal accumulation was observed of SAHA glucuronide at 50 mg/kg, and of N-phenyl-succinamic acid at 50 and 150 mg/kg/day for males on Week 26 after repeated daily oral dosing of SAHA Day 7 through Week 26.
- For the metabolite SAHA glucuronide, AUC values on Day 7 increased proportionally as dose, increased from 20 to 50 mg/kg for both sexes and between 50 and 150 mg/kg for males Increases were less than dose proportional for females between 50 and 150 mg/kg. On week 26, AUC increased proportionally for both sexes between 20 and 50 mg/kg and in a less than dose proportional fashion between 50 and 150 mg/kg.
- For N-phenyl-succinamic acid, AUC increased proportionally as doses increased from 20 to 50 mg/kg for both sexes on Day 7 and Week 26. Increases were less than dose proportional between 50 and 150mg/kg for males.

- No trends with respect to t1/2 values of SAHA, SAHA glucuronide and N-phenyl-succinamic acid were observed as doses increased from 20 to 150 mg/kg/day, and between Day 7 and Week 26.

TK Parameters in males and females following SAHA Administration to Rats			
Day	Dose		
	20 mg/kg	50 mg/kg	150 mg/kg
Dose normalized $AUC_{(0-24)}$ [(ng h/mL)/(mg/kg/day)] (Males/Females)			
SAHA			
Day 7	8.9/9.8	9.5/10.0	11.0/7.0
Week 26	11.7/13.3	13.4/11.4	8.2/7.8
SAHA Glucoronide			
Day 7	20.6/21.5	17.1/19.2	16.0/11.7
Week 26	23.1/25.6	24.1/21.8	14.8/14.8
N-Phenyl-Succinamic Acid			
Day 7	197/178	208/197	155/133
Week 26	264/235	297/191	214/150
Dose normalized Cmax [(ng/mL)/(mg/kg/day)] (males/females)			
SAHA			
Day 7	4.8/4.9	3.4/3.9	2.5/2.4
Week 26	8.3/13.0	4.9/6.3	3.1/2.9
SAHA Glucoronide			
Day 7	11.4/11.1	6.8/7.7	4.2/4.2
Week 26	15.8/25.1	12.3/12.3	6.8/8.2
N-Phenyl-Succinamic Acid			
Day 7	83.3/84	51.1/53	28.5/26.3
Week 26	124/154	73.2/78.1	37.6/30.1

Study title: 26-Week Repeat Dose Toxicity and Toxicokinetic Study in Beagle Dogs Administered AP390 Capsules with a 4-Week Recovery (TT#04-5504, Volume 4.2.3.2)

Key study findings:
The NOAEL of SAHA administered to dogs is 60 mg/kg/day for 26 weeks. Gastrointestinal toxicity caused by SAHA was associated with the high-dose regimen (particularly at the 160 mg/kg/day level) used in the study. For this regimen, dosing began at 80 mg/kg/day and was escalated in succession to 100, 125, and 160 mg/kg/day on Days 16, 30, and 97, respectively. Based on the 4 week repeat dose experiment, 80 mg/kg/day was chosen for the six month study. Additional changes to the study protocol included additional time to the feeding window (at least 12 hours) which appears to have abrogated dose limiting GI toxicity.

Conducting laboratory and location: —
Date of study initiation: March 16th, 2004
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AP390 (SAHA Capsules), 6002.001; –

Methods

Doses: 0, 20, 60, 80 mg/kg/day (80 mg/kg dose increased to 100, 125, and 160 mg/kg/day on days 16, 30 and 97, respectively

Species/strain: Dog/Beagle

Number/sex/group or time point (main study): 4/sex/dose

Route, formulation, volume, and infusion rate: oral, neat, gelatin capsule

Satellite groups used for toxicokinetics or recovery: 2/sex/dose for control and high dose

Age: 8-10 months

Weight: 9.8-12.4 kg for males; 7.7 -12.6 kg for females

Sampling times: Day 7, 56 and week 17 and 25 at 0, 30 min, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours post-dose.

Unique study design or methodology: Given a lack of toxicity, the dose in the HD group was incrementally increased throughout the study. EKG assessment occurred at 2 hours post-dose, but Tmax was calculated to be less than 2 hours for SAHA and its major metabolites.

Observations and times:

Mortality: Twice daily
Clinical signs: Cageside- once daily; detailed- once weekly
Body weights: prior to treatment, on day -1, day 1 and twice weekly thereafter
Food consumption: daily beginning 1 week before administration. (Food was provided at the PM room check and removed 1 hour prior to dosing)
Ophthalmoscopy: Prior to treatment, weeks 13, 26, and 30
EKG: Prior to treatment, weeks 13, 26, and 30 before dosing and 2 hours postdose. Tests included heart rate, PR, QRS, QT, QTc, and RR intervals, using Leads I, II, III, aVR, aVL, aVF, V1, V2, V3, and V4. In addition to the tabulation and reporting of quantitative measurements, a qualitative assessment of the trace for rhythm and abnormalities was performed and reported. QTc
Hematology: prior to treatment and Weeks 4, 13, 25 and 30.
Clinical chemistry: prior to treatment and Weeks 4, 13, 25 and 30.
Urinalysis: prior to treatment and Weeks 4, 13, 25 and 30
Gross pathology: All animals necropsied
Organ weights : See histopath table
Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( )

Results

Mortality: 1 HD female sacrificed week 24 due to recurring body weight loss (22.6%) and low food consumption during weeks 21-24. At necropsy, the animal had multiple red areas in the mucosa of the stomach that measured up to 0.2 cm in diameter. The lesions correlated microscopically with gastric erosions with acute inflammation and were considered test article-related. Test article-related microscopic findings were seen in the animal's bone marrow (sternum only), gastrointestinal tract, liver, and spleen. The finding in the bone marrow of the sternum was slight cellular depletion with erythroid

hypoplasia. Lesions in the gastrointestinal tract included slight erosions with acute inflammation in the stomach; slight villous blunting with crypt epithelium regeneration in the duodenum, jejunum, and ileum; minimal crypt cell necrosis in the jejunum; slight crypt epithelium regeneration in the cecum; and minimal acute inflammation in the ileum. In the liver, slight brown pigment was present within Kupffer cells, and moderately increased brown pigment was seen in the spleen. In addition, lymphocytic depletion affected multiple organs, including the thymus, spleen, and Peyer's patch. These macroscopic and microscopic pathology findings indicated that the animal's poor condition was due to a test article-induced gastroenteropathy.

Clinical signs: One HD male and female were observed to have nonformed or liquid feces.

Body weights: During the last week of dosing. The HD male with nonformed feces had a 10% reduction in body weight.

Food consumption: limited to the preterminal sacrifice animal

Ophthalmoscopy: no drug dependent observations

EKG: no drug dependent observations

Hematology: Slight, reversible, changes were note in HD females, but were limited to decreases in red cell mass (RBC/HG/HCT-≤15%, weeks 4 and 25) and WBC [29%-(primarily neutrophils-33% week 25) and increased platelets (27%, week 25).

Clinical chemistry: no drug dependent observations

Urinalysis: No drug dependent observations

Gross pathology: Test article related findings (multiple red pinpoint foci in the gastric mucosa, and multiple linear red areas in the mucosa of the cecum, colon, and rectum) were noted in the HD male with nonformed feces during the last week of dosing.

Organ weights: Not test article effects were observed

Histopathology: (excerpted from the sponsor's submission)

Incidence of Selected Microscopic Findings, Terminal and Unscheduled Sacrifice Animals[a]

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	20	60	80/100/125/160	0	20	60	80/100/125/160
Number Examined	4	4	4	4	4	4	4	4
Bone Marrow, Sternum								
Depletion, Cellular, with Erythroid Hypoplasia	0	0	0	1	0	0	0	1
Esophagus								
Erosion, with Acute Inflammation	0	0	0	1	0	0	0	0
Stomach								
Erosion with Acute Inflammation	0	0	0	0	0	0	0	1
Duodenum								
Villous Blunting with Crypt Epithelium Regeneration	0	0	0	0	0	0	0	1
Jejunum								
Inflammation, Acute	0	0	0	2	0	0	0	0
Necrosis, Crypt Cells	0	0	0	0	0	0	0	1
Villous Blunting with Crypt Epithelium Regeneration	0	0	0	1	0	0	0	1
Ileum								
Inflammation, Acute	0	1	0	2	1	0	0	1
Necrosis, Crypt Cells	0	0	0	2	0	0	0	0
Villous Blunting with Crypt Epithelium Regeneration	0	0	0	1	0	0	0	1
Cecum								
Hemorrhage, Lamina Propria	0	0	0	3	0	0	0	0
Inflammation, Acute	0	0	0	2	0	0	0	0
Necrosis, Crypt Cells	0	0	0	1	0	0	0	0
Regeneration, Crypt Epithelium	0	0	0	0	0	0	0	1
Colon								
Hemorrhage, Lamina Propria	0	0	0	1	0	0	0	0
Inflammation, Acute	0	0	0	3	0	0	0	0
Regeneration, Crypt Epithelium	0	0	0	1	0	0	0	0
Rectum								
Hemorrhage, Lamina Propria	0	0	0	2	0	0	0	0
Inflammation, Acute	0	0	0	3	0	0	0	0

a Includes data from Group 4 female, Animal No. H06049, which was sacrificed in a moribund condition during Week 24.

Toxicokinetics:

- Serum samples were assayed for SAHA and its metabolites (SAHA Glucuronide and N-Phenyl succinamic acid) using a validated mass spec detection method.
- In general, systemic exposure to SAHA is similar between males and females. Accumulation was observed on day 175 for both genders with a high degree of variability.
- Gender differences were not apparent in SAHA Glucuronide and N-phenyl succinamic acid. In general, AUC and Cmax were dose proportional for both metabolites.

TK Parameters in males and females following SAHA Administration to Dogs			
Day	Dose		
	20 mg/kg	60 mg/kg	80/100/125/160 mg/kg
Dose normalized $AUC_{(0\text{-}24)}$ [(ng h/mL)/(mg/kg/day)] (Males/Females)			
SAHA			
Day 7	4.2	4.1	0.9
Day 56	6.4	6.7	4.4
Day 116	9.2	3.4	3.3
Day 175	9.9	6.1	2.2
SAHA Glucoronide			
Day 7	24.9	18.1	11.6
Day 56	42.2	30.2	20.5
Day 116	25.9	14.2	13.1
Day 175	37.4	21.7	11.1
N-Phenyl-Succinamic Acid			
Day 7	105	62.4	48.5
Day 56	154	80.7	52.6
Day 116	105	46.4	41.5
Day 175	142	71.3	34.0
Dose normalized Cmax [(ng/mL)/(mg/kg/day)] (males/females)			
SAHA			
Day 7	2.1	1.2	0.9
Day 56	3.3	2.5	1.2
Day 116	3.8	1.5	1.0
Day 175	3.6	1.9	0.8
SAHA Glucoronide			
Day 7	7.1	3.9	2.6
Day 56	13.2	7.7	3.9
Day 116	7.8	4.3	2.8
Day 175	11.3	5.97	2.4
N-Phenyl-Succinamic Acid			
Day 7	18.6	9.81	8.74
Day 56	31.4	14.7	7.78
Day 116	23.2	12.3	8.16
Day 175	30.6	13.4	7.19

Histopathology inventory

Study	TT#04-5502	TT#04-5504
Species	Rats	Dogs
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
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
Gross lesions	X	X
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X	X*
Lachrymal gland		
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
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	X	X
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	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		
Peyer's Patch	X	

X, histopathology performed *, organ weight obtained

2.6.6.4 Genetic toxicology

Study Title: WIN 64652:*Salmonella*/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and *Escherichia Coli* WP2 uvrA Reverse Mutation Assay with a Confirmation Assay (TT935506; Volume 4.2.3.3.1) (Previously reviewed by Dr Lee Ham; IND 58915, review #3, modified herein)

Key Findings: Under the conditions of this assay, SAHA is positive for genotoxocity in tester strain TA100 in the presence of S9.

Conducting Laboratory:

Date of study initiation: March 30, 1993
GLP Compliance: Yes
QA reports: yes
Methodology

Strains: S. typhimurium TA98, TA100, TA1535, TA1537, TA1538; E. coli WP2uvrA

Concentration/Dose Selection Criteria: Bacterial Lawn inhibition

Range Finding Results: Neither precipitate nor appreciable toxicity was observed, therefore the maximum chosen for the definitive assay was 5000µg/plate.
Drug, lot #, and % purity: WIN64652, Lot C,
Metabolic Activation System: Aroclor 1254-induced rat liver S9
Vehicle: DMSO
Positive Controls:

Strain	Activation	Positive Control	Concentration (µg/plate)
TA98	+	2-aminoanthracene	1.0
	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	1.0
	-	sodium azide	1.0
TA1535	+	2-aminoanthracene	1.0
	-	sodium azide	1.0
TA1537	+	2-aminoanthracene	1.0
	-	9-aminoacridine	75
TA1538	+	2-aminoanthracene	1.0
	-	2-nitrofluorene	1.0
E. coli WP2 *uvrA*	+	2-aminoanthracene	10
	-	methyl methanesulfonate	1,000

Exposure Conditions:

Incubation and sampling times: 48 hours
Concentration/dose used in definitive study: 6.7 – 5000 µg/plate

Analysis:

 # Plates/replicates:
Counting Method: Entirely by Automated colony counter or entirely by hand

Criteria for Positive Results: positive and negative results within historical range, replicates show statistically significant and 2 fold increase in strains TA989, TA100 and Ecoli WP2*uvr*A and a 3 fold increase in strains TA1535, TA1537, and TA1538.

Results:

Study Validity: The study was valid

Study Outcome:

- Positive responses were observed with tester strains TA100 in the presence of S9 (2.3 fold) and TA1535 in the absence of S9 (3.6 fold). Dose responsive increases were observed in TA98 and TA100 but did not reach the 2 fold level of increases to indicate a positive response.

Test Article Id : WIN 64652 (Lot C)
Study Number : TC983.502060 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538	WP2 uvrA
0.0	32 ± 9	130 ± 5	14 ± 4	7 ± 1	6 ± 1	27 ± 7
100	40 ± 4	137 ± 31	25 ± 2	5 ± 4	9 ± 5	27 ± 10
333	45 ± 9	148 ± 10	46 ± 5	4 ± 3	7 ± 2	32 ± 3
1000	50 ± 6	170 ± 6	39 ± 11	6 ± 2	8 ± 1	34 ± 4
3333	61 ± 3	236 ± 23	50 ± 5	5 ± 2	11 ± 4	24 ± 4
5000	47 ± 13	223 ± 3	40 ± 8	5 ± 4	6 ± 3	20 ± 3
Pos	596 ± 150	832 ± 18	466 ± 9	2436 ± 157	360 ± 31	272 ± 81

Liver Microsomes: Rat liver S-9

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538	WP2 uvrA
0.0	32 ± 4	122 ± 40	11 ± 2	7 ± 5	14 ± 1	35 ± 5
100	38 ± 1	148 ± 22	12 ± 1	6 ± 3	13 ± 3	35 ± 5
333	39 ± 2	150 ± 21	12 ± 6	7 ± 4	17 ± 3	35 ± 1
1000	35 ± 6	187 ± 13	10 ± 5	9 ± 1	10 ± 3	33 ± 3
3333	40 ± 7	238 ± 13	16 ± 5	7 ± 0	14 ± 6	36 ± 8
5000	31 ± 4	283 ± 33	14 ± 2	3 ± 2	9 ± 3	35 ± 3
Pos	1090 ± 44	982 ± 42	119 ± 15	119 ± 15	1278 ± 170	668 ± 132

0.0 = Vehicle plating aliquot of 50 µL
Pos = Positive Control concentrations as specified in Materials and Methods section.

- Contamination of confirmatory assay resulted in multiple runs. A positive response was observed in tester strain TA100 in the presence of S9 (2.0 fold increase). Dose responsive increases were observed in TA98 in the presence of S9 and TA100 in the absence of S9 but did not reach the 2 fold magnitude.

APPEARS THIS WAY
ON ORIGINAL

Test Article Id : WIN 64652 (Lot C)
Study Number : TC983.502060 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA100	WP2 uvrA
0.0	131 ± 10	25 ± 6
100	128 ± 18	18 ± 6
333	157 ± 20	17 ± 2
1000	164 ± 15	18 ± 3
3333	234 ± 8	16 ± 6
5000	244 ± 3	11 ± 2
Pos	613 ± 25	404 ± 21

Liver Microsomes: Rat liver S-9

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538	WP2 uvrA
0.0	15 ± 3	147 ± 12	14 ± 4	5 ± 2	8 ± 1	20 ± 2
100	20 ± 5	151 ± 6	11 ± 3	5 ± 3	11 ± 7	20 ± 7
333	24 ± 3	167 ± 8	9 ± 2	7 ± 3	15 ± 2	24 ± 4
1000	22 ± 2	183 ± 16	15 ± 5	5 ± 1	15 ± 1	12 ± 0
3333	28 ± 5	250 ± 17	14 ± 4	7 ± 2	18 ± 7	16 ± 2
5000	30 ± 2	291 ± 23	18 ± 4	7 ± 2	17 ± 5	11 ± 6
Pos	385 ± 61	1338 ± 158	138 ± 18	186 ± 11	1650 ± 310	572 ± 73

0.0 = Vehicle plating aliquot of 50 µL
Pos = Positive Control concentrations as specified in Materials and Methods section.

- No positive reponses were observed with any tester strains in the presence or absence of S9. Dose responsive increases in TA100 were noted, but did not reach 2 fold magnitude.

APPEARS THIS WAY ON ORIGINAL

Test Article Id : WIN 64652 (Lot C)
Study Number : TC983.502060 Experiment No : B3

Average Revertants Per Plate ± Standard Deviation
Liver Microsomes: None

Dose (μg)	TA98	TA1535	TA1537	TA1538
0.0	19 ± 3	12 ± 2	6 ± 2	7 ± 4
100	14 ± 4	11 ± 1	6 ± 2	9 ± 2
333	16 ± 9	10 ± 5	6 ± 1	12 ± 3
1000	26 ± 2	11 ± 3	7 ± 1	9 ± 3
3333	31 ± 6	16 ± 2	7 ± 3	11 ± 2
5000	24 ± 1	13 ± 2	8 ± 3	10 ± 5
Pos	352 ± 27	284 ± 25	1680 ± 378	756 ± 24

Liver Microsomes: Rat liver S-9

Dose (μg)	TA98
0.0	23 ± 2
100	22 ± 2
333	27 ± 3
1000	25 ± 3
3333	34 ± 5
5000	36 ± 7
Pos	1695 ± 293

0.0 = Vehicle plating aliquot of 50 μL
Pos = Positive Control concentrations as specified in Materials and Methods section.

- This experiment was conducted to clarify disparate results between B1 and B3. The 3 fold increase observed in this strain in the B1 was not duplicated in this assay.

Test Article Id : WIN 64652 (Lot C)
Study Number : TC983.502060 Experiment No : B4

Average Revertants Per Plate ± Standard Deviation
Liver Microsomes: None

Dose (μg)	TA1535
0.0	11 ± 1
100	12 ± 1
333	15 ± 6
1000	11 ± 6
3333	14 ± 1
5000	13 ± 4
Pos	508 ± 62

0.0 = Vehicle plating aliquot of 50 μL
Pos = Positive Control concentrations as specified in Materials and Methods section.

Study Title: Microbial Mutagenesis Assay with Preincubation (TT048026; Volume 4.2.3.3.1)
Key Findings: Under the conditions of this assay, SAHA is positive for genotoxocity in tester strain TA97a in the absence of S9 (5000 µg/plate).

Conducting Laboratory: —

Date of study initiation: April 19, 2004
GLP Compliance: Yes
QA reports: yes
Methodology
Strains: S. typhimurium TA98, TA97a, TA100, TA1535; E. coli WP2uvrA
Concentration/Dose Selection Criteria: Bacterial Lawn inhibition
Range Finding Results: Based on results of TT935506; Volume 4.2.3.3.1. Neither precipitate nor appreciable toxicity was observed, therefore the maximum chosen for the definitive assay was 5000µg/plate.
Drug, lot #, and % purity: L-001079038, Lot 000K009; —
Metabolic Activation System: Xenobiotic-induced (Phenobarbital/Beta Naphthoflavone) rat liver
Vehicle: DMSO
Positive Controls: 2 aminoantracene

Exposure Conditions:
Incubation and sampling times: 48 hours
Concentration/dose used in definitive study: 30, 100, 300, 1000, 3000, 5000 µg/plate
Analysis:
 # Plates/replicates: triplicate
Counting Method: Entirely by Automated colony counter or entirely by hand
Criteria for Positive Results: positive and negative results within historical range, replicates show statistically significant and 2 fold increase compared to historical control with dose dependency
Results:
Study Validity: The study was valid
Study Outcome:
- SAHA produced a 2.2 fold increase with 5000µg/plate in revertants in TA97a without metabolic activation (see table excerpted from the sponsor's submission).

Salmonella his$^+$ or *Escherichia coli trp*$^+$ Revertants per Plate

	TA100 21-Apr-2004				TA1535 21-Apr-2004				TA97a 21-Apr-2004			
	Without S-9		With S-9		Without S-9		With S-9		Without S-9		With S-9	
Conc. µg/Plate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (DMSO)	160.2	11.7	189.6	16.3	23.5	7.4	34.8	6.4	201.7	13.2	318.0	18.4
30 µg	171.0	9.5	184.0	11.5	28.3	2.1	40.3	13.7	161.0	21.0	339.7	16.4
100 µg	160.0	19.9	197.3	18.6	28.3	4.9	34.3	3.5	205.7	9.5	323.7	11.9
300 µg	185.7	8.7	195.0	12.1	31.3	7.4	30.3	4.0	230.3	34.7	301.0	71.0
1000 µg	193.0	22.7	200.3	9.6	37.7	7.0	30.0	3.0	260.3	26.0	345.0	30.2
3000 µg	246.7†	6.4	251.7	16.5	32.7	6.7	37.3	6.6	292.3	47.3	389.0	6.1
5000 µg	235.0†*	23.6	310.7	9.8	25.3*	2.5	40.0	7.0	445.3*	10.1	503.7	21.0

Study Title: WIN64652: *In vitro* chromosome aberration analysis of CHO cells (TT935505; Volume 4.2.3.3.1) (Previously reviewed by Dr Lee Ham; IND 58915, review #3, modified herein)

Key Findings: WIN64652 was found to be mutagenic in the CHO cell assay at 100µg/mL with and without metabolic activation

Conducting Laboratory and Location:

Date of Study Initiation: March 24th, 1993
GLP Compliance: Yes
QA Reports: Yes
Drug, lot #, and % purity: WIN64652, Lot C.

Methods

Cell Line: CHO-K_1-BH_4 cells
Metabolic Activation: Aroclor 1254 induced rat liver S9
Controls:
Vehicle: DMSO
Positive: N-nitrosodimethylamine (DMN) and N-methyl N nitro N nitrosoguanidine
Exposure Conditions:
Incubation and sampling times: 5 hours with drug; 22 hours without in dose selection series. Cells harvested 18 hours post drug in Aberration Assay.
Doses used in definitive study:
0, 10, 25, 50, 100 and 500µg/mL in the presence and absence of S9.
Analysis:
No. or replicates: duplicates
Counting Method: 100 cells from each culture.

Basis of dose selection:
Cell cultures were exposed for 5 hours to 0 (solvent control) 0.49, 0.98, 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000µg WIN64652/mL of serum free culture medium in the presence and absence of S9. A precipitate was observed in the 2000 µg/mL treatment with and without S9. Additional cultures were treated to positive and negative controls.

Results

Study validity: In the absence of S9, MNNG resulted in an increase in the % cells with aberrations as did DMN in the presence of S9.

Study outcome: At 100 µg/mL WIN64652 (the highest dose analyzed), chromosome aberrations were induced in 28% of the analyzed cells with and without metabolic

activation without exceeding a 50% reduction in growth. There was a statistically significant increase in Gaps, and Aberrations (simple and complex).

CHROMOSOME ABERRATION ANALYSIS OF WIN 64652: IN VITRO CHROMOSOME ABERRATION ANALYSIS IN CHINESE HAMSTER OVARY (CHO) CELLS (Protocol 00108)

Chromosome Aberration Assay

Treatment (µg/ml)	S9 (+/-)	No. Cells Analyzed	Total Number			Aberrant Cells	% Aberrant Cells			
			Gaps[a]	Abs[b]	Abs/Cell		SI[c]	CX[d]	MS[e]	Total
0[f]	-	200	3	10	0.050	10	3.5	1.5	0	5.0[f]
10	-	200	7	10	0.050	9	2.0	2.5	0	4.5[f]
25	-	200	7	7	0.035	7	3.5	0	0	3.5[f]
50	-	200	15	14	0.070	13	5.0	1.5	0	6.5[f]
100	-	144	15[g]	98[g]	0.685[g]	41	12.5	15.3	0.7	28.5[f]
MNNG[h]	-	50	10	52	1.040	27	26.0	28.0	0	54.0**
0[f]	+	200	7	11	0.055	11	3.0	2.5	0	5.5[f]
10	+	200	7	10	0.050	10	4.0	1.0	0	5.0[f]
25	+	200	6	14	0.070	12	5.0	1.0	0	6.0[f]
50	+	200	11[g]	13[g]	0.065[g]	13	6.0	0	0.5	6.5[f]
100	+	158	14[g]	106[g]	0.675[g]	44	10.1	17.1	0.6	27.8[f]
DMN[i]	+	75	17	44	0.587	30	22.7	17.3	0	40.0**

Study Title: L001079038: Chromosomal aberrations i*n vitro*, in CHO cells TT#048633, TT#048639, TT048651 (TT048633; Volume 4.2.3.3.1)

Key Findings: L001079038 was found to be clastogenic (positive for chromosomal aberrations) under the conditions of these assays, in the presence and absence of metabolic activation.

Conducting Laboratory and Location: Merck Institute for Therapeutic Research, West Point, Pennsylvania
Date of Study Initiation: May 5th, 2004
GLP Compliance: Yes
QA Reports: Yes
Drug, lot #, and % purity: L001079038, Lot 000K009, Purity= —

Methods

Cell Line: CHO-WBL cells
Metabolic Activation: S9 from rats treated with beta-naphthoflavone and phenobarbital.

Controls:

Vehicle: DMSO
Positive: Cyclophosphamide with S9, Mitomycin C without S9

Exposure Conditions:

Incubation and sampling times:
Range finding: with and without S-9 (3-hour treatment): 62.5, 125.0, 250.0, 500.0, 1000.0, 2000.0, 4000.0, and 8000.0 µM; without S-9 (20-hour treatment): 1.0, 3.0, 10.0, 30.0, 90.0, 290.0, 870.0, 2640.0, and 8000.0 µM

Aberration Assay:with and without S-9 (3-hour treatment): 500, 750, 1000, 1250, 1500, 1750, and 2000 μM; without S-9 (20-hour treatment): 5, 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 μM
Confirmatory Assay: with S-9 (3-hour treatment): 450, 500, 550, 600, 650, 700, 750, and 800 μM; without S-9 (3-hour treatment): 250, 300, 350, 400, 450, 500, 550, and 600 μM

Analysis:

No. of replicates/counting method: 200 cells from each culture without duplication

Basis of dose selection:

Cell cultures were exposed for 20 hours with up to 8000 μM. There was marked suppression of growth in all series. The ranges selected for the aberration assay, up to 2000 μM for the 3-hour treatments with and without S-9, and up to 500 μM for the 20-hour treatment, were expected to include a dose with growth reduction not greatly exceeding 50% of concurrent solvent controls. Based on cell counts at 20 hours, cell growth after the 3-hour treatments was sharply reduced between 500 μM and 1000 μM with S-9 and between 250 μM and 500 μM without S-9 After the 20-hour treatment, growth was suppressed in a dose-related manner at 10 μM and above.

Results

Study validity: The tests with and without S-9 activation, and different treatment durations were considered separately. The tests were considered positive if there are statistically significant increases (p=0.05) over concurrent solvent controls in the percentages of cells with chromosomal aberrations at 2 or more separate concentrations of test article without greatly exceeding a 50% reduction in growth.

Study outcome:

Aberration Assay:

Cytotoxicity: There was clear dose-related cytotoxicity in all series. There was a reduction in the monolayer confluence with S-9 at 1250 μM and above, and without S-9 activation at ≥500 μM (3-hour treatment), and at ≥5 μM (20-hour treatment). Based on cell counts at 20 hours, growth after the 3-hour treatments was reduced from 81% of concurrent solvent controls at 500 μM to 46% at 750 μM with S-9 and to 43% at 500 μM, the lowest dose tested without S-9, therefore aberrations were not analyzed. After the 20-hour treatment, cell growth was reduced from 75% of concurrent solvent controls at 5 μM to 51% at 150 μM.

Aberrations:

Treatment	Cells Scored	% Cells With Abs	Frequency of Aberrations (Per 100 Cells)							
			Total Abs	Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	Severely Damaged	Pulverized Chromosome	Gaps
With S-9 (3-Hour Treatment)										
DMSO 1%	200	1.50	1.50	0.50	0.00	1.00	0.00	0.00	0.00	1.00
DMSO 1%	200	2.50	2.50	0.50	0.00	1.50	0.50	0.00	0.00	0.50
Cyclophosphamide 5 µM	200	13.00	13.50	2.00	4.50	6.50	0.50	0.00	0.00	0.50
Cyclophosphamide 10 µM	50	58.00	80.00	10.00	48.00	22.00	0.00	0.00	0.00	10.00
L-001079038										
500 µM	200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
750 µM	100	27.00	100.00	15.00	28.00	7.00	0.00	5.00	0.00	1.00
Without S-9 (20-Hour Treatment)										
DMSO 1%	200	1.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.50
DMSO 1%	200	1.50	1.50	0.00	0.00	1.00	0.50	0.00	0.00	0.50
L-001079038										
5 µM	200	1.50	1.50	1.00	0.00	0.00	0.50	0.00	0.00	1.00
50 µM	200	1.50	1.50	0.00	0.00	1.50	0.00	0.00	0.00	1.50
150 µM	200	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.50

Abs = Aberrations (Excluding Gaps, Pulverized Chromosome, Translocations, and abnormal monocentrics).
Severely damaged (SD) cell counted as 10 aberrations.

Confirmatory Assay:

Cytotoxicity: Only 3-hour treatments with and without S-9 were tested. There was clear dose-related cytotoxicity in both series. In cultures examined under the microscope before harvest, there was a reduction in the monolayer confluence at 750 µM and above with S-9 and at 250 µM and above without S-9 activation. Based on cell counts at 20 hours, growth was reduced from 88% of concurrent solvent controls at 550 µM to 55% at 700 µM with S-9, and from 90% of concurrent solvent controls at 250 µM to 46% at 600 µM without S-9.

Aberrations:

Treatment	Cells Scored	% Cells With Abs	Frequency of Aberrations (Per 100 Cells)							
			Total Abs	Chromatid Deletions	Chromatid Exchanges	Chromosome Deletions	Chromosome Exchanges	Severely Damaged	Pulverized Chromosome	Gaps
With S-9 (3-Hour Treatment)										
DMSO 1%	200	1.00	1.00	0.00	0.50	0.50	0.00	0.00	0.00	1.50
DMSO 1%	200	1.00	1.00	0.50	0.00	0.00	0.50	0.00	0.00	1.00
Cyclophosphamide 5 µM	100	15.00	36.00	3.00	14.00	9.00	0.00	1.00	0.00	3.00
Cyclophosphamide 10 µM	25	68.00	116.00	32.00	76.00	8.00	0.00	0.00	0.00	20.00
L-001079038										
500 µM	200	2.50	4.00	1.00	1.50	1.50	0.00	0.00	0.00	1.00
650 µM	200	5.00	26.50	1.50	3.50	1.00	0.50	2.00	0.00	2.50
700 µM	100	22.00	133.00	1.00	20.00	2.00	0.00	11.00	0.00	1.00
Without S-9 (3-Hour Treatment)										
DMSO 1%	200	1.50	6.00	0.50	0.00	0.00	0.50	0.50	0.00	0.50
DMSO 1%	200	2.00	2.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
Mitomycin C 0.5 µM	100	17.00	18.00	3.00	5.00	7.00	3.00	0.00	0.00	3.00
Mitomycin C 1.5 µM	50	48.00	108.00	36.00	58.00	12.00	2.00	0.00	0.00	10.00
L-001079038										
250 µM	200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
350 µM	100	18.00	126.00	5.00	8.00	3.00	0.00	11.00	0.00	2.00
600 µM	100	24.00	103.00	5.00	21.00	6.00	1.00	7.00	0.00	4.00

Abs = Aberrations (Excluding Gaps, Pulverized Chromosome, Translocations, and abnormal monocentrics).
Severely damaged (SD) cell counted as 10 aberrations.

Study title: **Screening Assay for Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes (TT035510; Volume 4.2.3.3.1)**

Key findings: Under the conditions of this study, there did not appear to be evidence of clastogenicity.

Conducting laboratory and location: —
Date of study initiation: December 9th, 2003
GLP compliance: no
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AP390, Lot No. AP-390-2, Purity not stated

Methods
Cell Line: Human peripheral lymphocytes (healthy adult donor)
Metabolic Activation: S9
Controls:
Vehicle (negative control): Cell Culture Grade Water
Positive: Mitomycin C (MMC)
Exposure Conditions:

Activation Condition	Test Article Added	Wash	Colcemid® Added	Harvest Started
- S9	0	--	20	22
+ S9	0	3	20	22

Doses used in definitive study: 0.5, 1.0, 2.0, 3.00, 4.00, 6.00, 8.00, 10.0, and 12.0 µg/mL were utilized. Aberrations were analyzed from 2.0 and 4.0 µg/mL, which had 17 and 56% reductions in mitotic index.

Basis of dose selection: 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL were utilized with and without S9 in the range finding assay. Cytotoxicity was observed without metabolic activation (22 hour treatment), at 7.81 µg/mL, (a 71% mitotic reduction was observed). Cytotoxicity was observed with activation (3 hour treatment) at ≥250 µg/mL. In a confirmatory assay, cytotoxicity was observed without activation (22 hour treatment) at 3.0 µg/mL (66% mitotic reduction).

Incubation and sampling times: see table above.

Results

Study validity : This was a non-GLP range-finding assay without confirmation.

Study outcome: There was no evidence of clastogenicity as evidenced by increases in polyploid cells or cells with endoreduplication.

Assay No.: 25711-0-449OECD Trial No.: A1 Date: 12/18/03 Lab No.: CY121403 Test Article: AP390

					# Cells Scored for Aberrations	% Mitotic Index Reduction[a]	# Cells Scored for pp and er	# of pp Cells	# of er Cells	Judgement (+/-)[b]	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: gaps	simple breaks	chte	chre	mab	Totals[c] -g-	Totals[c] +g+	Judgement (+/-)[d]
Controls																		
Vehicle:	CCGW	100	µL/mL	A	100		100	0	0		2	1				1	3	
				B	100		100	0	0			2				2	2	
				Total	200		200				2	3				3	5	
				Average	%	0		0.0	0.0		1.0	1.5				1.5	2.5	
Positive:	MMC	0.300	µg/mL	A	50		100	0	0		4	12	7			17	20	
				B	50		100	0	0		10	16	7			21	28	
				Total	100		200				14	28	14			38	48	
				Average	%	-		0.0	0.0	-	14.0	28.0	14.0			38.0	48.0	+
Test Article		7.81	µg/mL		33		--	--	--		5	2				2	6	
				Average	%	71	-	-	-	-	15.2	6.1				6.1	18.2	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations pp: polyploidy er: endoreduplication

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$.

[e] Insufficient data for analysis CCGW = cell culture grade water MMC = Mitomycin C

Study Title: Chromosomal Aberrations in Cultured Purified Human Blood Lymphocytes with AP390 (TT045501; Volume 4.2.3.3.1)

Key Findings: AP390 was not found to be clastogenic (positive for chromosomal aberrations) under the conditions of these assays, in the presence and absence of metabolic activation, however a confirmatory assay was not completed.

Conducting Laboratory and Location:
Date of Study Initiation: May 28th, 2004
GLP Compliance: Yes
QA Reports: Yes
Drug, lot #, and % purity: AP390, 104C/N051CP, Purity=

Methods

Cell Line: Human peripheral lymphocytes (healthy adult donor)
Metabolic Activation: Aroclor 1254 S9

Controls:

Vehicle: DMSO
Positive: Cyclophosphamide with S9, Mitomycin C without S9

Exposure Conditions:

Incubation and sampling times:

Range finding: with and without S-9 (3-hour treatment- 22 hour sampling) and without S-9 (22-hour treatment): 0.061, 0.122, 0.244, 0.488, 0.977, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL

Aberration Assay: with and without S-9 (3-hour treatment-22 hour sampling): 150, 300, 600, 800, 1000, 1100, 1250, 1400, 1550, 1700, 1850 and 2000 µg/mL; without S-9 (22-hour treatment): 3.13, 6.25, 12.5, 15.0, 17.5, 20.0, 25.0, 30.0, 40.0, 50.0, 75.0, 10 and 150 µg/mL

Analysis:

No. of replicates/counting method: One hundred cells, from each duplicate culture from four concentrations of the test article, the negative and vehicle controls, and one dose level from the positive.

Basis of dose selection: In the assay without metabolic activation (3-hour treatment), reductions of 0%, 22%, and 43% were observed in the mitotic indices of the cultures treated with 250, 500, and 1000 µg/mL, respectively, as compared with the vehicle control cultures. In the assay without metabolic activation (~22-hour treatment), reductions of 0%, 21%, 43%, 64%, 80%, 87%, 90%, 91%, and 92% were observed in the mitotic indices of the cultures treated with 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL, respectively, as compared with the vehicle control cultures. In the assay with metabolic activation, reductions of 0% and 35% were observed in the mitotic indices of the cultures treated with 500 and 1000 µg/mL, respectively, as compared with the vehicle control cultures.

Results

Study validity: The tests with and without S9 activation, and different treatment durations were considered separately. The tests were considered positive if there are statistically significant increases (p=0.01) over concurrent solvent controls in the percentages of cells with chromosomal aberrations at 2 or more separate concentrations of test article without greatly exceeding a 50% reduction in growth. Although this study is valid, confirmatory assays were not conducted within this study.

Study outcome:

Without Activation 3 Hour Incubation

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	RPMI 1640		8.9	8.4	8.7	--
Vehicle Control	DMSO	10.0 µL/mL	10.3	9.6	10.0	0
Test Article		600 µg/mL	10.3	9.4	9.9	1
		800 µg/mL	8.9	5.1	7.0	30
		1000 µg/mL	5.0	4.4	4.7	53
		1100 µg/mL[a]	4.4	3.0	3.7	63
		1250 µg/mL[a]	4.8	5.3	5.1	49
		1400 µg/mL[a]	0.0	3.8	1.9	81
		1550 µg/mL[a]	1.9	4.2	3.1	69
		1700 µg/mL[a]	0.4	0.2	0.3	97
		1850 µg/mL[a]	0.0	1.1	0.6	94
		2000 µg/mL[a]	0.1	0.0	0.1	99

[a] Precipitate observed at dose and wash.

Dose concentrations of 150 and 300 µg/mL were not analyzed.

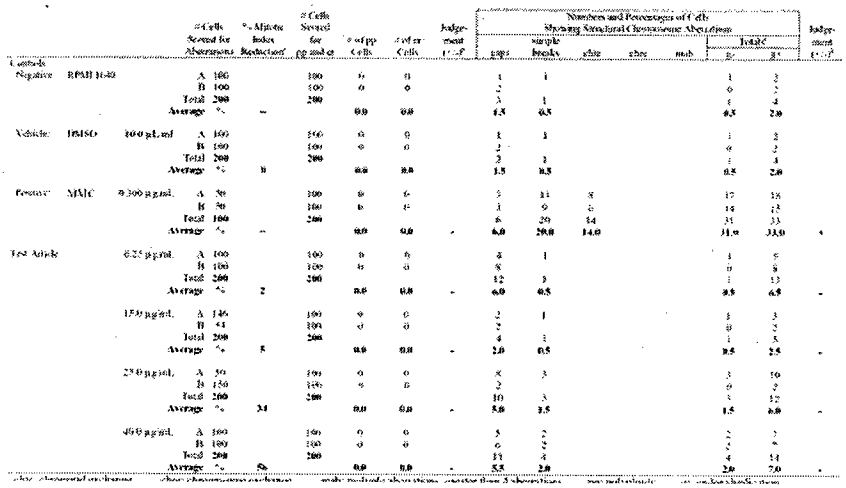
RPMI 1640 = culture medium DMSO = dimethylsulfoxide

				# Cells Scored for Aberrations	%-Mitotic Index Reduction*	# Cells Scored for gaps and/or	# of pp Cells	# of er Cells	Judgement (+/-)*	Numbers and Percentages of Cells Showing Structural Chromosome Aberrations: simple: gaps	simple: breaks	chte	chre	mab	Totals*: #-	Totals*: #+	Judgement (+/-)*
Controls: Negative:	RPMI 1640		A	100		100	0	0							0	0	
			B	100		100	0	0							0	0	
			Total	200		200									0	0	
			Average	%	--		0.0	0.0							0.0	0.0	
Vehicle:	DMSO	10.0 µL/ml	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0			1				0	1	
			Total	200		200				1	2				1	3	
			Average	%	0		0.0	0.0		0.5	1.0				0.5	1.5	
Positive:	MMC	1.50 µg/ml.	A	75		100	0	0		6	12	5			16	20	
			B	75		100	0	0		4	11	9		1	19	22	
			Total	150		200				10	23	14		1	35	42	
			Average	%	--		0.0	0.0	-	6.7	15.3	9.3		0.7	23.3	28.0	+
Test Article		300 µg/ml.	A	100		100	0	0		1					0	1	
			B	100		100	0	0		4	3	2			5	8	
			Total	200		200				5	3	2			5	9	
			Average	%	--		0.0	0.0	-	2.5	1.5	1.0			2.5	4.5	-
		600 µg/ml.	A	100		100	0	0		1		1			1	2	
			B	100		100	0	0							0	0	
			Total	200		200				1		1			1	2	
			Average	%	1		0.0	0.0	-	0.5		0.5			0.5	1.0	-
		800 µg/ml.	A	100		100	0	0		1	1				1	2	
			B	100		100	0	0		2		1			1	3	
			Total	200		200				3	1	1			2	5	
			Average	%	30		0.0	0.0	+	1.5	0.5	0.5			1.0	2.5	+
		1000 µg/ml.	A	100		100	0	0			2				0	2	
			B	100		100	0	0		3	2	1			3	6	
			Total	200		200				3	4	1			3	8	
			Average	%	53		0.0	0.0	-	1.5	2.0	0.5			1.5	4.0	-

Without Activation, 22 Hour Incubation

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	RPMI 1640		6.2	4.8	5.5	--
Vehicle Control	DMSO	10.0 µL/mL	4.8	3.3	4.1	0
Test Article		3.13 µg/mL	4.3	4.9	4.6	0
		6.25 µg/mL	3.8	4.1	4.0	2
		12.5 µg/mL	3.2	2.1	2.7	34
		15.0 µg/mL	3.6	4.2	3.9	5
		17.5 µg/mL	2.3	2.7	2.5	39
		20.0 µg/mL	2.4	3.5	3.0	27
		25.0 µg/mL	2.8	2.5	2.7	34
		30.0 µg/mL	3.0	2.1	2.6	37
		40.0 µg/mL	1.8	1.8	1.8	86
		50.0 µg/mL	2.0	2.2	2.1	49
		75.0 µg/mL	1.4	1.7	1.6	61
		100 µg/mL	1.0	1.7	1.4	66
		150 µg/mL	0.8	1.6	1.2	71

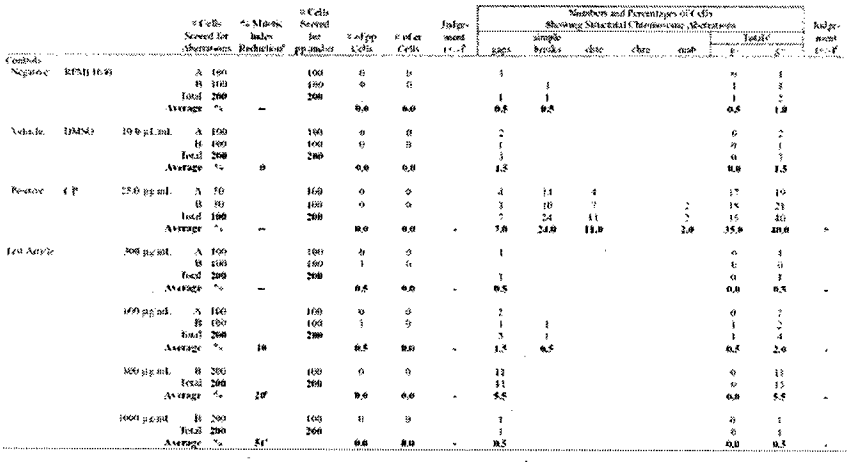
RPMI 1640 = culture medium DMSO = dimethylsulfoxide



With Activation, 3 Hour Incubation:

Treatment			% Mitotic Index A Culture	% Mitotic Index B Culture	Average % Mitotic Index	% Mitotic Reduction
Negative Control	RPMI 1640		8.6	11.2	9.9	--
Vehicle Control	DMSO	10.0 µL/mL	14.0	15.8	14.9	0
Test Article		600 µg/mL	16.6	10.1	13.4	10
		800 µg/mL	5.1	11.9	8.5	43
		1000 µg/mL	5.1	7.3	6.2	58
		1100 µg/mL[a]	6.9	2.0	4.5	70
		1400 µg/mL[a]	1.7	2.8	2.3	85
		1700 µg/mL[a]	0.1	0.1	0.1	99
		1850 µg/mL[a]	0.0	0.0	0.0	100
		2000 µg/mL[a]	0.0	0.0	0.0	100

[a] Precipitate observed at dose and wash.
Dose concentrations of 150, 300, 1250, and 1550 µg/mL were not analyzed.
RPMI 1640 = culture medium DMSO = dimethylsulfoxide



L-001079038: Exploratory Assay for Chromosomal Aberrations In Vitro, in Chinese Hamster Ovary Cells (TT #05-8662) and Exploratory Flow Cytometric Assay for Measuring DNA Synthesis Inhibition and Cell Cycle Kinetics (TT #05-8818) in Chinese Hamster Ovary Cells. (TT058662; Volume 4.2.3.3.1)

Study Summary:
The purpose of this non GLP report was to determine if chromosomal aberrations observed in the CHO cell assay might have a secondary mechanism such as inhibition of DNA synthesis. Aphidicolin was used as the positive control for direct action of a clastogenic compound on DNA. DNA synthesis was assessed by incorporation of bromodeoxyuridine (BrdUrd) into S-phase cells in cultures treated for 3 hours with L-001079038 without S-9 activation. Alterations in cell cycle kinetics were assessed in cells harvested immediately after treatment or allowed to recover for 5 hours and 17 hours.

Marked suppression of DNA synthesis was observed at the end of the 3 hour treatment period, as would be expected based on the mechanism of action. Significant accumulation in S-phase, as seen with aphidicolin was observed with ≥200µM. At 5 hours post treatment at doses of ≥200 µM there was slowed cell cycle progression but recovery at 17 hours with less than 400 µM (see figures excerpted from the sponsor's submission).

1 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

_ § 552(b)(5) Deliberative Process

_ § 552(b)(4) Draft Labeling

Summary: Structural Chromosomal Aberrations and DNA Synthesis Inhibition

Treatment	Chromosomal Aberration at 20 Hours			DNA Synthesis Inhibition at 3 Hours[b]	
	PD (% Control)	% Aberrant Cells	Freq. Abs per 100 Cells[a]	% Cells Labeled w. BrdUrd	BrdUrd Uptake per S-phase Cell (% Control)
3 Hours Without S-9					
DMSO 1%	100	0.50	0.50		100
DMSO 1%	100	1.50	1.50	58.5	100
APC 50 µM	ND	NS		56.3	14
MMC 0.5 µM	85	8.00**	11.00		
MMC 1.5 µM[c]	86	30.00**	38.00		
L-001079038 (µM)					
200	96	2.00	3.00	65.0	76
300	88	NS		64.7	40
400[c]	66	26.00**	226.0[e]	61.1	17
500[d]	53	60.00**	456.00[e]	50.7	9
600	45	NS		24.7	5

PD = Population doubling.
DMSO = Dimethylsulfoxide.
MMC (mitomycin C) is the positive control for aberrations.
APC (aphidicolin) is the positive control for DNA synthesis inhibition.
NS = Not scored.
ND = Not done.
[a] The total number of aberrations per 100 cells, since a cell may have more than one aberration.
[b] Data are averages from duplicate cultures.
200 cells scored for aberrations per point except where noted.
[c] 50 cells scored for aberrations.
[d] 25 cells scored for aberrations.
[e] Includes severely damaged cells (≥10 Aberrations per cell).
** $p \leq 0.01$ compared to the relevant control group using a one-sided Fisher's exact test. Since several comparisons with a common control were made, an adjustment procedure of Dunnett was used to assess the overall significance of each comparison for doses of test compound.

L-001079038: Assay for Micronucleus Induction in Mouse Bone Marrow (TT048626; Volume 4.2.3.3.2)

Key Findings:

- SAHA was found to be clastogenic in vivo in the mouse micronucleus assay

Conducting Laboratory and Location: Merck Research Laboratories; West Point, PA
Date of Study Initiation: April 12, 2004
GLP compliance: yes
QA reports: yes
Drug, Lot, Purity: L001079038; Lot 000K009; —
Formulation/Vehicle: 1.0% (w/v) carboxymethylcellulose sodium and 0.5% (v/v) Tween® 80 in deionized water.

Methods:

Species: - .CD-1®(ICR)BR mice, male

Dose selection criteria: maximum testable dose 2000 mg/kg; In an acute toxicity test, the ~lethal dose was >2000 mg/kg.

Positive Controls: Mitomycin C

Exposure Conditions:

Incubation and Sampling times: 24 and 48 hours

Doses used in the definitive study: 500, 1000, 2000 mg/kg, PO

Analysis:

No. of replicates: 5-7 mice/dose, 2000 PCEs counted

Counting Method: Manual

Criteria for positive results: The assay would usually be considered positive if a significant increase in the frequency of micronucleated PCE ($p<0.05$) occurred at a minimum of 2 dose levels when compared with the concurrent vehicle control mean: either 2 doses at a given sacrifice time or 1 dose at each of 2 sacrifice times. Evidence for a positive dose relation would also be taken into account. Judgment would not depend upon statistical analysis alone but would also take into account whether there is a biologically meaningful response and the historical control range.

Summary of individual study findings:

Study validity: The study was conducted at the maximal "testable" dose, which was defined by the Standard Operating Protocol as 2000 mg/kg.

Study outcome:

- No animals exhibited clinical signs at any doses.
- There was a 2-3 fold increase in micronucleated PCEs compared to control at all dose levels at 24 hours and at ≥1000 mg/kg at 48 hours. There was evidence (statistical) for a increasing trend in micronucleus frequency at both sacrifice times.

Treatment Dose	Harvest Time (Hr)	Males Total PCE	Total MN-PCE	MN-PCE/ 1000 PCE[a]	% PCE[a]
1.0% (w/v) Carboxymethylcellulose sodium (CMC; medium viscosity) and 0.5% (v/v) Tween® 80 in Deionized Water 20 mL/kg L-001079038	24	10,000	8	0.8 ± 0.3	40.2 ± 2.1
500 mg/kg L-001079038	24	10,000	22	2.2 ± 0.5	46.9 ± 3.4
1000 mg/kg L-001079038	24	10,000	25	2.5 ± 0.3	47.3 ± 1.5
2000 mg/kg Mitomycin C 0.35 mg/kg	24	10,000	26	2.6 ± 0.5	38.7 ± 3.9
Mitomycin C 2.0 mg/kg	24	10,000	98	9.8 ± 2.3	44.1 ± 1.9
1.0% (w/v) Carboxymethylcellulose sodium (CMC; medium viscosity) and 0.5% (v/v) Tween® 80 in Deionized Water 20 mL/kg L-001079038	48	10,000	486	48.6 ± 10.1	43.8 ± 1.2
500 mg/kg L-001079038	48	10,000	11	1.1 ± 0.2	38.0 ± 2.3
1000 mg/kg L-001079038	48	10,000	11	1.1 ± 0.5	35.7 ± 3.6
2000 mg/kg	48	10,000	27	2.7 ± 0.3	35.6 ± 3.9
			23	2.3 ± 0.4	29.6 ± 4.5

PCE = Polychromatic erythrocytes.
MN-PCE = Micronucleated PCE.
[a] Group mean and standard error

2.6.6.5 Carcinogenicity

Carcinogenesis studies have not been performed with SAHA.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **L-001079038 Oral Fertility Study in Female Rats (TT# 04-7360, Volume 4.2.3.5.1)**

Key study findings:

- Based on a dose dependent increase in the number of corpus luteum, the NOEL for effects on reproductive performance is <15 mg/kg/day. Based on the treatment related decreases in implants and live births, the NOEL for embryonic development is 15 mg/kg/day.

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: August 5, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-001079038; Lot 000K009. —

Methods

Doses: 15, 50, 150 mg/kg/day
Species/strain: Rat, Sprague Dawley
Number/sex/group: 24/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics: none
Study design: Females were dosed for 14 days prior to cohabitation, during cohabitation, and through GD 7.
Parameters and endpoints evaluated:

Physical Examinations	Daily from initiation of treatment through GD8, on GD12 and GD15
Body Weight	Females were weighed on Premating Days (PMD) 1, 4, 8, 11, 14; during cohabitation on Days 22 and 29; and on GD 0, 2, 4, 6, 8, 12, and 15.
Food Consumption	Measured for all females over 4-day intervals beginning on PMD 1 and PMD 8, and GD 1 and GD 8
Estrous Cycle Monitoring	After 14 days of dosing, females were housed with untreated males of the same strain in a 1:1 ratio for a maximum of 12 nights. During the cohabitation period vaginal lavage samples were examined microscopically each morning for the presence of sperm. If no sperm was noted, vaginal cell types were recorded (but are not reported). The day of finding a copulatory plug and/or sperm was considered GD 0.
Necropsy	All females were euthanized on presumed GD 15 through 17 by CO2 asphyxiation. The uterus was removed and opened, and pregnancy status recorded. Corpora lutea were counted. Any

	apparently nonpregnant uterus was briefly stained with approximately 9% ammonium sulfide in order to visualize early resorptions; however, none were seen. Implants were counted and each classified as live fetus, dead fetus, or resorption. Fetuses were euthanized by rapid induction of hypothermia. A gross examination, limited to macroscopic examination of thoracic and abdominal viscera, was performed on all F0 females.

Results

Mortality: No mortality observed

Clinical signs: No clinical signs observed

Body weight: In the 150-mg/kg/day group there was a treatment-related decrease in mean body weight gain during PMD 1 and 14 (-67% below control). During the remainder of the dosing period (GD 0 through 8) there were no treatment-related effects on maternal body weight gain in the 150-mg/kg/day group.

Food consumption: Slight (↓9.5%) decrease PMD 1-5 in the high dose group.

Toxicokinetics: Not conducted.

Necropsy: No treatment related effects observed

Fertility parameters:

- There was no significant change in time to mating (in 4-day periods), the mating index (mated females/females cohabited, percent), the fecundity index (pregnant females/mated females, percent), or the fertility index (pregnant females/females cohabited, percent).
- There was a treatment-related increase in the mean number of corpora lutea per pregnant female at all doses relative to concurrent controls. There was also an increase in the incidence of pregnant females with increases in corpora lutea above the highest concurrent and historical control values (22 and 23 corpora lutea, respectively) in the 15, 50, and 150 mg/kg/day groups (1/22, 2/23, and 21/22, respectively). Potentially due to an increase in luteinizing hormone receptor (LHR) expression in the corpora lutea since LHR gene promoter activity can be substantially up-regulated in cultured JAR cells (human placental carcinoma cell line) in the presence of the histone deacetylase inhibitors trichostatin A or sodium butyrate (Zhang and Dufau, 2003). Secondary to the increase in corpora lutea values, there were treatment-related increases in the peri-implantation loss (≥50-mg/kg/day) and a treatment-related increase in postimplantation loss (150 mg/kg/day) secondary to increases in the percentage of resorptions and dead fetuses per implants. Decreases in the mean number of live fetuses/pregnant female (150-mg/kg/day) were also observed. (see table excerpted from the sponsor's submission).

Treatment Group	Control	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Mated Females	24	24	24	24
Pregnant	22	22	23	22
Examined Live Litter	22	22	23	17
Resorbed or Dead Litter	0	0	0	5
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Not Pregnant	2	2	1	2
Live	2	2	1	2
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	349	391	453	686
Corpora Lutea/Pregnant Female ± SD	15.9 ± 2.6	17.9 ± 3.3	19.7 ± 4.7	31.2 ± 7.2
% Peri-Implantation Loss, Litter Mean ± SD	5.4 ± 7.1	6.3 ±12.6	12.4 ±10.9	45.0 ±14.6
Implants	329	362	388	359
Implants/Pregnant Female ± SD	15.0 ± 2.1	16.5 ± 2.2	16.9 ± 2.4	16.3 ± 7.6
Resorptions	34	21	42	251
% Resorptions/Implants, Litter Mean ± SD	10.3 ±15.3	5.7 ± 5.4	12.0 ±16.0	72.0 ±29.6
Dead Fetuses	0	1	3	14
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.3 ± 1.2	0.7 ± 3.5	3.9 ± 8.5
% Postimplantation Loss, Litter Mean ± SD	10.3 ±15.3	6.0 ± 5.5	12.7 ±15.8	75.9 ±26.3
Live Fetuses	295	340	338	94
Sex Not Examined	295	340	338	94
Live Fetuses/Pregnant Female ± SD	13.4 ± 3.0	15.5 ± 3.1	14.7 ± 3.3	4.3 ± 5.1
Live Fetuses/Live Litter ± SD	13.4 ± 3.0	15.5 ± 2.1	14.7 ± 3.3	5.5 ± 5.1

% Peri-Implantation Loss = [(No. Corpora Lutea - No. Implants) / No. Corpora Lutea] X 100.

Study title: L-001079038 Oral Fertility Study in Male Rats (TT#03-7440, Volume 4.2.3.5.1)

Key study findings: The NOEL of SAHA in male rats for effects on fertility was 150 mg/kg/day, the highest dose utilized in this study. The NOEL for general toxicity was 50 mg/kg/day.

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: September 9th, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L-001079038; Lot 000K009, —

Methods

Doses: 20, 50, 150 mg/kg/day
Species/strain: Rat, Sprague Dawley
Number/sex/group: 24/males/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics:
Study design: L-001079038 (PO) was administered for approximately 10 weeks prior to cohabitation, during cohabitation, and until the day prior to scheduled sacrifice (approximately 14 weeks total).
Parameters and endpoints evaluated:

Physical Examinations	Males: Daily Females: First day of Cohabitation, GD0, 7, 15.
Body Weight	Males: Twice weekly Females: First day of cohabitation on GD0, 7 and 15.
Food Consumption	Prior to and following cohabitation interval, twice weekly for the presence of remaining food.

Mating	Prior to study start, all males were examined for testes descent and penile alterations. Males were housed with untreated females of the same strain in a 1:1 ratio following approximately 10 weeks of treatment. Mating was confirmed by daily examination of females for the presence of a copulatory plug in the vagina or sperm in a vaginal lavage. The cohabitation interval was limited to a maximum of 10 nights. Females that had no indication of having bred during the first 5 nights of cohabitation (3, 4, 1, and 2 females in the control, 20-, 50-, and 150-mg/kg/day groups, respectively) were removed and replaced with a new female for an additional maximum 5 nights of cohabitation. The day of observing a seminal plug and/or sperm was considered Gestation Day (GD) 0.
Necropsy	Females: euthanized on presumed GD 15 through 17 by CO2 asphyxiation. The uterus was removed and opened, and pregnancy status recorded. Corpora lutea were counted. Any apparently nonpregnant uterus was briefly stained with approximately 9% ammonium sulfide in order to visualize early resorptions; however, none were seen. Implants were counted and each classified as live fetus, dead fetus, or resorption. Fetuses were euthanized by rapid induction of hypothermia. Males: 1) Gross Examination Male rats surviving to study termination were anesthetized by CO2 asphyxiation and euthanized by exsanguination at necropsy. The gross examination of thoracic and abdominal viscera, epididymides, and testes was performed. 2) Organ Weights The terminal body weight and weights of the testes and left cauda epididymis from male rats surviving to study termination were recorded. Testes weight data were expressed as absolute weight and weight as percent of body weight. 3) Tissue Fixation The testes and the right epididymis from animals surviving to study termination were fixed in Bouin's solution. Epididymis and testes from animals found dead or sacrificed early were fixed in 10% Neutral/Buffered Formalin. The testes and epididymis of the control and high-dose groups were processed for histomorphologic examination. 4) Histomorphologic Examination Sections of both testes and the epididymis from all male rats in the control and 150-mg/kg/day groups were prepared by routine methods, stained with hematoxylin and eosin, and examined microscopically. All macroscopic findings of the testes and epididymides were examined microscopically.

Results

Mortality:

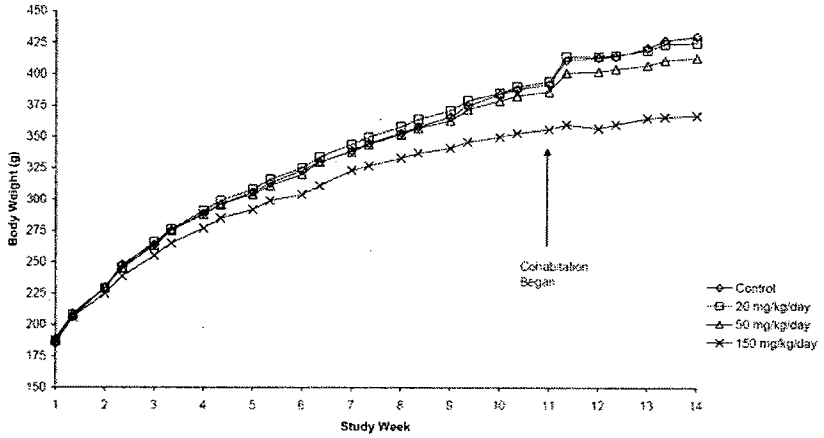
- 4 treatment related deaths in the HD males.
    - 2 early sacrifices due to clinical signs (unkempt fur, ocular/nasal discharge, abnormal feces, decreased activity, decreased skin turgor, mucoid discharge, abnormal respiratory sounds and/or over-responsiveness to touch)

- 2 found dead, similar signs were noted prior to death
- No mortality in females

Clinical signs: see mortality.

Body weight: 150 mg/kg M- up to an 18% decrease beginning as early as week 1. (see figure excerpted from the sponsor's submission. No changes observed in females.

Figure A-1. L-001679038: Oral Fertility Study in Male Rats. IT #03-7440
Average Body Weights (g) of Males



Food consumption: In the HD males, 13 of 24 were observed to have food remaining on 2 or more occasions. No changes observed in females.

Toxicokinetics: not conducted.

Necropsy: No drug related findings

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- No drug dependent changes in the mating index (percent of mated females/females cohabited), fecundity index (percent of pregnant females/mated females), and fertility index (percent of pregnant females/females cohabited) were observed.
- Embryonic/fetal survival parameters as assessed by the average numbers of implants and live fetuses per pregnant female and by the derived peri- and postimplantation loss calculations were similar to those seen in untreated pregnant rats in this laboratory and were unrelated to treatment.
- There were no drug dependent changes in embryonic/fetal survival parameters (average numbers of implants, live fetuses/pregnant female, peri/post implantation loss).
- No drug dependent changes in mean sperm count per cauda epididymis, mean sperm count/g cauda epididymis, and mean percent sperm motility.

Embryofetal development

Study title: L-001079038: Oral Range-Finding Reproduction Study in Female Rats (TT#04-7295, Volume 4.2.3.5.2)
SAHA (20, 50, 150, 300 mg/kg/day) was administered to pregnant female Sprague-Dawley rats (10/group) GD 6- LD20 to evaluate the potential effects on embryo and fetal development. Due to treatment-related body weight loss (-44% on GD 16), rats in the 300 mg/kg/day dose group were terminated before scheduled sacrifice between GD 15 and 17. Due to treatment-related decreased body weight gain during gestation (-26%) and increased pup deaths (100% from 4 dams), the rats in the 150 mg/kg/day dose group was terminated between GD 22 and LD 1. Treatment-related findings in the 50 mg/kg/day (300 mg/m2/day) dose group included decreased mean body weight gain (↓21%), decreases in leukocytes (mainly neutrophils-↓20%, lymphocytes-↓21%, eosinophils-↓29%, and basophils-↓40%) and alkaline phosphatase (27%) on GD 14, and slight-to-moderate decreases in mean pup body weights (5-20%) during lactation. Treatment-related findings in the 20 mg/kg/day (120 mg/m2/day) dose group included a slight decrease in mean maternal body weight gain during gestation (↓7%) and a slight decrease in leukocytes (↓11%) on GD 14. Based on these results, the recommended high-dose level of SAHA for the developmental toxicity study in rats was 50 mg/kg/day.

Study title: L001079038 Oral Developmental Toxicity Study in Rats with Prenatal Evaluation (TT#04-7290, Volume 4.2.3.5.2)

Key study findings: In the absence of maternal toxicity, developmental toxicity was observed with SAHA, 50 mg/kg/day, when administered GD6-20 to rats. Findings consisted of marked decreases in fetal weight and increases in fetuses with skeletal variations (cervical ribs, supernumerary ribs and vertebral count, and sacral arch variations) and sites of incomplete ossifications (skull, thoracic vertebra and sternebra). NOAEL: 15 mg/kg/day (90 mg/m2)

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: July 26, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L001079038, Lot 000K009. ——

Methods

Doses: 5, 15, 50 mg/kg/day
Species/strain: Rat, Sprague Dawley
Number/sex/group: 22/females/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics: none
Study design: Females were dosed GD 6 through GD20

Parameters and endpoints evaluated:

Physical Examinations	GD0 and daily from GD6-GD21
Body Weight	GD0, 6, 8, 10, 12, 14, 16, 18, 20, 21
Food Consumption	Recorded over the following 2 day intervals beginning on GD 3, 6, 10, 14 and 18
Necropsy	F0 female rats were euthanized on GD 21 by CO2 asphyxiation. The uterus of each female was examined to determine pregnancy status. The number of corpora lutea was counted and recorded as the total number per female. Placental morphology was evaluated by gross examination. Uterine implants were counted and each was classified as a live fetus, dead fetus, or resorption. All fetuses were weighed and examined externally. The sex of each fetus was determined by external examination of the genitalia and recorded. Following external examinations, fetuses were euthanized by oral administration of sodium pentobarbital. The viscera of approximately one half of all fetuses in each litter were examined by fresh dissection. The heads from approximately one half of all fetuses in each litter were removed, fixed in Bouin's solution, and examined after freehand coronal sectioning. Fetuses were fixed in ethanol, cleared in potassium hydroxide, and stained with alizarin red for subsequent skeletal examination. Following cesarean section, a gross examination of the thoracic and abdominal viscera of the F0 females was performed. Females were subsequently discarded.

Results

Mortality (dams): None

Clinical signs (dams): None

Body weight (dams): There was an apparent decrease in mean maternal body weight gain between GD 6 and 21 in the 50-mg/kg/day group; however, when the GD 21 body weight of each dam was adjusted for total fetal weight (i.e., individual GD 21 maternal body weight minus total fetal weight), the adjusted mean body weight gain during this period was comparable to the control (60 g and 69 g gain, respectively). The adjusted mean GD 21 maternal body weight in the 50-mg/kg/day group was only 2% below the adjusted mean control body weight.

Food consumption (dams): No drug related effects.

Toxicokinetics: not conducted

Terminal and necroscopic evaluations: there were no treatment related effects on embryonic or fetal survival as assessed by percent peri-implantation loss, implants per

pregnant female, percent post-implantation loss and live fetuses per pregnant female. (See table excerpted from the sponsor's submission).

Treatment Group	Control	5 mg/kg/day	15 mg/kg/day	50 mg/kg/day
Mated Females	22	22	22	22
Pregnant	22	22	20	20
Examined Live Litter	22	22	20	20
Resorbed or Dead Litter	0	0	0	0
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Not Pregnant	0	0	0	2
Live	0	0	0	2
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	346	347	303	303
Corpora Lutea/Pregnant Female ± SD	15.7 ± 1.6	15.6 ± 1.1	15.2 ± 1.7	15.2 ± 1.6
% Peri-Implantation Loss, Litter Mean ± SD	3.8 ± 8.1	3.7 ± 4.9	1.5 ± 2.7	7.2 ±24.9
Implants	335	336	299	285
Implants/Pregnant Female ± SD	15.2 ± 1.5	15.3 ± 1.1	15.0 ± 1.7	14.2 ± 2.7
Resorptions	12	6	4	10
% Resorptions/Implants, Litter Mean ± SD	3.5 ± 4.5	1.8 ± 3.0	1.3 ± 2.8	4.4 ±12.2
Dead Fetuses	0	0	1	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.4 ± 1.7	0.0
% PostImplantation Loss, Litter Mean ± SD	3.5 ± 4.5	1.8 ± 3.0	1.7 ± 4.0	4.4 ±12.2
Live Fetuses	323	324	294	273
Females	161	161	141	135
Males	172	163	153	138
Sex Ratio, Litter Mean ± SD	0.46 ± 0.09	0.49 ± 0.15	0.48 ± 0.13	0.50 ± 0.11
Live Fetuses/Pregnant Female ± SD	14.7 ± 1.6	14.7 ± 1.1	14.7 ± 1.9	13.7 ± 3.3
Live Fetal Weight (g), Litter Mean ± SD				
Females	4.85 ± 0.39	4.92 ± 0.32	4.73 ± 0.27	3.70 ± 0.56
Males	5.11 ± 0.27	5.19 ± 0.20	5.02 ± 0.29	4.04 ± 0.26

% Peri-Implantation Loss = [(No. Corpora Lutea - No. Implants) / No. Corpora Lutea] X 100.
% Postimplantation Loss = [(No. Resorptions + No. Dead Fetuses) / No. Implants] X 100.
Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).

Offspring (malformations, variations, etc.):

- There were no drug related effects on fetal sex ratios, placental morphology, external morphology, or visceral/coronal morphology.
- There was a marked decrease in mean live fetal weight in the 50 mg/kg group (24% and 21% below control in males and females, respectively).
- There was a treatment related increase in the incidence of fetuses with a number of skeletal variations (malformations were not affected) and incomplete ossifications in the 50 mg/kg group. See table excerpted from the sponsor's submission. Litter mean for fetuses with 1 or more of these findings was 61% compared to 17% in the control.

Treatment-Related Skeletal Variations and Incomplete Ossifications
(Litter Means, Percent)

		L-001079038 (mg/kg/day)		
	Control	5	15	50
Vertebral count variation	0.32	–	–	12
Sacral vertebra variation	0.30	–	–	1.4
Cervical rib	1.8	–	–	7.6
Supernumerary rib	11	–	–	41
Inc. Oss. skull bone	0	–	–	2.5
Inc. Oss. thoracic vertebra	0	–	–	2.8
Inc. Oss. sternebra	3.0	–	–	31

– = No treatment-related change.
Inc. Oss. = Incomplete ossification.

Study title: L001079038 Oral TK Study in Rats with Evaluation of Placental Transfer (TT#04-7450, Volume 4.2.3.5.2)

Key study findings:

- For the parent drug SAHA, transplacental equilibrium appears to be reached after 30 min postdose at both dose levels (15 and 50 mg/kg/day). The ratios of fetal versus maternal SAHA serum concentrations were comparable at 0.5 and 2 hours post-dose.
- For the metabolites, the ratios of the fetal versus maternal serum levels increased between both time points at 15 and 50 mg/kg/day of SAHA, probably due to a slower rate of transplacental transfer.

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: September 29, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: : L001079038, Lot 000K009,

Methods

Doses:15, 50 mg/kg/day
Species/strain: Rat, Sprague Dawley
Number/sex/group: 15/females/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics: none
Study design: Females were dosed GD 6 through GD20
Parameters and endpoints evaluated:

Mortality	Daily
Body Weight	GD0, 6, 8, 10, 12, 14, 16, 18, 20, 2 collected solely for the purpose of dose calculation
Maternal and Fetal Blood Collection	Beginning on GD 20, maternal blood samples were collected at 0.5, 1, 2, 4, 6, and 24 hours after dosing. Fetal blood samples were collected at 0.5 and 2 hours after dosing. To obtain these samples 4 females per group were anesthetized with isoflurane and bled from the posterior vena cava at 0.5 hour after dosing. All live fetuses from these dams were individually removed from the uterus and blood was collected from the umbilical vessels and pooled by litter (approximately 1 mL per litter). Another 4 females per group were anesthetized and bled from the orbital sinus at 1 hour after dosing. These same females were again anesthetized at 2 hours postdose and blood was collected from the posterior vena cava. All live fetuses from these dams were similarly bled. Another 4 females per group were anesthetized with isoflurane and bled from the orbital sinus at 4, 6, and 24 hours after the GD 20 dose.

Results

Mortality (dams): None observed

Toxicokinetics:

- For the parent drug, SAHA, transplacental equilibrium appeared to be reached after 30 min post-dose at both dose levels studied (15 and 50 mg/kg/day). The ratios of fetal versus maternal SAHA serum concentrations were comparable at 0.5 and 2 hours post-dose (see table below).
- For the metabolites, the ratios of the fetal versus maternal serum levels increased between time points at 15 and 50 mg/kg/day of SAHA. This is consistent with a slower rate of transplacental transfer for the metabolites due to their physicochemical properties.
- Following 15 and 50 mg/kg, respectively, the mean AUC_{SAHA} was 0.13 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{SAHA\ glucuronide}$ was 0.04 and 0.61x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic\ acid}$ was 0.62 and1.92x the observed clinical concentration.

Table B: Summary of Toxicokinetic Parameters of SAHA, SAHA Glucuronide and N-Phenyl-Succinamic Acid in Maternal (Pregnant) Rats (Gestation Day 20) Following Repeated Once Daily Oral Gavage Administrations of L-001079038 From Gestation Days 6 Through 20 – Dose Levels 15 and 50 mg/kg/day

Analyte	Dose Level of L-001079038 (mg/kg/day)	$AUCss_{(0\text{-}t)}$ (ng•h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)	$AUCss_{(0\text{-}t)}$ / Dose	Cmax / Dose
SAHA	15	229	164.5	0.50	0.687	15.3	11.0
SAHA	50	749	320.8	0.50	1.24	15.0	6.42
SAHA glucuronide	15	286	220.0	0.50	0 908	19.1	14.7
SAHA glucuronide	50	827	319.8	0.50	1.08	16.5	6.40
N-phenyl-succinamic acid	15	5374	1897.5	0.50	2.53	358	127
N-phenyl-succinamic acid	50	16554	2860.0	0.50	2.85	331	57.2

Mean Maternal and Fetal Serum Drug and Metabolite Concentration (ng/mL)Following Repeated Once Daily Oral Gavage Administration of SAHA from GD6 through GD20 to Rats						
	0.5 hours Post-dose			**2.0 hours Post-dose**		
	Maternal	**Fetal**	**M/F Ratio**	**Maternal**	**Fetal**	**M/F Ratio**
15 mg/kg						
SAHA	164.5 ± 35.41	74.93 ± 17.38	2.2	45.05 ± 10.32	23.25 ± 5.4	1.9
SAHA Glucoronide	220 ± 129.4	19.93 ± 3.56	10.1	49.13 ± 6.78	21.63 4.68	2.2
N-Phenyl-succinamic acid	1898 ± 535.0	338.5 ± 46	5.6	548 ± 113.7	464.3 ± 65.1	1.2
50 mg						
SAHA	320.8 ± 112.5	122.7 ± 33.8	2.6	183.5 ± 34.3	61.3 ± 6.5	3.0
SAHA Glucoronide	319.8 ± 46.6	25.1	12.8	205.8 ± 46.1	44.9 ± 3.1	4.5
N-Phenyl-succinamic acid	2860 ± 364.1	475.3 ± 90.4	6.0	1510 ± 179.6	744 ± 60.7	2.0
Maternal or Fetal Serum Concentration to Human Serum Cmax Concentration Ratios						
15 mg/kg						
SAHA	0.68	0.31	NA	0.19	0.10	NA

Mean Maternal and Fetal Serum Drug and Metabolite Concentration (ng/mL)Following Repeated Once Daily Oral Gavage Administration of SAHA from GD6 through GD20 to Rats						
	0.5 hours Post-dose			2.0 hours Post-dose		
	Maternal	Fetal	M/F Ratio	Maternal	Fetal	M/F Ratio
SAHA Glucoronide	0.16	0.01	NA	0.04	0.02	NA
N-Phenyl-succinamic acid	2.0	0.36	NA	0.58	0.49	NA
50 mg						
SAHA	1.33	0.51	NA	0.76	0.26	NA
SAHA Glucoronide	0.24	0.02	NA	0.15	0.03	NA
N-Phenyl-succinamic acid	3.02	0.50	NA	1.59	0.79	NA

Study title: L-001079038: Oral Range-Finding Study in Pregnant Rabbits (TT#04-7305, Volume 4.2.3.5.2)

SAHA (20, 50, 150, 300 mg/kg/day) was administered to pregnant female Dutch Belted rabbits (10/group) GD 7- GD20 to evaluate the potential effects on embryo and fetal development. Due to treatment-related body weight loss (-13 g on GD 16/17 versus +79 g in controls), rabbits in the 300 mg/kg/day dose group were terminated before scheduled sacrifice between GD 16 and 17. Changes in hematology and clinical chemistry are summarized in the tables below (excerpted from the sponsor's submission).

Treatment-Related Hematological Changes
(Percent Difference in Mean Values from Concurrent Controls - GD 21)

	L-001079038 (mg/kg/day)		
Parameter	20	50	150
Erythrocyte count	-	-	-20
Reticulocytes	-	-	+83
Hemoglobin	-	-	-16
Hematocrit	-	-	-10
Mean corpuscular volume	-	-	+11
Leukocytes	-	-20	-22
Neutrophils	-27	-37	-54
Lymphocytes	-	-13	-16
Monocytes	-	-35	-41

- = No treatment-related change

Treatment-Related Serum Biochemical Changes
(Percent Difference in Mean Values from Concurrent Controls - GD 21)

	L-001079038 (mg/kg/day)		
Parameter	20	50	150
Aspartate aminotransferase	-	-	+33
Alanine aminotransferase	-	-25	-47
Triglycerides	-	-	+45

- = No treatment-related change

There were no treatment-related effects on mean live fetal body weight, fetal external morphology, placental morphology or on embryonic/fetal survival as assessed by the numbers of corpora lutea, implants, and live fetuses per pregnant female and the derived peri- and postimplantation loss values. The mean value for the incidence of peri-implantation loss in the 150-mg/kg/day groups was 20.2% in comparison with 4.6% in the concurrent control group. This increase was due primarily to 2 out of 10 females with values of 50% and 67%. Based on these results, the recommended high-dose level of SAHA for the developmental toxicity study in rabbits was 150 mg/kg/day.

Study title: L001079038 Oral Developmental Toxicity Study in Rabbits (TT#04-7300, Volume 4.2.3.5.2)

Key study findings: The NOEL for developmental toxicity (↓fetal weight, incomplete ossification of the metacarpals, increased incidence in 13th rib, gall bladder malformations) was not determined given that gall bladder malformations occurred in the lowest treatment group.

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: July 29th, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L001079038, Lot 000K009.

Methods

Doses: 20, 50, 150 mg/kg/day
Species/strain: Rabbits, Dutch Belted
Number/sex/group: 18/females/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics: none
Study design: Females were dosed GD7 through GD20
Parameters and endpoints evaluated:

Physical Examinations	Daily GD1-GD28
Body Weight	GD0, 7, 9, 11, 13, 15, 17, 19, 21, and 28
Food Consumption	2 day intervals beginning GD4
Necropsy	F0 female rabbits were euthanized on GD 28 pentobarbital. The uterus of each female was examined to determine pregnancy status. The number of corpora lutea was counted and recorded as the total number per female. Placental morphology was evaluated by gross examination. Uterine implants were counted and each was classified as a live fetus, dead fetus, or resorption. All fetuses were weighed and examined externally. The sex of each fetus was determined by external examination of the genitalia and recorded. Following external examinations, fetuses were euthanized by oral administration of sodium pentobarbital. The viscera of all fetuses in each litter were examined by fresh dissection. The heads of all fetuses in each litter were removed, fixed in Bouin's solution, and examined after freehand coronal sectioning. Following cesarean section, a gross examination of the thoracic and abdominal viscera of the F0 females was performed. Females were subsequently discarded.

Results

Mortality (dams): No treatment related mortality

Clinical signs (dams): No treatment related findings

Body weight (dams): No treatment related findings

Food consumption (dams): No treatment related findings

Toxicokinetics: Not conducted as part of this study

Terminal and necroscopic evaluations:

- There were no drug related findings in placental morphology, embryonic/fetal survival (corpora lutea, % peri-implantation loss, resorption, post-implantation loss) or fetal sex ratios.
- Mean live fetal weights were decreased by 8 and 11% for male and female fetuses, respectively, in the 150 mg/kg dose group.

Offspring (malformations, variations, etc.):

- Dose dependent external alterations were limited to 1 incidence of each, Encephalomeningocele and Anasarca in the 150 mg/kg group.
- Visceral Malformations included microcardia (n=1, 150 mg/kg), absent kidney (n=2, 150 mg/kg), absent ureter (n=2, 150 mg/kg), gall bladder malformation (n=5, 20 mg/kg; n=9, 50 mg/kg; n=8, 150 mg/kg). These findings were observed in 7 litters in the HD group in comparison to 2 litters in the control group with malformations.

Summary of Fetal Visceral Examinations

Treatment Group		Control	20 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Total Litters Examined		16	16	16	16
Live Fetuses/Litters Examined		104/ 16[a]	119/ 16	116/ 16	106/ 16
Dead Fetuses/Litters With Dead Fetuses		0/ 0	0/ 0	1/ 1	0/ 0
Fetuses with Malformations (%L.M.± SD)		2(2.5 ± 6.8)	5(4.8 ± 7.5)	11(11 ±14.9)	11(10 ±16.9)
Litters with Malformations (%)		2(13)	5(31)	8(50)	7(44)
Fetuses with Variations (%L.M.± SD)		5(5.1 ± 8.4)	4(3.3 ± 6.0)	3(3.0 ± 9.0)	4(3.9 ± 8.9)
Litters with Variations (%)		5(31)	4(25)	3(19)	4(25)
Type and Number of Fetal Alterations (%L.M.± SD)	Class				
Microcardia	M	0	0	0	1(0.69± 2.8)e
Ventricular Septal Defect	M	2(2.5 ± 6.8)[b,c]	0	0	0
Persistent Truncus Arteriosus	M	1(1.3 ± 5.0)[b]	0	0	0
Abnormal Origin Subcr. Art.	M	1(1.3 ± 5.0)[c]	0	0	0
Cor Triloculare	M	0	0	1(0.69± 2.8)[d]	0
Absent Kidney	M	0	0	0	2(1.8 ± 7.1)[f]
Absent Ureter	M	0	0	0	2(1.8 ± 7.1)[f]
Absent Gallbladder	M	0	0	1(0.86± 3.6)	0
Gallbladder Malformation	M	0	5(4.8 ± 7.5)	9(9.5 ±14.7)	8(8.0 ±15.4)
Small Lungs	M	0	0	1(0.69± 2.8)[d]	1(0.69± 2.8)[e]
Carotid Branching Variation	V	1(1.3 ± 5.0)[c]	0	0	0
Small Gallbladder	V	1(0.78± 3.1)	2(1.5 ± 4.2)	0	3(0.88± 3.6)
Gallbladder Variation	V	3(3.5 ± 7.2)	1(1.0 ± 4.2)	3(3.6 ± 8.0)	2(1.8 ± 6.3)
Cyst	V	0	1(0.69± 2.8)	0	1(1.0 ± 4.2)

L.M. = Litter Mean. M = Malformation. V = Variation.
[a] See individual table for exclusions.
[b] Multiple alterations observed for Litter #04-0479, Fetus 1.
[c] Multiple alterations observed for Litter #04-0482, Fetus 4.
[d] Multiple alterations observed for Litter #04-0543, Fetus 5.
[e] Multiple alterations observed for Litter #04-0528, Fetus 5.
[f] Multiple alterations observed for Litter #04-0532, Fetuses 1 and 4.

- No evidence of developmental toxicity was observed in fetal coronal examinations.
- There was a treatment related increase in the incidence of fetuses with short 13th ribs (2.4 fold increase) and incomplete ossifications of the metacarpals (11 fold increase) in the 150 mg/kg group. See tables below for additional findings with n=1 or 2 occurences in the high dose group

Summary of Fetal Skeletal Examinations

Treatment Group		Control	20 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Type and Number of Fetal Alterations (%L.M.± SD)	Class				
Absent Skull Bone	M	1(1.3 ± 5.0)	0	1(0.69± 2.8)	2(2.1 ± 8.3)[b,c]
Small Skull Bone	M	0	0	0	1(1.0 ± 4.2)[b]
Cervical Vertebra Malformation	M	0	0	0	1(0.88± 3.6)[d]
Thoracic Vertebra Malformation	M	0	0	0	1(0.69± 3.6)[d]
Caudal Vertebra Malformation	M	1(1.6 ± 5.3)	0	1(0.78± 3.1)	1(0.76± 3.1)[e]
Fused Ribs	M	0	0	0	1(0.69± 3.6)[d]
Hypoplastic Rib	M	1(1.3 ± 5.0)	0	0	1(0.69± 3.6)[d]
Branched Rib	M	0	0	1(0.78± 3.1)	1(0.78± 3.1)[e]
Sternebral Malformation	M	0	1(1.3 ± 5.0)	0	0
Cervical Vertebra Variation	V	0	0	0	1(1.0 ± 4.2)
Cervical Rib	V	0	0	0	1(0.78± 3.1)
Knobby Rib	V	0	0	0	1(0.69± 3.6)[d]
Short 13th Rib	V	16(34 ±20.5)	23(17 ±17.2)	25(23 ±24.3)	35(23 ±20.6)[b,c,d,e]
Sternebral Variation	V	0	0	2(1.6 ± 6.7)	4(4.3 ± 8.0)[b,c]
Pelvic Bone Variation	V	0	0	1(0.78± 3.1)	2(1.8 ± 4.9)

L.M. = Litter Mean. M = Malformation. V = Variation.
[b] Multiple alterations observed for Litter #04-0525, Fetus 3.
[c] Multiple alterations observed for Litter #04-0525, Fetus 6.
[d] Multiple alterations observed for Litter #04-0552, Fetus 1.
[e] Multiple alterations observed for Litter #04-0529, Fetus 7.

Summary of Fetal Ossification Data

Treatment Group		Control	25 mg/kg/day	50 mg/kg/day	150 mg/kg/day
Torso and Limb Examination					
Total Litters Examined		16	16	16	16
Live Fetuses/Litters Examined		105/ 16	112/ 16[a]	116/ 16	106/ 16
Dead Fetuses/Litters With Dead Fetuses		0/ 0	0/ 0	1/ 1	0/ 0
Fetuses with Incomplete Ossification (%L.M.± SD)		22(23 ±25.8)	29(24 ±16.6)	26(24 ±29.9)	46(43 ±31.9)
Litters with Incomplete Ossification (%)		11(69)	13(81)	13(81)	13(81)
Number of Ossified Sacrocaudal Vertebrae ± SD		19.3 ± 0.4	19.3 ± 0.5	19.3 ± 0.5	18.9 ± 0.6
Head Examination					
Total Litters Examined		16	16	16	16
Live Fetuses/Litters Examined		105/ 16	112/ 16	116/ 16	106/ 16
Dead Fetuses/Litters With Dead Fetuses		0/ 0	0/ 0	1/ 1	0/ 0
Fetuses with Incomplete Ossification (%L.M.± SD)		8(6.6 ±11.7)	3(2.8 ± 6.8)	4(2.4 ± 6.5)	6(5.8 ±11.2)
Litters with Incomplete Ossification (%)		5(31)	2(13)	2(13)	4(25)
Site and Number of Fetuses with Incomplete Ossification (%L.M.± SD)	Class				
Incomp. Oss. Cervical Vertebra	O	1(1.3 ± 5.0)	0	0	0
Incomp. Oss. Thoracic Vertebra	O	0	1(0.63± 2.5)	0	0
Incomp. Oss. Lumbar Vertebra	O	18(19 ±26.9)	16(14 ±17.2)	18(18 ±21.1)	14(12 ±17.8)
Incomp. Oss. Skull Bone	O	1(0.89± 3.6)	0	0	2(2.1 ± 8.3)
Incomp. Oss. Sternebra	O	2(2.8 ± 7.3)	1(1.1 ± 5.0)	2(1.6 ± 4.0)	5(4.7 ± 9.5)
Incomp. Oss. Metacarpal	O	3(2.7 ± 6.1)	11(8.3 ±13.7)	6(4.4 ± 9.9)	30(29 ±26.5)
Incomp. Oss. Pelvic Bone	O	0	0	0	1(1.0 ± 4.2)
Incomp. Oss. Talus-Calcaneus	O	1(1.3 ± 5.0)	0	0	4(4.0 ± 9.4)
Inc. Oss. Hyoid	O	7(5.7 ±11.0)	3(2.5 ± 6.8)	4(2.4 ± 6.5)	4(3.7 ± 8.5)

L.M. = Litter Mean. O = Ossification.
[a] See individual table for exclusions.

Study title: L001079038 Oral TK Study in Rabbits with Evaluation of Placental Transfer (TT#04-7480, Volume 4.2.3.5.2)

Key study findings: SAHA, SAHA Glucuronide and N-phenyl-succinamic acid drug concentrations were found to cross the placenta to the fetus.

Conducting laboratory and location: Merck Research Laboratories; West Point, PA
Date of study initiation: September 30, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: L001079038, Lot 000K009, —.

Methods

Doses:50, 150 mg/kg/day
Species/strain: Rabbit, Dutch Belted
Number/sex/group: 15/females/group
Route, formulation, volume, and infusion rate: Oral, 1.0% (v/v) carboxymethylcellulose sodium / 0.5% (v/v) Polysorbate 80 in deionized water, 5 mL/kg
Satellite groups used for toxicokinetics: none
Study design: Females were dosed GD 7 through GD20
Parameters and endpoints evaluated:

Mortality	Daily
Body Weight	GD0, 7, 9,11, 13, 15, 17, 19, 20 collected solely for the purpose of dose calculation
Maternal and Fetal Blood Collection	Beginning on GD 20, maternal blood samples were collected at 0.5, 1, 2, 4, 6, and 24 hours after dosing. Fetal blood samples were collected at 0.5 and 6 hours after dosing. To obtain these samples 4 females per group were anesthetized with isoflurane and bled from the vena cava at 0.5 hour after dosing. All live fetuses from these

	dams were individually removed from the uterus and blood was collected from the great vessels and pooled by litter. Another 4 females per group were bled 2 hours after dosing and again at 6hours. All live fetuses from these dams were similarly bled. Another 4 females per group were bled 1, 4, and 24 hours after the GD 20 dose.

Results

Mortality (dams): One 150 mg/kg animal (non-pregnant) was found dead on day 8, not deemed due to treatment.

Toxicokinetics:

- For the parent drug, SAHA, concentrations were higher at the 0.5 hr timeoint compared to the 6 hour timepoint. (See table below).
- For the metabolites, the ratios of the fetal versus maternal serum levels increased between both time points at 50 and 150 mg/kg/day of SAHA. This is consistent with a slower rate of transplacental transfer for the metabolites due to their physicochemical properties.
- Following 600 and 1800 mg/m2, respectively, the mean AUC_{SAHA} was 0.12 and 0.43x the systemic exposure in patients administered the recommended human dose, $AUC_{SAHA\ glucuronide}$ was 0.19 and 0.52x the observed clinical AUC and $AUC_{n\text{-}phenyl\text{-}succinamic\ acid}$ was 0.94 and4.5x the observed clinical AUC.

Table B: **Summary of Toxicokinetic Parameters of SAHA, SAHA Glucuronide and N-Phenyl-Succinamic Acid in Maternal (Pregnant) Rabbits (Gestation Day 20) Following Repeated Once Daily Oral Gavage Administrations of L-001079038 From Gestation Days 7 Through 20 – Dose Levels 50 and 150 mg/kg/day**

Analyte	Dose Level of L-001079038 (mg/kg/day)	$AUCss_{(0-t)}$ (ng•h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)	$AUCss_{(0-t)}$ / Dose	Cmax / Dose
SAHA	50	212	114.8	1.0	.	4.24	2.30
SAHA	150	742	324.9	0.5	1.09	4.95	2.17
SAHA glucuronide	50	1326	651.0	1.0	0.768	26.5	13.0
SAHA glucuronide	150	3666	1375.9	0.5	1.18	24.4	9.17
N-phenyl-succinamic acid	50	8110	2007.5	1.0	3.60	162	40.2
N-phenyl-succinamic acid	150	38458	4115.0	2.0	2.69	256	27.4

Mean Maternal and Fetal Serum Drug and Metabolite Concentration (ng/mL)Following Repeated Once Daily Oral Gavage Administration of SAHA from GD6 through GD20 to Rabbits						
	0.5 hours Post-dose			**6 hours Post-dose**		
	Maternal	**Fetal**	**M/F Ratio**	**Maternal**	**Fetal**	**M/F Ratio**
50 mg/kg						
SAHA	75.6 ± 63.8	8.9 ± 8.2	8.5	0	1.9 ± 3.7	-
SAHA Glucoronide	429.8 ± 137.7	1.8 ± 3.5	312	7.6 ± 8.9	13.3	1.8
N-Phenyl-succinamic acid	1082 ± 203	25.4 ± 10.0	42.5	171.9 ± 145.5	356.6 ± 421	2.1

Mean Maternal and Fetal Serum Drug and Metabolite Concentration (ng/mL)Following Repeated Once Daily Oral Gavage Administration of SAHA from GD6 through GD20 to Rabbits						
	0.5 hours Post-dose			6 hours Post-dose		
	Maternal	Fetal	M/F Ratio	Maternal	Fetal	M/F Ratio
150 mg						
SAHA	324.9 ± 244.6	31.5 ± 22.1	10.3	12.8 ± 9.4	6.5 ± 6.0	2.0
SAHA Glucoronide	1375 ± 889.1	9.1 ± 6.1	154.6	96.4 ± 87.4	11.3 ± 22.5	8.5
N-Phenyl-succinamic acid	2422 ± 1660.7	74.4 ± 39.8	32.5	2532 ± 1806.5	466.5 ± 483.9	5.4
Maternal or Fetal Serum Concentration to Human Serum Cmax Concentration Ratios						
50 mg/kg						
SAHA	0.32	0.04	NA	0	0.01	NA
SAHA Glucoronide	0.32	<0.01	NA	1.27	<0.01	NA
N-Phenyl-succinamic acid	1.14	0.02	NA	0.18	0.01	NA
150 mg						
SAHA	1.35	0.13	NA	0.05	0.03	NA
SAHA Glucoronide	1.01	<0.01	NA	0.07	<0.01	NA
N-Phenyl-succinamic acid	2.55	0.08	NA	2.6	0.49	NA

2.6.6.7 Local tolerance

L-001079038-000K: Acute Dermal Irritation Study in Rabbits (TT#04-5517, Volume 4.2.3.6, GLP)

The skin irritation/corrosive potential and reversal of dermal effects of L-001079038 (pH=5, 0.5 g in 0.4 mL deionized water) following a 4-hour dermal exposure of L-001079038 was evaluated. Dermal erythema and edema were evaluated and scored at approximately 60 minutes and 24, 48, and 72 hours following the removal of the test substance at the end of the 4-hour exposure in each of 3 rabbits. The rabbit that was initially treated is also examined immediately after test substance removal. The reversibility of any dermal effects was assessed for up to 14 days, if necessary. No dermal irritation was observed following L-001079038 exposure but was observed with the positive control.

L-001079038-000K: Local Lymph Node Assay (LLNA) in Mice (TT#04-5518, Volume 4.2.3.6, GLP)

The dermal sensitization potential of L-001079038 (1 g/mL in methyl ethyl ketone) following a 4-hour dermal exposure of L-001079038 was evaluated. See the table below for parameters evaluated (Excerpted from the sponsor's submission).

Study Parameter	Frequency
Body Weight	Test days 0 and 5
Mortality/Moribundity Checks	At least once daily
Dosing	Test days 0-2
Days of Rest	Test days 3-4
Injection of Radioactivity	Test day 5
Removal of Lymph Nodes	At sacrifice (test day 5)
Disintegrations per minute (dpm) data	Test day 6

The positive control was hexylcinnamaldehyde (HCA) in a 4:1 mixture of acetone:olive oil. 25 µL of L-001079038-000K were administered topically to the dorsum of each mouse ear for 3 consecutive days (test days 0-2) at 0, 1 ,10, or 100% L-001079038 or the

positive control and/or vehicle. On day 5, 20 μCi of ^{3}H-Thymidine (IV) per mouse was administered. One mouse (601 in the 10% test substance group) was accidentally killed during dosing and the lymph node data for this mouse were excluded from the statistical analysis.

Approximately 5 hours after the injection, animals were sacrificed by carbon dioxide asphyxiation, draining auricular lymph nodes were removed, and single cell suspensions were prepared. The single cell suspensions were incubated at 2-8°C overnight. On test day 6, the single cell suspensions were counted on a beta counter. The counts per minute (cpm) data were converted to disintegrations per minute (dpm). A stimulation index (SI) was derived for each experimental group by dividing the mean dpm of each experimental group by the mean dpm of the vehicle control group. Statistically significant increases in cell proliferation in the test concentration groups compared to the vehicle control group and/or SIs of greater than or equal to 3.0 indicated a positive response.

Dermal sensitization was observed with the positive control, however L-001079038 did not produce a dermal sensitization response in mice.

Bovine Corneal Opacity and Permeability (TT#05-5503, Volume 4.2.3.6, GLP)

The ocular irritancy potential of L-001079038 [750 μL of a 20% (w/v); pH=not determined] following a 4 hour incubation of L-001079038 was evaluated in bovine eyes (n=3-4). The change in opacity and permeability for each cornea (including the negative control corneas) was calculated by subtracting the initial reading from the final reading. These values were then corrected by subtracting from each the average change in opacity or permeability observed for the negative control corneas. The mean value of each treatment group was calculated by averaging the corrected values of each cornea for that treatment condition.

Occular irritation was observed with the positive control; however L-001079038 did not appear to produce an ocular irritation response in bovine eyes.

L-001079038-000K: Skin Irritation Test (SIT) Using th — Skin Model with Optional IL-1α (TT#05-5504, Volume 4.2.3.6, GLP)

The — skin model bioassay kit was used to assess the potential dermal irritation of L-001079038 (25 mg dosed directly on top of the tissue and 25μL of sterile water added, pH not determined) following a 15 minute exposure and 42 hour post-exposure expression incubation in triplicate cultures. The positive control, 1% triton®-X-100 was tested at 4 and 8 hours, with a corresponding 8 hour negative control.

Although the mean viability of the three treated tissues was >50% (54%), which would result in a non-irritant prediction, the individual viability values of the 2 of 3 tissues were less than 50%, which is suggestive of an equivocal prediction.

2.6.6.8 Special toxicology studies
None

2.6.6.9 Discussion and Conclusions
2.6.6.10 Tables and Figures
See Toxicology Tabulated Summary section 2.6.7

2.6.7 TOXICOLOGY TABULATED SUMMARY
Toxicology

	6 month oral toxicity study in SD Rats	Six month oral toxicity study in beagle dogs
Study #	TT#04-5502	TT#04-5504
Doses (mg base/kg)	0, 20, 50, 150	0, 20, 60, 80 (HD increased to 100, 125, and 160 on day 16, 30, and 97)
Doses (mg base/m2)	0, 120, 300, 900	0, 400, 1200, 1600 (2000, 2500, 3200)
Died or Sacrificed Moribund (drug dep)	2 LD M, 1MD M/2MD F, 1HD F	1 HD male during week 24
Body weight (%)	50 mg/kg: ↓8-9 % beginning week 6 150 mg/kg: ↓14 (F) and 22% (M) beginning week 2. Partial recovery	10% decrease in HD male that was sacrificed moribund
Food Consumption (%)	50 mg/kg: ↓~15% beginning week 6-9 150 mg/kg: ↓~15% beginning week 2	Changes limited to preterminal sacrifice animal
Clinical Observations	150 mg/kg: missing teeth, masses in the maxillary region or abdomen	HD: nonformed or liquid feces
Ophthalmoscopy	No effect	No effect
Hematology	≥20 mg/kg-↓Red cell mass (20%) ↑Reticulocytes (≤2fold) ↓WBC,lympocytes, neutrophils, monocytes, and eosinophils (up to 75%)	HD Females: ↓RBC/HCT/Hg (up to 15%; week 4/25) ↓WBC (29%; week 25) ↓Neutrophils(33% week 25) ↑Platelets (27%, week 25)
Clinical Chemistry	≥20 mg/kg in M, 150 mg/kg in females: ↓Globulin (40%), ↓cholesterol (57%), ↓K (up to 19%) 150 mg/kg F: ↑CK (93% females)	No effect
Urinalysis	≥50 mg/kg (males) pH decreased (↓12%), ↑ excreted calcium (up to 4x) ≥20 mg/kg (males)↑Urinary Calcium (3 fold)	No effect
Organ weights	Partially reversible ↓liver, kidney, thymus and seminal vesicles at 150 mg/kg	No effect
Gross pathology	≥150 mg/kg: Dark/Red Focus of the stomach.	Limited to multiple red pinpoint foci in gastric mucosa and multiple linear red areas in the mucosa of the cecum, colon, and rectum in the preterminal sacrifice animal.
Histopathology	≥20 mg/kg: Marrow: Erythroid/myeloid hyperplasia Spleen: pigment increased Adrenal cortex: loss of distinct zona fasiculata and reticularis	*Bone marrow: depletion Esophagus/stomach: erosion with inflammation GI (Duodenum, Jejunum, Ileum, Cecum, Colon, and/or Rectum):

	6 month oral toxicity study in SD Rats	Six month oral toxicity study in beagle dogs
	Stomach: erosion Ovary: corpora lutea decreased 150 mg/kg: Thymus/Spleen: lymphocytic depletion, inflammation Stomach: ectasia, ulcer Kidney: pyelonephritis, hyperplasia Mineralization increased, cyst Following recovery: (150 mg/kg) Bone: degeneration (cartilage), Eye: inflammation Kidney: mineralization Spleen: pigment increased Thymus: lymphocytic depletion Adrenal: degeneration (cystic/hemorrhagic) Pancreas: Infiltrate	Villous blunting, acute inflammation, necrosis (crypt cells), hemorrhage, and/or regeneration of crypt epithelium

*findings limited to the high dose.

Genotoxicity

Title	Study #	Without Metabolic Activation	With Metabolic Activation
WIN 64652: *Salmonella*/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and *Escherichia Coli* WP2 uvrA Reverse Mutation Assay with a Confirmation Assay	TT935506-definitive	Positive in TA100 (5000µg/plate)	Positive in TA1535 ≥333 µg/plate
	Confirmatory assay 1	Negative all strains	Negative all strains
	Confirmatory assay 2	Not conducted	Negative TA1535
Microbial Mutagenesis Assay with Preincubation	TT048026	Positive in TA97a (5000 µg/plate)	Negative all strains
WIN64652: *In vitro* chromosome aberration analysis of CHO cells	TT935505	10 fold ↑ in aberrations with 100 µg/mL	10 fold ↑ in aberrations with 100 µg/mL
L001079038: Chromosomal aberrations *in vitro,* in CHO cells TT#048633, TT#048639, TT048651	TT048633	Positive for clastogenicity ≥350 µM	Positive for clastogenicity ≥650µM
Screening Assay for Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes	TT035510	Negative for clastogenicity, but a confirmatory assay was not conducted and the study was Non-GLP	
Chromosomal Aberrations in Cultured Purified Human Blood Lymphocytes with AP390	TT045501	Negative for clastogenicity, but a confirmatory assay was not conducted (GLP)	
Assay for Micronucleus Induction in Mouse Bone Marrow	TT048626	Positive for clastogneicity in the mouse (≥500 mg/kg-24 hours and 1000 mg/kg-48 hours.	

Reproductive and Developmental Toxicity

Study #	TT047360	TT037440	TT047290	TT047300
Title	L-001079038 Oral Fertility Study in Female Rats	L-001079038 Oral Fertility Study in Male Rats	L001079038 Oral Developmental Toxicity Study in Rats with Prenatal Evaluation	L001079038 Oral Embryofetal Development in Rabbits
Methods	SAHA administered 14 days prior to mating through GD 7. (n=24/group)	SAHA administered for approximately 10 weeks prior to cohabitation, during cohabitation, until day prior to scheduled sac (14 weeks total).	Administered GD 6-20 to presumed pregnant females (n=22)	Administered GD 7-20 to presumed pregnant females (n=18/group)
Key Findings	↑ in corpus luteum at ≥15 mg/kg, NOEL for reproductive performance is <15 mg/kg. ↓ in implants and live births (≥50 mg/kg) yields a NOEL for embryonic development	NOEL>150 mg/kg/day, the highest dose utilized in the study.	$NOAEL_{embryolethality}$: 50 mg/kg $NOAEL_{development\ abnormality}$:15 mg/kg Skeletal variations and incomplete ossifications observed at 50mg/kg.	$NOAEL_{embryolethality}$: 150 mg/kg $NOAEL_{development\ abnormality}$: 50 mg/kg
Species	Sprague Dawley Rat	Sprague Dawley Rat	Sprague Dawley Rat	New Zealand White Rabbit
Doses	0, 15, 50, 150 mg/kg (0, 90, 300, 900 mg/m2)	0, 20, 50, 150 mg/kg (0, 120, 300, 900 mg/m2)	0, 5, 15, 50 mg/kg/day (0, 30, 90, 300 mg/m2)	0, 20, 50, 150 mg/kg/day (0, 240, 600, 1800 mg/m2)
Mortality and Clinical Signs	Obtained daily during dosing No mortality observed; no clinical signs	Males: Daily Females: First day of Cohabitation, GD0, 7, 15. 4 treatment related deaths in the HD males, signs included unkempt fur, ocular/nasal discharge, abnormal feces, decreased activity, decreased skin turgor, mucoid discharge, abnormal respiratory sounds and/or over-responsiveness to touch	daily during dosing and GD0 No unscheduled deaths during study and no drug dependent clinical signs	daily GD1-28 No mortality observed. No treatment related clinical signs
Body Weight/	Obtained twice weekly HD- 67% decrease in weight gain	Males: Twice weekly Females: First day of cohabitation on GD0, 7 and 15.	BW- GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 21. Food Consumption: GD3, 6, 10, 14,	BW: GD 0, 7, 9, 11, 13, 15, 17, 19, 21 and 28.

Study #	TT047360	TT037440	TT047290	TT047300
Food Consumption	compared to control premating, no difference during gestation HD-↓9.5% food consumption PMD1-5	HD M-BW↓ 18% (as early as week 1), with qualitative ↓ in food consumption. No changes observed in females.	18. BW: No drug dependent changes on maternal body weight when adjusted for fetal weight No change in food consumption	No drug dependent changes on maternal body weight or food consumption
Necropsy	↑13, 30 and 97% in corpora lutea respectively with a 2 fold increase % periimplantation loss in the MD group and a 8.5 fold increase in the HD group. 150 mg/kg/day- ↑7fold post-implantation loss and resorptions, with a >10 fold increase in dead fetuses/litter and ~70%↓ in live fetuses per female and per live litter.	No drug dependent changes on fertility indices.	No treatment related effects on embryonic or fetal survival 50 mg/kg ~20% ↓ in live fetal weight ↑ in fetuses with skeletal variations (vertebral count/sacral verterbra variations, cervical rib, supernumery ribs), and incomplete ossifications of the skull, thoracic vertebra and sternebra.	No drug related findings in placental morphology, embryonic/fetal survival, or fetal sex ratios. 150 mg/kg: ↓live fetal weight (8-11%) Malformations: 150 mg/kg: microcardia (n=1), absent kidney (n=2), absent ureter (n=2) ≥20 mg/kg: bladder (n=5, 9, 8 respectively) Variations: 150 mg/kg: Short 13th rib (2.4x control); Incomplete ossification of metacarpals (11 fold)

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
The nonclinical studies submitted to this NDA provide sufficient information to support the use of vorinostat (ZOLINZA®) for the treatment of patients with cutaneous T-cell lymphoma who have progressive persistent or recurrent disease.

Suggested labeling: See separate labeling review

Signatures:

Reviewer Signature S. Leigh Verbois , Ph.D.

Supervisor Signature David Morse, Ph.D. Concurrence Yes X No ___

APPENDIX/ATTACHMENTS

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

/s/

Leigh Verbois
9/13/2006 05:22:57 PM
PHARMACOLOGIST

David Morse
9/18/2006 03:41:17 PM
PHARMACOLOGIST

MEMO TO FILE

FROM: Houda Mahayni, R.Ph., Ph.D.
TO: NDA 21-991, Vorinostat Capsule, 100 mg
DATE RECEIVED: July 31, 2006
SUBJECT: Proposed dissolution specification

BACKGROUND: The sponsor is proposing the following specification for release and shelf life:
15 min ≥ — but —
60 min ≥ —

Dissolution Parameters:

Apparatus:	Paddle
Rotation Speed:	100 rpm
Dissolution Medium:	2% Tween 80
Medium Volume:	900 mL
Medium Temperature:	37±0.5°C
Sampling Times:	15 minutes, 60 minutes

Vorinostat is very slightly soluble in water at physiologic pH and sparingly soluble in solvents with pH > 8.5 and slightly soluble in ethanol. The vorinostat drug substance is cohesive in nature, with low aqueous solubility and relatively poor flow. Based on the Biopharmaceutics Classification System (BCS), vorinostat has low solubility and low permeability (Class IV compound). — , is the primary factor controlling in vitro capsule dissolution. —

— The sponsor used the Biobatch (Lot No. 0683DFC004A001) using the similarity factor f2 to guide formulation development —

— was selected for capsule formulation. The drug product is manufactured by — process. Excipients used were the following:

- Microcrystalline cellulose —
- Croscarmellose Sodium —
- Magnesium stearate —

The reviewer, Josephine Jee had two questions:

1. Whether testing this product using one media is sufficient since it has low solubility
2. Whether the proposed specification is proper

ASSESSMENT:
The sponsor used 2% Tween 80 as a dissolution media. Based on the low solubility of the drug substance, it is acceptable to use surfactant in the media. The choice of two point specification for the dissolution of Vorinostat capsules is acceptable for poorly water soluble drug. As to the proposed specification, it will difficult to comment in absence of data. However, based on a preliminary observation, the proposed dissolution specification seems to be loose. For additional help with setting dissolution specification, please provide additional information and raw data from the Biobatch, stability batch, and commercial batch.

Houda Mahayni, R.Ph., Ph.D.

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

/s/

Houda Mahayni
8/2/2006 09:01:34 AM
BIOPHARMACEUTICS