CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205353Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Farydak (panobinostat)

Date: September 2, 2014
To: File for NDA 205353
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Farydak conducted by Drs. Place and Ringgold, and secondary memorandum and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Farydak may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
09/02/2014

MEMORANDUM

Date: August 28, 2014
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 205353
Drug: FARYDAK (panobinostat)
Indications: Treatment of patients with multiple myeloma, who have received at least 1 prior therapy
Applicant: Novartis Pharmaceuticals Corp.

Panobinostat is a small molecule histone deacetylase (HDAC) inhibitor. It also inhibited deacetylation of some non-histone proteins in *in vitro* and/or *in vivo* studies. Treatment of normal and transformed cells with panobinostat resulted in apoptosis and/or cell cycle arrest; however, higher activity was observed in transformed cells. Anticancer activity of panobinostat was demonstrated *in vitro* using different types of cells (including multiple myeloma cells) and in xenograft models of cancer.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Animal toxicology studies were conducted in appropriate species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. Panobinostat-related toxicities in rats and/or dogs included the following: thyroid toxicities (reduced T_3, T_4, and TSH, C-cell hyperplasia, follicular hypertrophy, and one case of follicular cell adenoma); decreased WBCs, differentials, and platelets; hemorrhage in multiple organs including brain and lungs; inflammation in multiple organs including liver and lungs; bone marrow abnormalities such as hyperostosis and plasmacytosis; skin hyperplasia and papilloma; and toxicities in male reproductive organs. Osseous metaplasia of the lung was observed in 3 high dose dogs in the 39-week study. This finding may be secondary to the inflammation and injury to the lungs of the animals; however, a direct pharmacologic effect cannot be excluded at this time.

Panobinostat and/or its metabolites crossed the blood-brain barrier in tissue distribution studies. Safety pharmacology studies further showed potential for CNS effects as indicated by reduced motor activity, wobbly gait, convulsion, and reduced grip strength in mice, 15-60 min post-dose. Based on cardiovascular assessments, panobinostat has the potential to prolong QT_C. In telemetered dogs, QTc prolongation of up to 25 msec was observed in panobinostat treated animals.

QTc prolongation has been reported in patients treated with panobinostat. Other adverse findings in patients which correlate with findings in the animals include

thrombocytopenia, neutropenia, anemia, bleeding, and skin disorders. Adverse findings observed in animals and not detected (or detected with low incidence) in patients, have been included in Section 13.2 of the label. These are mainly findings in the thyroid, bone marrow, and skin.

Panobinostat was genotoxic in the battery of genetic toxicology studies conducted and was teratogenic in rats and rabbits. Teratogenicity in rats was observed at a dose that did not cause maternal toxicity. Pregnancy Category D is recommended for FARYDAK.

FARYDAK may impair male and female fertility. In a combined male and female fertility study, both male and female rats received panobinostat. Female rats had reduced mating index, fertility index and conception rate. In toxicology studies conducted in the dog, panobinostat caused oligospermia, reduced secretory granules, and testicular degeneration.

The nonclinical studies were reviewed by Drs. Emily Place and Kimberly Ringgold. The nonclinical findings are summarized in the "Executive Summary" and "Integrated Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with Drs. Place and Ringgold that from a nonclinical perspective, FARYDAK may be approved and that no additional nonclinical studies are needed to support approval of FARYDAK for the proposed indication.

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/s/

HALEH SABER
08/28/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205353
Supporting document/s:	1
Applicant's letter date:	March 22, 2014
CDER stamp date:	March 24, 2014
Product:	Panobinostat
Indication:	Multiple Myeloma (for patients who have received at least one prior therapy)
Applicant:	Novartis Pharmaceuticals Corp.
Review Division:	Division of Hematology Products
Reviewer:	Emily Place, PhD MPH Kimberly Ringgold, PhD
Supervisor/Team Leader:	Haleh Saber, PhD
Division Director:	Ann Farrell, MD
Project Manager:	Diane Hanner, CAPT

Disclaimer

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1 Executive Summary

1.1 Recommendations

Panobinostat is a histone deacetylase (HDAC) inhibitor of both histone and non-histone proteins. The applicant is seeking approval for the oral route of administration and the proposed clinical dose is 20 mg once daily. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted. Chronic toxicology studies in the rat and dog, genotoxicity, and reproductive toxicity studies were reviewed by Kimberly Ringgold, Ph.D. (b) (4)

The pharmacology/toxicology studies conducted support approval of panobinostat for the proposed indication (in combination with bortezomib and dexamethasone, for the treatment of patients with multiple myeloma, who have received at least one prior therapy).

1.2 Discussion of Nonclinical Findings

Panobinostat is a histone deacetylase (HDAC) inhibitor with activity to HDAC isoforms in class I, II and IV at low nanomolar concentrations in vitro. Treatment of cells with panobinostat resulted in accumulation of acetylated histones and non-histone proteins as well as cell death and cell cycle arrest, including human multiple myeloma cells. Panobinostat also caused cell death ex vivo in cells taken from patients with multiple myeloma and in both xenograft and disseminated mouse models of myeloma. Tumor tissues dissected from mice xenografts that were treated with panobinostat showed elevated levels of acetylated histones. Panobinostat in combination with bortezomib and dexamethasone reduced tumor burden and increased survival of animals.

Safety pharmacology studies showed no adverse respiratory findings. Neurological findings were evident in mice and presented as decreased motor activity, wobbly gait, convulsion, and decreased grip strength. The IC_{50}s in the hERG assay for panobinostat and a human metabolite BJB432 were 3.5 μM and 1.6 μM, respectively, suggesting week inhibition of the potassium channel. However, QTc prolongation was observed when panobinostat was given orally to dogs in a cardiovascular telemetry study.

After oral dosing, bioavailability of panobinostat was 6% in the rat and 50% in the dog. Plasma protein binding of panobinostat and its carboxylic metabolite (formed in mice and rats only, at 37ºC) was independent of the dose in the species tested. The average free panobinostat (and the carboxylic metabolite in mice and rats) was 40% in the mouse, 20% in rat and dog plasma, and 10% in human plasma. Tissue distribution was extensive following administration of oral panobinostat. The highest exposure (by C_{max} or AUC) was in the bile, GI tract, uveal tract, skin, pituitary, and liver. The longest

observed half-life was for the skin with a $t_{1/2}$ of up to 600 hours. Panobinostat and/or its metabolites distributed to the fetus, placenta, uterus and mammary glands of pregnant rats. Panobinostat was extensively metabolized following oral exposure in both rats and dogs; the %AUC of parent drug in the plasma was at 4% and 9% in the rat and dog respectively. One unique metabolite was detected in rat, 4 unique metabolites in dog and most prominent human metabolite "BJB432" was detected in both species (0.83% in dogs, 6.95% in rats). The main route of elimination is hepatobiliary. In the rat, 83% of elimination occurred in the feces, and less than 1% in the urine. In the dog, 60% was eliminated through the feces with 34% in the urine. Based on the data collected in general toxicology studies, there were no gender differences in exposure, and increased in C_{max} and AUC values were dose proportional.

The general toxicology studies were conducted in the rat and dog via oral (gavage), which is the intended route of administration. The 4, 13 and 26 week repeat dose toxicity studies in rat and 4 and 39 week repeat dose toxicity studies in dogs are reviewed. The 13 week repeat dose toxicity study in dogs was not reviewed but summarized to show findings related to thyroid toxicity and the male reproductive system. Nonclinical studies also included genotoxicity and developmental and reproductive toxicology studies. All appropriate studies were conducted in compliance with Good Laboratory Practice (GLP) regulations.

General Toxicology studies

Nonclinical findings in the rat and dog show that panobinostat targets the bone marrow, hematopoietic/lymphatic systems, liver, lung, kidney, thyroid, mammary gland (atrophy; rat only) GI tract, skin (dog only) and male reproductive organs (dog only). Findings include:

- Thyroid: decreased T_3, T_4, and TSH, follicular cell hypertrophy; follicular cell adenoma in one animal (rat); focal C cell hyperplasia; decreased colloid; vacuolation
- Hematopoietic/lymphatic systems: ↓RBCs; ↓ WBCs, differentials, and platelets; lymphoid depletion/atrophy; granulocytic aplasia or hypoplasia in the bone marrow; plasmacytosis in lymph nodes and bone marrow; erythrophagocytosis in lymph nodes and bone marrow; increased number of granulocytic cells and presence of abnormal cytoplasmic granulation in bone marrow, irregular nuclear shaped and enlarged cell size in marrow; hyperostosis of femur.
- Male reproductive organs): oligospermia; attenuation of prostatic epithelium with reduced secretory granules, testicular degeneration, degeneration of seminiferous tubules, increased epididymal debris
- Skin: wart-like growth, papilloma and hyperplasia.
- Hemorrhage: lacrimal gland, brain, spinal cord, GI tract, and lung.
- Inflammation: inflammatory cells in kidney, pancreas, GI tract, liver, and lung.

Osseous metaplasia was observed in the 39-week dog study in 2 main study high-dose animals (1 male and 1 female) and 1 recovery high-dose female. The finding is likely drug-related. The finding may be related to the pharmacology of HDAC inhibitors as there are several reports in the scientific literature that demonstrate that HDAC

inhibitors alter bone homeostasis (e.g., osteoblast and osteoclast differentiation/suppression).

Genotoxicity
Panobinostat was mutagenic in the in vitro bacterial reverse mutation assay (AMES test). Panobinostat tested negative for chromosome aberrations; however, endo-reduplication (increased number of chromosomes) in human peripheral blood lymphocytes in vitro was observed. Panobinostat also was positive for DNA damage in a COMET assay in mouse lymphoma cells.

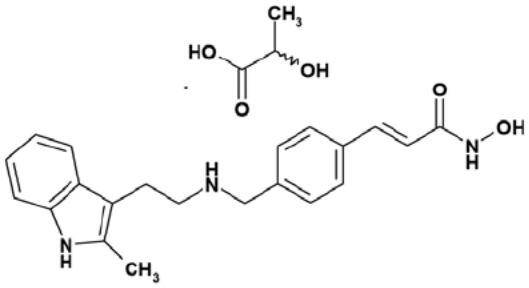
Reproductive and Development Toxicity
Panobinostat may impair male and female fertility. Panobinostat elicited toxicity towards male reproductive organs in the dog in both the 4 and 13 week repeat dose studies. Toxicities included prostatic atrophy, reduced secretory granules in the prostate, testicular degeneration and oligospermia and epididymal debris. In a combined male and female fertility study in rats, females had reduced mating index, fertility index, and conception rate at 100 mg/kg (600 mg/m^2). Increased resorption and post-implantation loss were seen at ≥ 10 mg/kg (60 mg/m^2) and reduced number of live embryos was observed at doses ≥ 30 mg/kg (180 mg/mg/m^2).

Panobinostat was teratogenic in the rat and rabbit. In the rat, embryo-fetal malformations (cleft palate and short tail), and variations or anomalies (e.g. incomplete ossifications, extra presacral vertebrae, and extra ribs) occurred at 30 mg/kg (180 mg/m^2) in the absence of maternal toxicities. There were no live fetuses at the 100 mg/kg dose. In the rabbit, maternal toxicity was observed at 80 mg/kg (960 mg/m^2). Embryo-fetal toxicities included decreased fetal weight ≥40 mg/kg and malformations at 80 mg/kg. Malformations included absent digits, cardiac interventricular septal defects and aortic arch interruption, and missing gall bladder. Other skeletal variations or anomalies included incomplete ossification (≥10 mg/kg), and extra ribs (80 mg/kg). Thus, administration of panobinostat during pregnancy may pose a risk to the human fetus.

2 Drug Information

2.1 panobinostat

CAS Registry Number:	404950-80-7 (free base), 960055-56-5
Generic Name:	Panobinostat lactate
Code Name:	LBH-589
Chemical Name:	(E)-N-hydroxy-3-[4-({[2-(2-methyl-1H-indol-3-yl)ethyl]amino}methyl)phenyl]acrylamide 2-hydroxypropanoic acid salt prolinamide acetate (salt)

Molecular Formula/Molecular Weight	$C_{21}H_{23}N_3O_2$. $C_3H_6O_3$; 439.5
Structure 	
Pharmacologic class:	histone deacetylase inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 69,862 (Oral); IND 67091 (Intravenous)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Composition of one LBH589 15mg and 20mg Hard capsules

Ingredient	Theoretical amount [mg]		Function	Reference to standards
	15mg	20mg		
LBH589 lactate salt, anhydrous[1,2]	(b) (4)		Active	Novartis monograph
Mannitol			(b) (4)	Ph.Eur., USP
Microcrystalline cellulose/ Cellulose, microcrystalline				Ph.Eur., NF
Pregelatinized starch				Ph.Eur., NF
Magnesium stearate[3]				Ph.Eur., NF
(b) (4)				Ph.Eur., USP

2.3.2 Comments on Novel Excipients: There are no Pharmacology/Toxicology concerns with the excipients or their levels in the drug formulation.

2.3.3 Comments on Impurities/Degradants of Concern: There are no Pharmacology/Toxicology concerns with the levels of impurities in the drug substance or drug product.

2.4 Proposed Clinical Population and Dosing Regimen

Panobinostat in combination with bortezomib and dexamethasone, is being proposed for patients with refractory/relapsed Multiple Myeloma who have received at least one prior therapy. Panobinostat is administered at a dose of 20 mg once daily orally. Cycles consist of dosing on days 1, 3, 5 each week for 2 weeks, 1 week off, for 8 cycles. Patients can continue for 8 additional cycles if they are receiving clinical benefit.

2.5 Regulatory Background

The IND for oral capsules (69,862) was submitted in 2004. (b) (4) NDA 205353 was submitted on March 22, 2014 for refractory multiple myeloma.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.	Module
Primary Pharmacodynamics		
In vitro Pharmacology		
Preclinical characterizations of the potent deacetylase inhibitor NVP-LBH589	RD-2008-51291	4.2.1.1
In vitro sensitivity across the cancer cell line encyclopedia panel	RD-2013-50424	4.2.1.1
In vivo Pharmacology		
Histone acetylation as a pharmacodynamic marker for NVP-LBH589	RD-2010-50113	4.2.1.1
Secondary Pharmacodynamics		
In vivo activity of NVP-LBH589 and bortezomib in the MM1.S-luciferase human systemic multiple myeloma xenograft model	RD-2008-51313	4.2.1.2
Safety Pharmacology		
Neuropharmacological profile in male mice	(b) (4) r0280108	4.2.1.3
A pharmacological assessment of the effect of LBH589 on the respiratory system of the male albino rat	(b) (4) r0280118	4.2.1.3
Effects of LBH589 and BJB432 on cloned hERG potassium channels	(b) (4) r0870294	4.2.1.3

Study Title	Study No.	Module
expressed in human embryonic kidney cells		
A pharmacological assessment of the effect of repeated oral doses of LBH589 on the cardiovascular system of the beagle dog using telemetry	(b) (4) 0680202-01	4.2.1.3
Pharmacokinetics		
Absorption		
Absorption, distribution, metabolism and excretion of [14C]LBH589 following oral and intravenous dosing, intact and bile duct cannulated rats	DMPK-r0201550-01/02	4.2.2.2.1
Absorption, metabolism, and excretion of [14C]LBH589 following oral and intravenous dosing in dogs	DMPK-r0300092	4.2.2.2.1
Distribution		
Tissue distribution of [14C]LBH589 in male and female rats after an oral dose as determined by quantitative whole autoradiography	DMPK-r0500724	4.2.2.3.1
Absorption, metabolism, and excretion of [14C]LBH589 following oral and intravenous dosing in dogs	DMPK-r0300092	4.2.2.2.1
Tissue distribution of radioactivity following an oral dose of [14C]LBH589 in the pregnant rat	DMPK-r0700906	4.2.2.3.1
Blood distribution and plasma and serum protein binding of [14C]LBH589 in the mouse, rat, dog, and human	DMPK-r00200414	4.2.2.3.1
Metabolism		
Analysis of metabolite profiles in dog plasma following oral administration of [14C]LBH589	DMPK-r0900382	4.2.2.4.1
Analysis of metabolite profiles in rat plasma following oral administration of [14C]LBH589	DMPK-r0900383	4.2.2.4.1
Excretion		
Absorption, distribution, metabolism and excretion of [14C]LBH589 following oral and intravenous dosing, intact and bile duct cannulated rats	DMPK-r0201550-01/02	4.2.2.2.1
Absorption, metabolism, and excretion of [14C]LBH589 following oral and intravenous dosing in dogs	DMPK-r0300092	4.2.2.2.1
Repeat dose toxicology: *(including supportive toxicokinetics)*		
4-week (3 doses/week) oral (gavage) toxicity study in rats with a 4-week recovery period	0370121	4.2.3.2.1
13-week (3 doses/week) oral (gavage) toxicity study in rats followed by a 4-week recovery period	0680019	4.2.3.2.1
26 Week (3 doses/week) Oral (Gavage) Toxicity Study in Rats Followed by a 4 Week Recovery Period	0680134	4.2.3.2.1
4-week (3 doses/week) oral toxicity study in dogs with a 4-week recovery period	0370122-01	4.2.3.2.1
39 Week (3 dose/week) Oral (Gavage) Toxicity Study in Dogs with a 4 Week Recovery Period	0680133	4.2.3.2.1
Genotoxicity		
Mutagenicity test using *S. typhimurium*	0212012	4.2.3.3.1
Chromosome aberration test in cultured human peripheral blood lymphocytes	0212210	4.2.3.3.1
Comet assay in vitro with L5178Y mouse lymphoma cells	(b) (4) lr0115032	4.2.3.3.1

Study Title	Study No.	Module
Reproductive and development Toxicity		
LBH589: An oral (gavage) fertility and early embryonic development study in the rat	0670759-01	4.2.3.5.1
An Oral Embryo-fetal development study in rat	0670511	4.2.3.5.2
An Oral Embryo-fetal development study rabbit	0670512	4.2.3.5.2

3.2 Studies Not Reviewed

Study Title	Study No.	Module
Primary Pharmacodynamics		
In vitro Pharmacology		
Effect of deacetylase inhibitor LBH589 on preclinical Hodgkin Lymphoma models	RD-2010-50107	4.2.3.2.1
In vitro Pharmacology		
In vivo antitumor activity of NVP-LBH589 against human HCT 116 colon tumor subcutaneously implanted into athymic mice	RD-2001-50288	4.2.1.1.1
In vivo anti-tumor activity of NVP-LBH589 in the HH human cutaneous T-cell lymphoma xenograft model	RD-2007-50247	4.2.1.1.1
Safety Pharmacology		
Effects of hERG tail currents in stably transfected HEK293 cells	0516281	4.2.1.3.1
Effects of hERG tail currents in stably transfected HEK293 cells	0616811	4.2.1.3.1
Electrophysiological study of combination with LBH589 and Taxotere in isolated rabbit heart	0618520	4.2.1.3.1
Electrophysiological study of combination with LBH589 and 5-azacytidine in isolated rabbit heart	0618521	4.2.1.3.1
Electrophysiological investigations in the isolated rabbit heart	0618524	4.2.1.3.1
Electrophysiological study of BJB432 in isolated heart	0618585	4.2.1.3.1
Electrophysiological study of taxotere in the isolated heart	0718512	4.2.1.3.1
Intravenous safety pharmacology screening study in telemetered dogs	0110024	4.2.1.3.1
Intravenous safety pharmacology screening study in telemetered dogs	0210083	4.2.1.3.1
Intravenous safety pharmacology telemetry study in dogs amendments 01 and 02	0210083-02	4.2.1.3.1
Effects of LBH589 on cloned hERG channels expressed in mammalian cells	0280136	4.2.1.3.1
Electrophysiological investigations in the isolated rabbit heart	0350418	4.2.1.3.1
Measurements of hERG channel surface expression in stably transfected HEK293 cells	0516287	4.2.1.3.1
Effects of (b) (4) and metabolite M40.8 on cloned hERG potassium channels expressed in human embryonic kidney cells	0870532	4.2.1.3.1
Effects of five test articles on cloned hERG channel surface expression in mammalian cells	0870542	4.2.1.3.1

Study Title	Study No.	Module
Effects of M36.9 and T24.0 on cloned hERG potassium channels expressed in human embryonic kidney cells	0970190	4.2.1.3.1
Effects of LBH589 and metabolites: 315-02, 519-07, 539-08, 541-08 in SM-HERG-lite assay	0970523	4.2.1.3.1
Electrophysiological investigations in the isolated rabbit heart	0618523	4.2.1.3.1
Electrophysiological study of AMN107 and LBH589 in isolated heart	0718532	4.2.1.3.1
Evaluation of NVP-LBH589 in the Langendoff heart	RD-2001-50377	4.2.1.3.1
Pharmacokinetics		
Analytical Methods and Validation		
[14C]LBH589 Synthesis and Analysis	DMPK-r0500221	4.2.2.1.1
[14C]LBH589 Synthesis and Analysis	DMPK-r0600474	4.2.2.1.1
[M+6]LBH589 Synthesis and Analysis	DMPK-r0700846	4.2.2.1.1
[14C]LBH589 Synthesis and Analysis	DMPK-r0101750	4.2.2.1.1
[14C]LBH589 Synthesis and Analysis (new label)	DMPK-r0300680	4.2.2.1.1
[M+6]LBH589 Synthesis and Analysis	DMPK-r0300657	4.2.2.1.1
Quantitative determination of LBH589 (panobinostat) in rat plasma by LC-MS/MS	DMPK-r0101758c	4.2.2.1.1
Quantitative determination of LBH589 (panobinostat) and its metabolite BJB432 in rat plasma by LC-MS/MS	DMPK-r0600958a	4.2.2.1.1
Quantitative determination of LBH589 (panobinostat) in rabbit plasma by LC-MS/MS	DMPK-r0600957	4.2.2.1.1
Quantitative determination of LBH589 (panobinostat) in dog plasma by LC-MS/MS	DMPK-r0101758b	4.2.2.1.1
Quantitative determination of LBH589 (panobinostat) and its metabolite BJB432 in dog plasma by LC-MS/MS	DMPK-r0600958b	4.2.2.1.1
Stability of [14C]LBH589 in plasma from human, rat, mouse, dog and monkey	DMPK-r0201360	4.2.2.1.1
Absorption		
Absorption, metabolism, and excretion in the rabbit following intravenous or oral administration of [14C]LBH589	DMPK-r0700878	4.2.2.2.1
Distribution		
Distribution, metabolism, and excretion following a single intravenous dose of [14C]LBH589 in the rat	DMPK-r0101753	4.2.2.3.1
Pharmacokinetic, distribution and preliminary protein binding data for NVP-LBH589	DMPK-r01-1477	4.2.2.3.1
Metabolism		
Metabolism of [14C]LBH589 by human, monkey, dog, and rat liver slices	DMPK-r0101754	4.2.2.4.1
Other Pharmacokinetic Studies		
A comparison of two oral doses of LBH589 in the rat	DMPK-r0500726	4.2.2.7.1
Plasma concentrations of LBH589 following a HCL tablet in the dog	DMPK-r0300072	4.2.2.7.1
Plasma exposure to LBH589 in nine dogs following dosing with three oral formulation	DMPK-r0600179	4.2.2.7.1

Study Title	Study No.	Module
Absolute bioavailability of LBH589 in the dog	DMPK-r002130	4.2.2.7.1
Toxicology		
Single dose toxicity		
An acute intravenous toxicity study in mice	0270147	4.2.3.1.1
An acute intravenous toxicity study in rats	0270146	4.2.3.1.1
Repeat dose toxicity		
2-week oral (gavage) dose range-finding toxicity study in rats (7 doses)	0370080	4.2.3.2.1
1 cycle (3 days dosing) intravenous toxicity/batch comparison study in male rats with a 2-week recovery period	0270151	4.2.3.2.1
4 cycle (3 days dosing/cycle) intravenous toxicity study in rats with a 4-week recovery period	0270103	4.2.3.2.1
An intravenous rising dose/2-week dose range-finding toxicity study in rats	0270059	4.2.3.2.1
13-week (once weekly) intravenous toxicity study of LBH589 with a 4-week recovery period	0670757	4.2.3.2.1
An oral (gavage) rising dose study in dogs	0270176	4.2.3.2.1
2-week (3 doses/week) oral (gavage) dose range-finding toxicity study in dogs	0370089	4.2.3.2.1
13 week (3 doses/week) oral (gavage) toxicity study in dogs with a 4-week recovery period	0680020-01	4.2.3.2.1
An intravenous rising-dose study in dogs with ECG monitoring	0170106	4.2.3.2.1
4 cycle (3 day dosing/cycle) intravenous study in dogs with a 4-week recovery period	0270069	4.2.3.2.1
13-week (once weekly) intravenous toxicity study of LBH589 with a 4-week recovery period in the beagle dog	0670758	4.2.3.2.1
Genotoxicity		
Ames Test	0113105	4.2.3.3.1
Reproductive and Developmental Toxicity		
An oral (gavage) embryo-fetal development dose range-finding study in rats	0570309-01	4.2.3.5.2
An oral (gavage) embryo-fetal development dose range-finding study in rabbits	0670018	
Local Tolerance		
Local tolerance test after intravenous, intra-arterial and perivenous administration in the rabbit	0770115	4.2.3.6.1
Other Toxicity Studies		
A 4-week (3 doses/week) oral (gavage) investigative study in the male rat with 1- and 2-week interim sacrifices: LBH589 vs. PTU comparative gene expression profiling analysis of the thyroid pituitary and liver	0770979	4.2.3.7.3
UV/vis absorption spectrum for initial phototoxicity assessment	0517503	4.2.3.7.7
In vitro 3T3 NRU phototoxicity assay	0580320	4.2.3.7.7
Modified local lymph node assay (LLNA) in mice (identification of contact allergens), Assessment of the contact sensitizing	0670352	4.2.3.7.7

Study Title	Study No.	Module
potential with the murine local lymph node assay (LLNA tier 1)		
Modified local lymph node assay (LLNA) in mice (identification of contact allergens), Assessment of the contact sensitizing potential with the murine local lymph node assay (LLNA tier 1)	0670584	4.2.3.7.7

3.3 Previous Reviews Referenced

Non-clinical reviews under IND 69862, IND 67091 (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

Panobinostat (LBH-589) is an orally administered cinnamic hydroxamic acid histone deacetylase inhibitor (HDACI). Histone deacetylase enzymes target lysine groups on histones and various non-histone proteins such as p53 α-tubulin, heat shock protein 90 (HSP90) and HIF-Iα.

Pharmacology Key Findings

- Panobinostat inhibited purified recombinant HDAC enzymes (HDACs 1, 3, 5, 6, 9, 10 and 11) in vitro with IC50s ≤15nM. Panobinostat inhibited HDACs 4, 7 and 8 with IC50s ≤550nM. Panobinostat demonstrated increased activity for HDAC isoform inhibition when compared to vorinostat, belinostat and mocetinostat.
- Panobinostat treatment of cultured normal and transformed cells resulted in apoptosis and cell cycle arrest. Apoptosis was increased in 3 cancer cell lines tested but not in untransformed cells. Panobinostat induced a dose-dependent increase in apoptosis in plasma cells collected from patients with refractory multiple myeloma.
- In experiments using a myeloma cell line, panobinostat inhibition of cell cycle progression correlated with changes in cell cycle regulatory protein expression including increased p21 and p53 levels, and decreased Cdk2/4 and CyclinD1.
- Panobinostat inhibits histone deacetylation in cutaneous T cell lymphoma and Hodgkin's lymphoma cells in vitro.
- Panobinostat increased histone acetylation in tumors excised from mouse xenografts. Panobinostat decreased tumor volume and increased survival in mouse tumor xenograft models of subcutaneous plasmacytoma or disseminated myeloma.
- Human primary multiple myeloma cells treated in vitro with panobinostat, bortezomib and dexamethasone showed increased apoptosis when compared to treatment with any drug alone or doublet combination.

- Panobinostat in combination with bortezomib and dexamethasone showed the greatest effect on tumor burden and survival in the human xenograft model of subcutaneous plasmacytoma.

Study title: Preclinical characterizations of the potent deacetylase inhibitor NVP-LBH589
Study number: rd-2008-51291
Conducting Laboratory: Novartis Pharmaceuticals Corp.
Location: 4.2.1.1

The ability of panobinostat to (1) inhibit HDAC isoform activity, (2) activate transcription of the cell cycle inhibitor p21, (3) affect cell viability, and (4) induce apoptosis, was evaluated. Experiments compared panobinostat to three other HDAC inhibitors: vorinostat (SAHA), belinostat (PXD-101) and mocetinostat (MGCD0103).

HDAC activity was measured using recombinant proteins expressed in either HEK293 cells stably expressing the FLAG-tagged HDAC isoforms 1, 3 or 6 or purified from baculovirus expression system (HDACs 2, 4, 5, 7, 8, 9, 10 or 11). Activity of HDAC isoforms (HDAC 1-11) was measured by cleavage dependent fluorescence of Bis(BOC-(ac)Lys)-rhodamine 110 (acetylated peptide substrate). Transcriptional activation of cell cycle protein p21was measured by luciferase activity in a promoter driven expression construct in H1299 cells.

Panobinostat inhibited HDAC isoforms with IC50 values in the nanomolar range. When compared to the three other HDAC inhibitors, panobinostat had lower IC50s against all 11 enzymes tested. HDACs 1, 2, 3 (Class I), HDACs 5, 6, 9, 10 (Class II) and HDAC 11 (Class IV) were inhibited at concentrations ≤10nm. The concentration of panobinostat for 50% activation (AC50) was determined relative to the psammaplin A reference (p21 activator) using linear regression between data points. Promoter activation of p21 as measured by AC50 was achieved at 46nM of panobinostat.

Table 1. IC50 values for Panobinostat against HDAC isoforms in vitro

		NVP-LBH589	(b) (4) (SAHA)	(b) (4) (PXD-101)	(b) (4) MGCD0103
Inhibition of Enzyme Activity IC_{50} [nM]	HDAC1	2.5	75.5	17.6	142
	HDAC2	13.2	362	33.3	147
	HDAC3	2.1	57.4	21.1	205
	HDAC4	203	15,056	1236	> 30,000
	HDAC5	7.8	163	76.3	1889
	HDAC6	10.5	27.1	14.5	> 30,000
	HDAC7	531	12,522	598	> 30,000
	HDAC8	277	1,069	157	28,167
	HDAC9	5.7	78.1	44.2	1,177
	HDAC10	2.3	88.4	31.3	54.9
	HDAC11	2.7	109	44.2	104
p21 Promoter Activation AC_{50} [nM]		46	9,800	>10,000	12,900

IC50 given for HDAC enzymatic activity. Mean concentration values relative to DMSO control from at least 4 individual titrations is shown.

(Excerpted from the submission)

Panobinostat inhibited cell proliferation in a panel of 8 cancer cell lines and normal human epithelial cells grown in monolayer as measured by using a CellTiter-Glo or MTS assay. IC50 and LD50 values were calculated. Panobinostat inhibited 50% growth in cultures at concentrations that were ~10-fold greater or more than in normal cells.

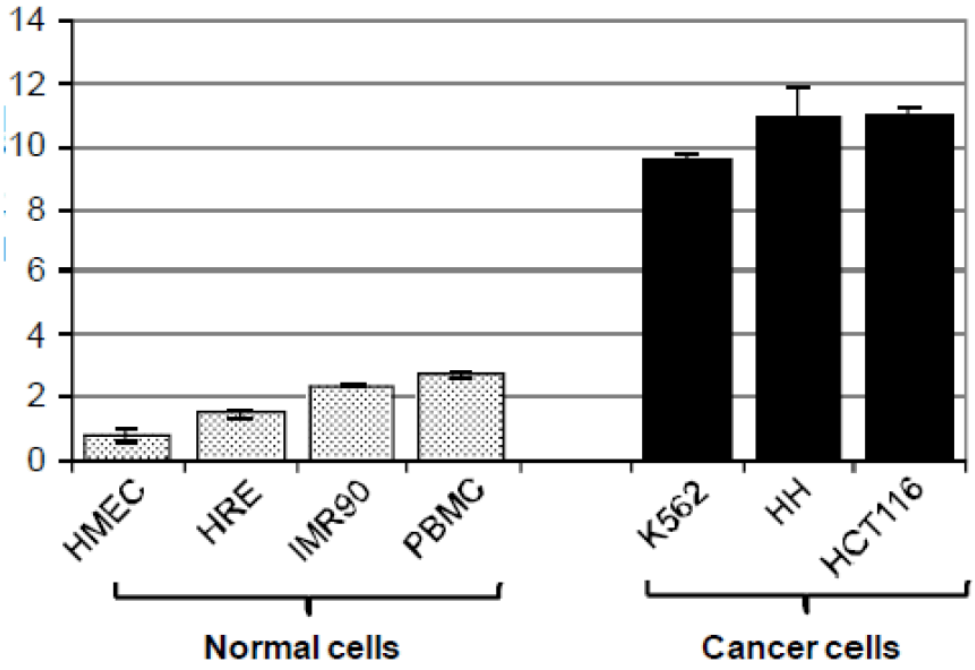
Table 2. Panobinostat inhibits cell proliferation and viability in cancer and normal cell lines

		NVP-LBH589		
Cell Lines	**Cell Type**	**IC_{50} [nM]**	**LD_{50} [nM]**	**LD_{90} [nM]**
HH	CTCL	0.7	4.3	14
K562	CML	3.8	9.3	20.6
KG-1a	AML	3	7.1	14.8
L-428	HL	10.5	26.1	57.5
BT-474	Breast Cancer	2.6	22.4	306
LNCaP	Prostate Cancer	1.4	16.6	134
HCT116	Colon Cancer	7.1	51.7	344
Bx-PC3	Pancreatic Cancer	15.9	105	541
HMEC	Normal Human Mammary Epithelial	97.3	1526	> 5,000
HRE	Normal Human Renal Epithelial	186	653	> 5,000

Cell numbers were measured before and 3 days after compound treatment by CellTiter Glo proliferation assay. Data are representative of at least three independent experiments. CTCL: Cutaneous T Cell Lymphoma; CML: Chronic Myelogenous Leukemia; AML: Acute Myeloid Leukemia; HL: Hodgkin's Lymphoma.

Exposure to 100nM of panobinostat for 24 hours resulted in increased apoptosis relative to vehicle control as measured by caspase 3/7 activation (Caspase-Glo®). Panobinostat showed increased apoptosis in cancer cell lines in vitro (K562, CML; HH CTCL; HCT116, colon) when compared to non-transformed cells (HMEC, HRE, IMR90, PBMC). The y-axis shows fold induction of caspase activity by panobinostat over vehicle-treated controls (y-axis) in the cell lines (x-axis) in the figure below:

Figure 1. Panobinostat promotes apoptosis in cancer cells

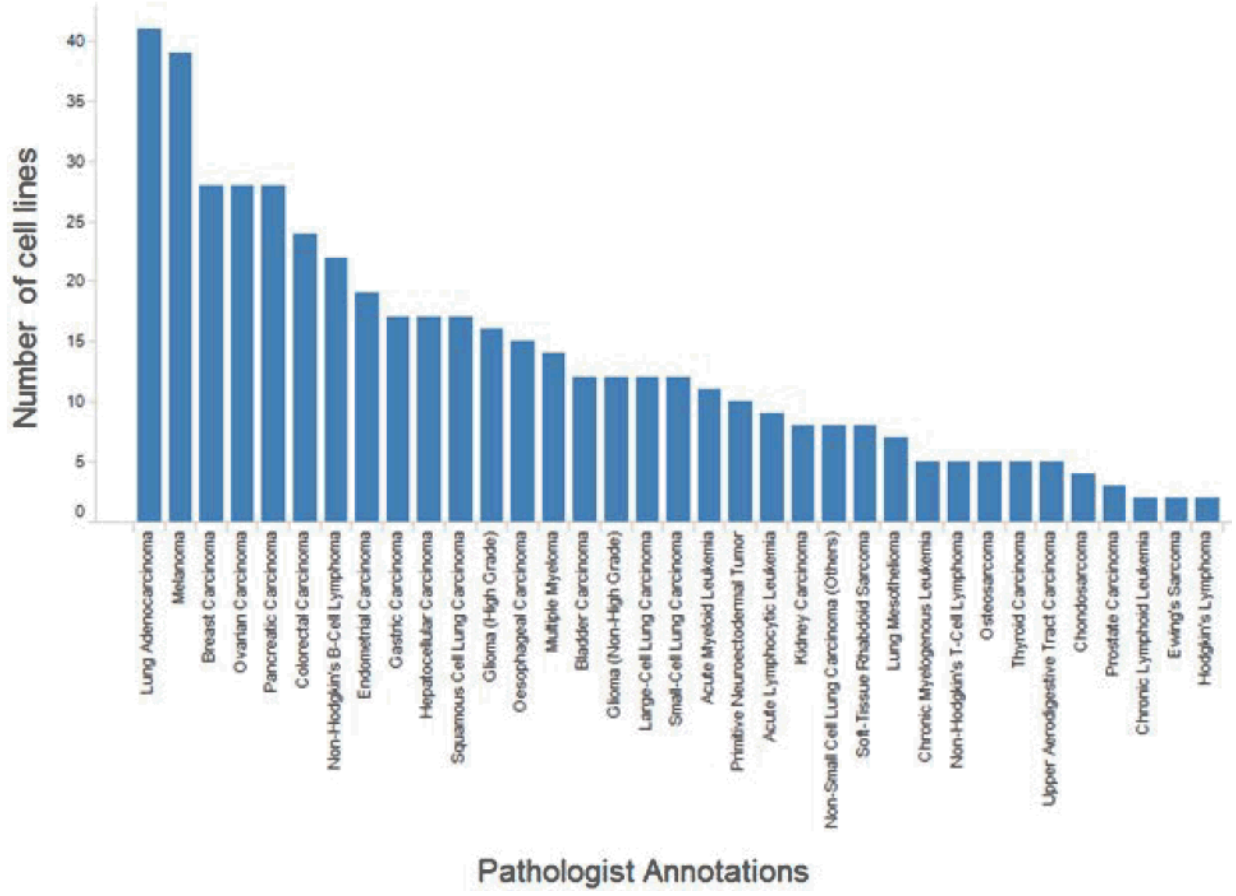


(Excerpted from the submission)

Study title: LBH589 In vitro sensitivity across the cancer cell line encyclopedia panel
Study number: rd-2013-50424
Conducting laboratory: Novartis Pharmaceuticals Corp.
Location: 4.2.1.1

Cell lines were obtained from commercial sources as part of the Novartis Cancer Cell Line Encyclopedia Project. A total of 472 cell lines were assayed. High throughput technology was used where dividing cells were treated with a range of panobinostat concentrations (2.5 nM – 8 μM) for 72 to 84 hours. Cell viability was determined using a luminescence-based measure of ATP (Cell Titer Glo; Promega). Raw values of luminescence were normalized to vehicle controls, and then further manipulated using a proprietary surface pattern model described to remove edge and region effects (presumably from the plate readers). The numbers of cell lines per cancer type is presented in the figure below:

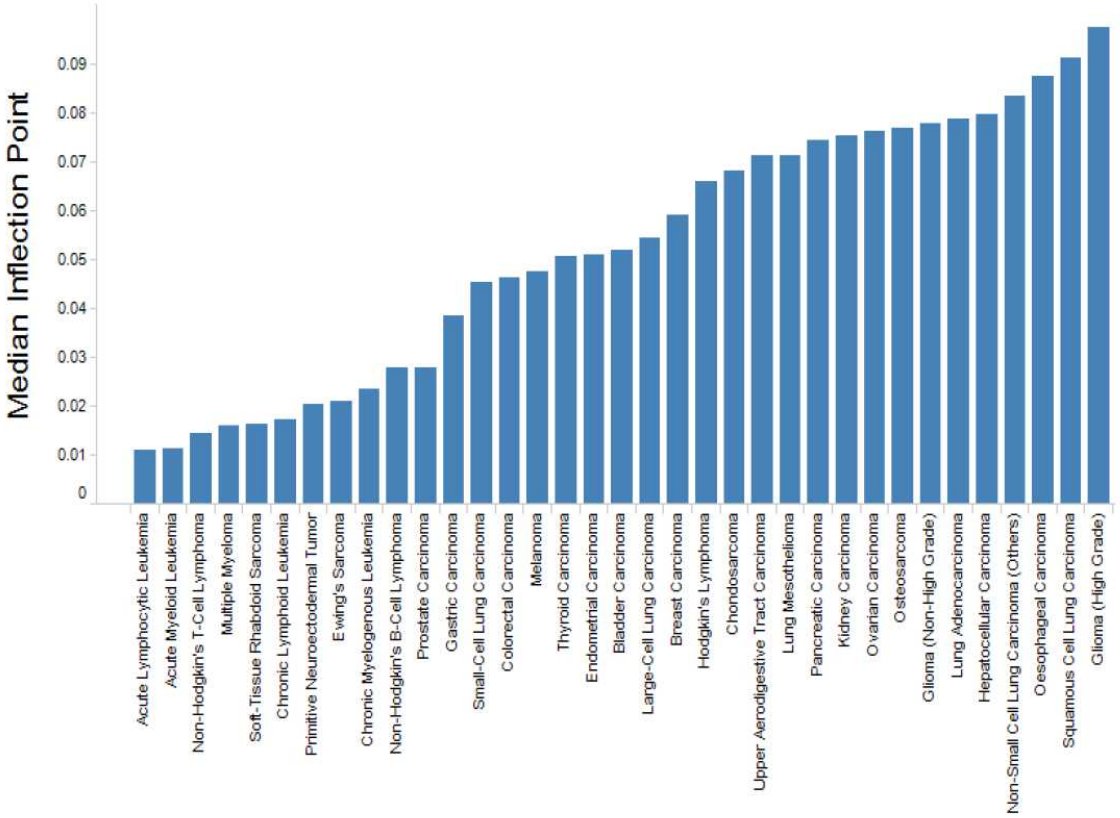
Figure 2. Cancer type distribution in the Novartis Cancer Cell Line Encyclopedia Project



(Excerpted from the submission)

Methods to determine sensitivity to panobinostat across hundreds of cancer cell lines were performed using the same high throughput screening assay as noted above. Dose response data was fitted to a model using NIH/NCGC guidelines and median inflection points (IP) were calculated by pathologist annotations. Potency of drug concentration was depicted by the median inflection point below (i.e. low inflection point, low concentration causing sensitivity in listed cell line). The bar graph shows median inflection points sorted in increasing order. Hematologic cancers, including multiple myeloma (FDR<0.05), showed sensitivity to panobinostat.

Figure 3. In vitro sensitivity in a panel of cancer cell lines

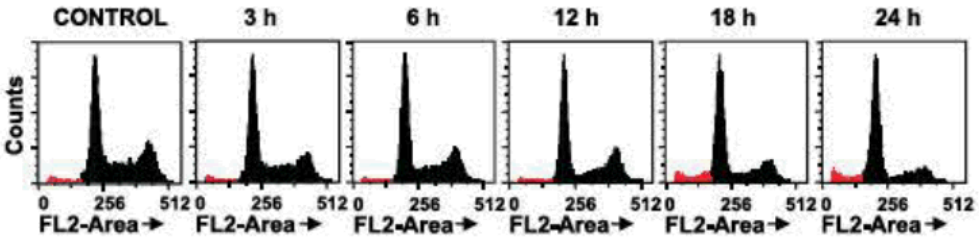


(Excerpted from the submission)

Supporting literature: Maiso et al. 2006 The histone deacetylase inhibitor LBH589 is a potent antimyeloma agent that overcomes drug resistance.
Study number: N/A
Location: 4.3; references, Maiso2006p5781

MM1S human multiple myeloma cells were treated with panobinostat at 100nM for up to 24 hours. Cell cycle changes were determined by measuring propidium iodide staining of cell DNA content by flow cytometry. Following exposure to panobinostat, cells accumulated in G0/G1 phases of the cell cycle with a coordinate drop in S-phase and G2 cell cycle phase populations. The effect on cell cycle correlates with changes in the expression of cell cycle checkpoint genes including: p21, CyclinD1, cdk2 and cdk4 as measured by immunoblotting (100nM Panobinostat, same time points). Panobinostat promotes changes in proliferative potential mediated in part through changes in the expression of cell cycle regulatory genes.

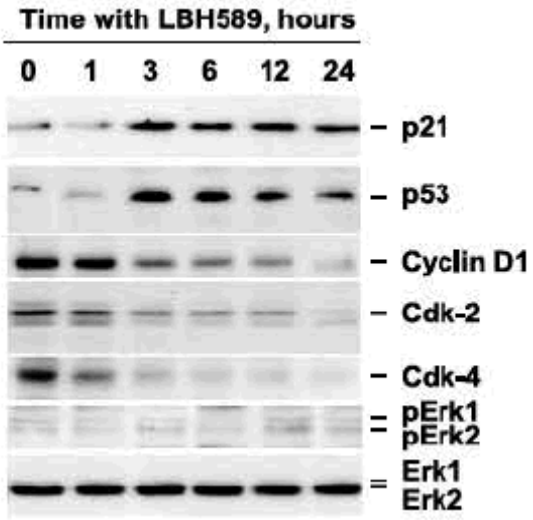
Figure 4. Panobinostat promotes cell cycle arrest in multiple myeloma cells



	CONTROL	3 h	6 h	12 h	18 h	24 h
G_0/G_1	43.0	49.0	49.9	53.9	63.5	72.2
S	38.7	35.7	30.1	20.9	20.5	14.0
G_2/M	18.3	15.3	20.0	25.2	16.0	13.8

(Excerpted from the submission)

Figure 5. Panobinostat induced cell cycle arrest is mediated through gene expression changes

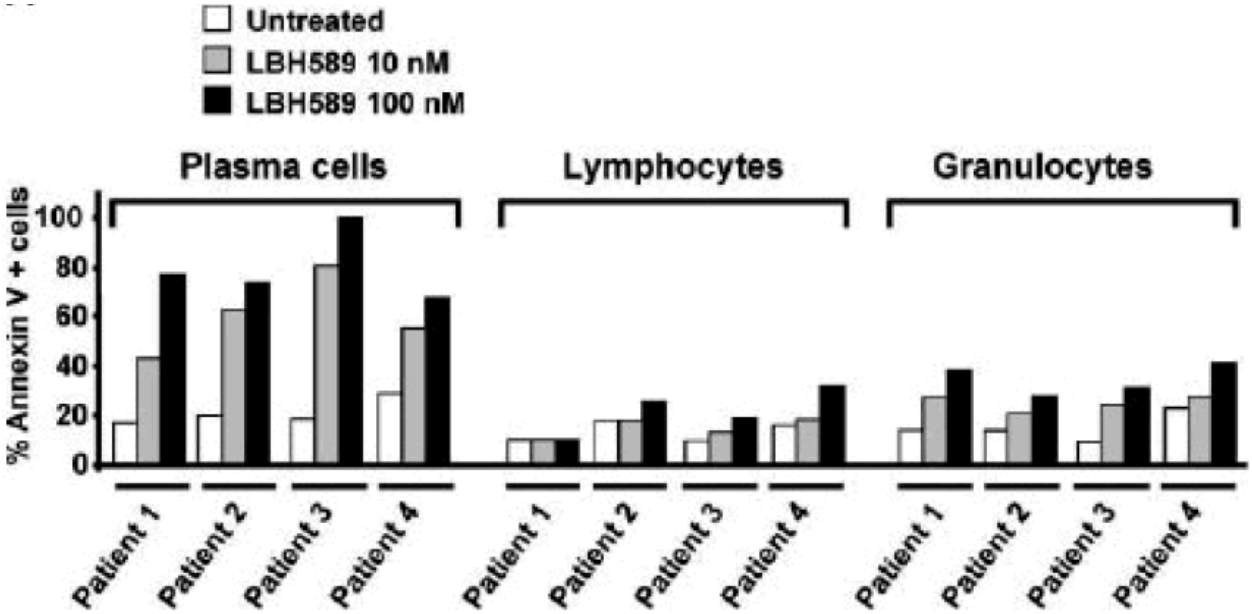


(Excerpted from the submission)

Cell viability was measured following exposure to panobinostat (10nM and 100nM concentrations for 24hours) on 4 patient samples harvested from bone marrow. Two patients were newly diagnosed (patients 3 and 4). Two patients were refractory to multiple lines of treatment (patients 1 and 2; high dose dexamethasone, alkylating agents, doxorubicin, and autologous stem cell transplantation). Annexin V staining of cytosolic phosphatidylserine, was measured by flow cytometry as a marker for loss of cell viability/apoptosis to compare plasma cells, lymphocytes and granulocyte populations. Panobinostat induced dose dependent increase in apoptosis in plasma

cells, but not lymphocytes or granulocytes (untreated compared to 10nM and 100nM concentrations).

Figure 6. Panobinostat promotes apoptosis in human myeloma cells ex vivo

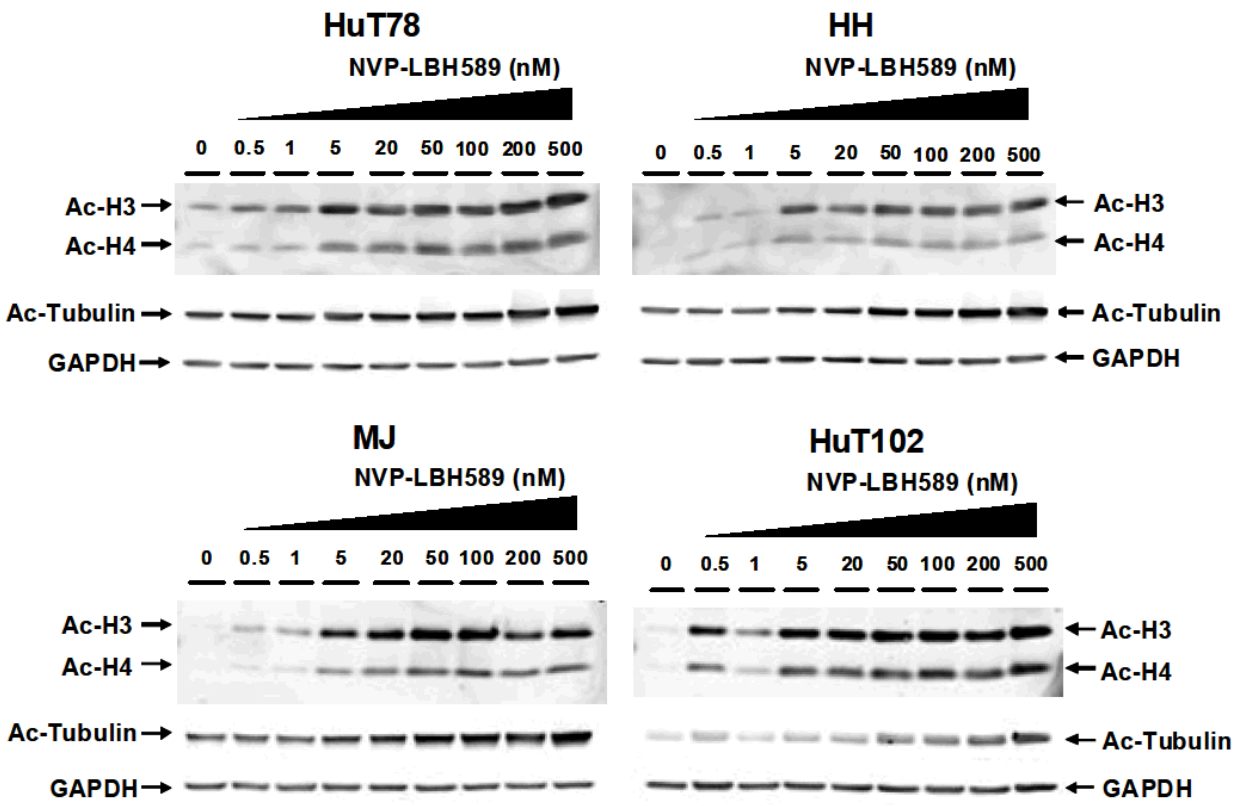


(Excerpted from the submission)

Study title: Histone acetylation as a pharmacodynamic marker for NVP-LBH589
Study number: rd-2010-50113
Conducting laboratory: Novartis Pharmaceuticals Corp.
Location: 4.2.1.1

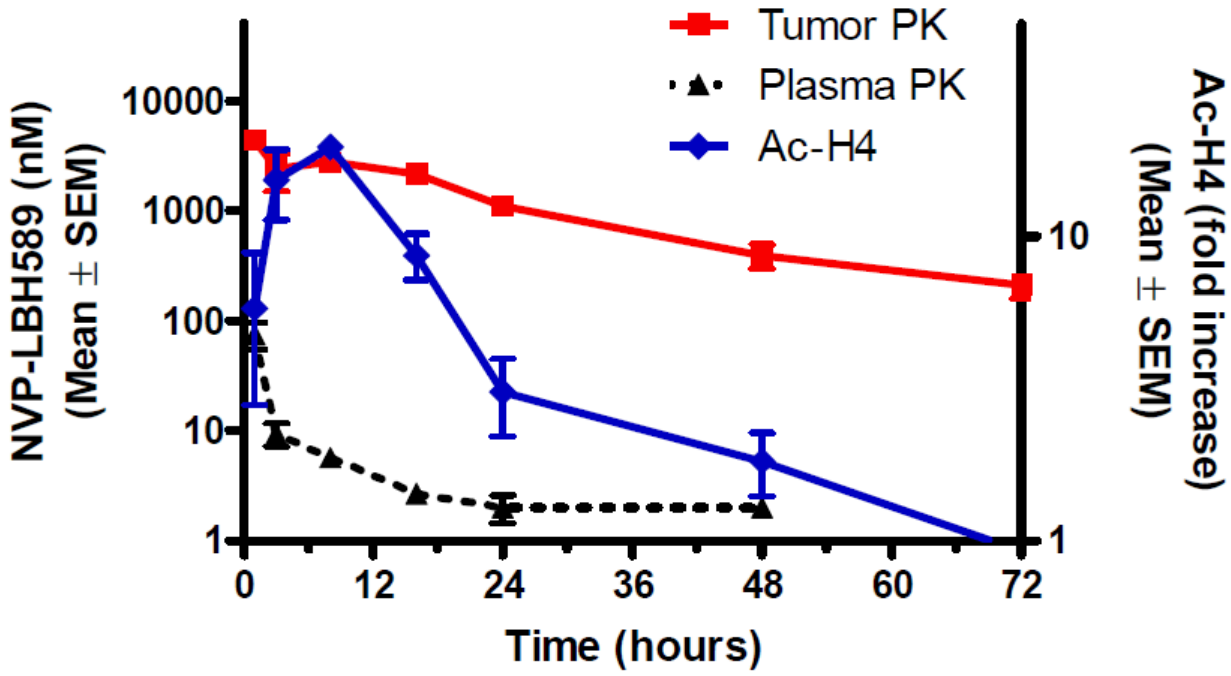
Four human cutaneous T cell lymphoma (CTCL) cell lines were treated with panobinostat (0.5-500nM) for four hours. Changes in histone acetylation (histone H4, Ac-H4; histone H3, Ac-H3) were determined by western blot analysis as compared to a DMSO control. GAPDH was included as a loading control. At increasing concentrations of panobinostat, there was an increase in levels of acetylated histones (Ac-H4, Ac-H3) and acetylated tubulin 4 hours post treatment.

Figure 7. Histone and tubulin acetylation in CTCL cell lines following treatment with panobinostat



Mice bearing HCT116 subcutaneous xenograft tumors were treated with a single i.v. 19.8 mg/kg dose of panobinostat. Plasma and tumor samples were collected at 1, 3, 8, 16, 24, 48 and 72 hours following dosing (n=3 animals/time point). Acetylated histone H4 levels (Ac-H4) were determined by western blot analysis and band intensity was quantified using the ODYSSEY Infrared Imaging System (Li-Cor). Band intensity was normalized to GAPDH, and was displayed relative to vehicle treated controls. Levels of Ac-H4 were correlated to exposure to panobinostat in both the plasma and the tumor (AUC 0-72). Panobinostat rapidly cleared from the plasma and were undetected 48hours post-treatment (systemic AUC 0-72hr = 371nM). Tumor levels remained elevated suggesting elevated tissue distribution following i.v. treatment (tumor AUC0-72hrs= 82,831nM). Ac-H4 in dissected xenografts from mice increased following a single 19.8mg/kg dose i.v. with max levels reached 8 hours post treatment and almost undetectable at 72 hours. Exposure to a single dose of panobinostat resulted in tumor distribution and changes in levels of acetylated histones in the sampled tumor lysate.

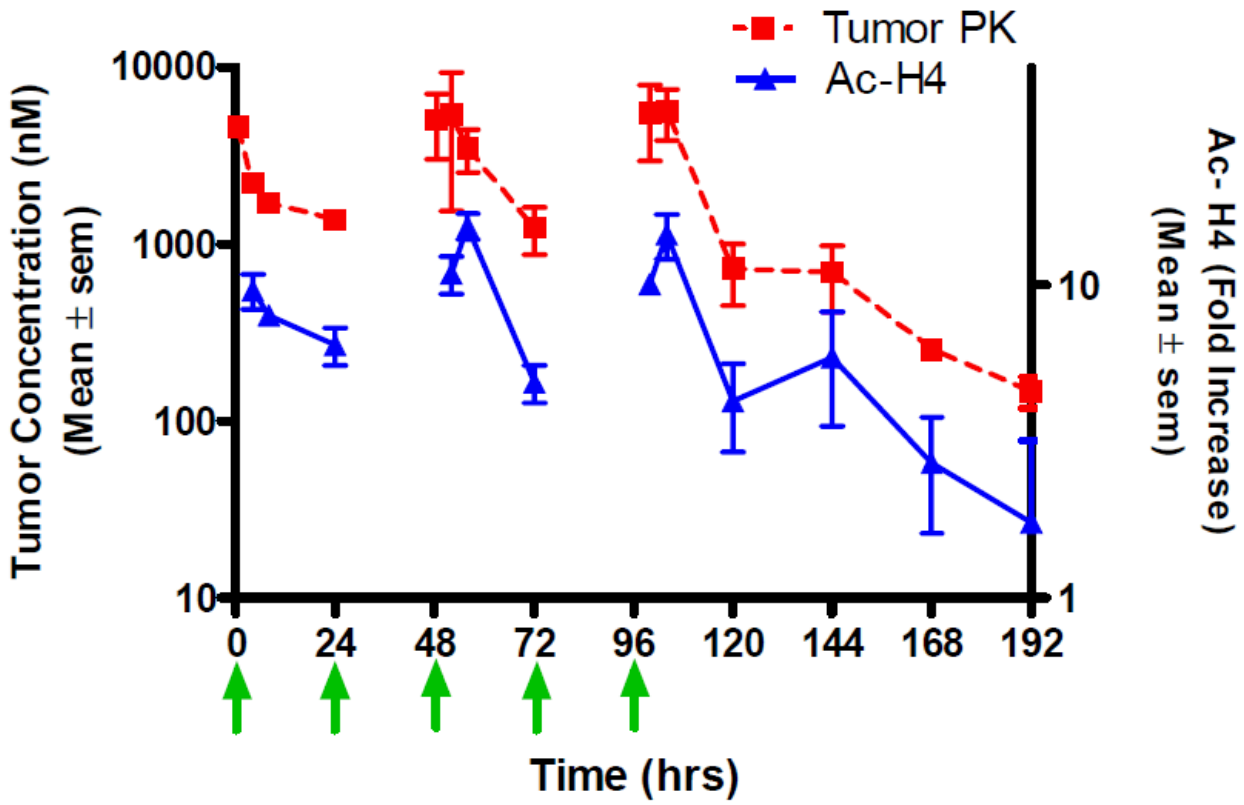
Figure 8. PK/PD for single dose panobinostat and Ac-H4



(Excerpted from the submission)

In a separate experiment, mice bearing HCT116 subcutaneous xenograft tumors were treated with i.v. 11.9mg/kg panobinostat once daily for 5 consecutive days. Plasma samples were collected and tissue was dissected from tumors after dosing on days 1 and 3 at 0.5, 4, 8 and 24 hours post-dose for analysis of panobinostat concentration and levels of Ac-H4 (n=3 animals/time point). Ac-H4 levels were determined by Western blot analysis and band intensity was quantified using the ODYSSEY Infrared Imaging System (Li-Cor). Band intensity was normalized to GAPDH, and was displayed relative to vehicle treated controls. Tumor levels of acetylated histone H4 reached peak levels 4-8 hours after dosing on days 1, 3, 5 and reached levels comparable to vehicle 96hrs following the last dose. Repeat exposure to panobinostat correlates with changes in levels of acetylated histones from HCT116 (colon cancer cell) tumors over time.

Figure 9. Repeat dose PK/PD of panobinostat and Ac-H4

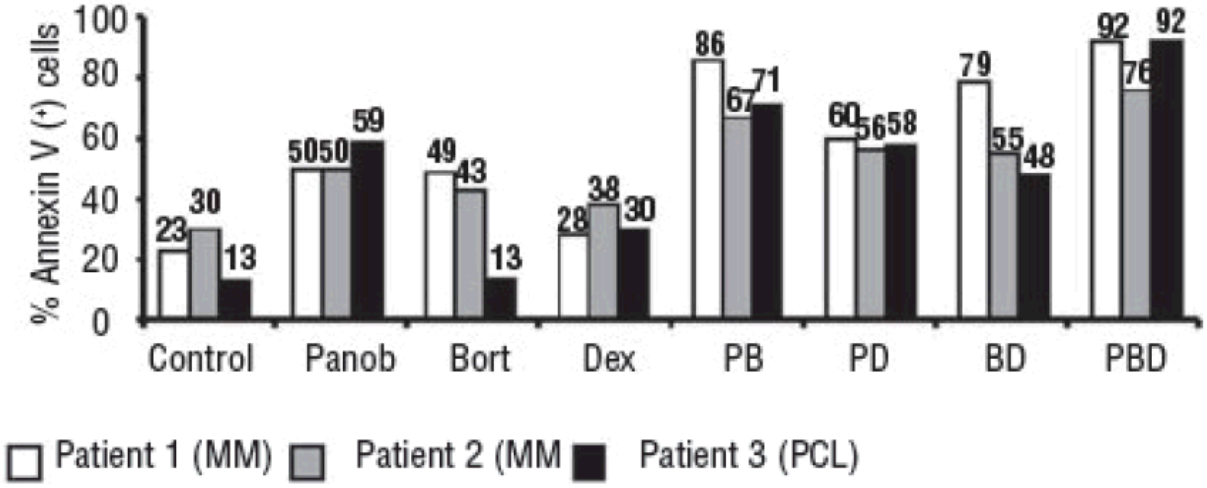


(Excerpted from the submission)

Supporting literature: Ocio et al. 2010. In vitro and in vivo rationale for the triple combination of panobinostat (LBH589) and dexamethasone with either bortezomib or lenalidomide in multiple myeloma.
Study number: N/A
Location: 4.3; references, Ocio2010p794

Plasma cells were isolated from three multiple myeloma patients including one patient with highly resistant plasma cell leukemia (patient 3) and treated with panobinostat (P, 20nM), bortezomib (B, 5nM), dexamethasone (D, 40nM) or a combination for 24 hours. Loss of cell viability/apoptosis was measured using Annexin V positivity by flow cytometry. Within the three patients there is noticeable variability in response to treatment combinations, however all three drugs (PBD) combined results in the largest population of Annexin V/apoptosis positive cells following treatment relative to vehicle controls.

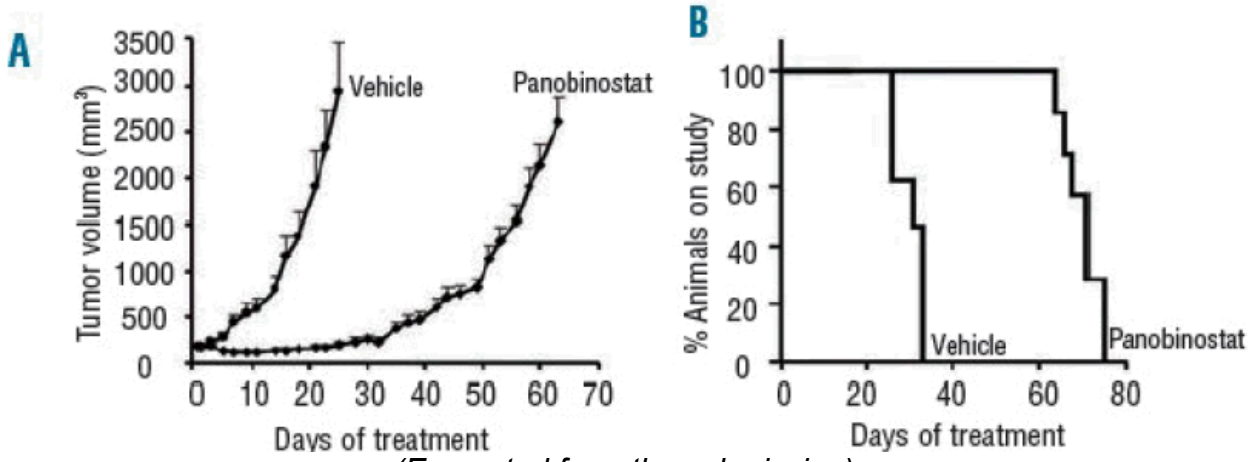
Figure 10. Combination treatment of panobinostat, bortezomib and dexamethasone



(Excerpted from the submission)

Treatment of panobinostat on a subcutaneous human plasmacytoma model was examined using CB-17 NOD-SCID mice injected subcutaneously with 3e6 human multiple myeloma cells (MM1.S) into the right flank. Following development of palpable tumors, mouse were randomized to vehicle control (10% HPBCD in water) or panobinostat (10 mg/kg 5 days/week, 5mg/kg subsequent days, for 21 days). Panobinostat delayed tumor growth (A) and promoted survival as measured by time to endpoint (TTE, median 30 versus 70 days). The TTE period captured initiation of treatment until euthanasia due to disease progression.

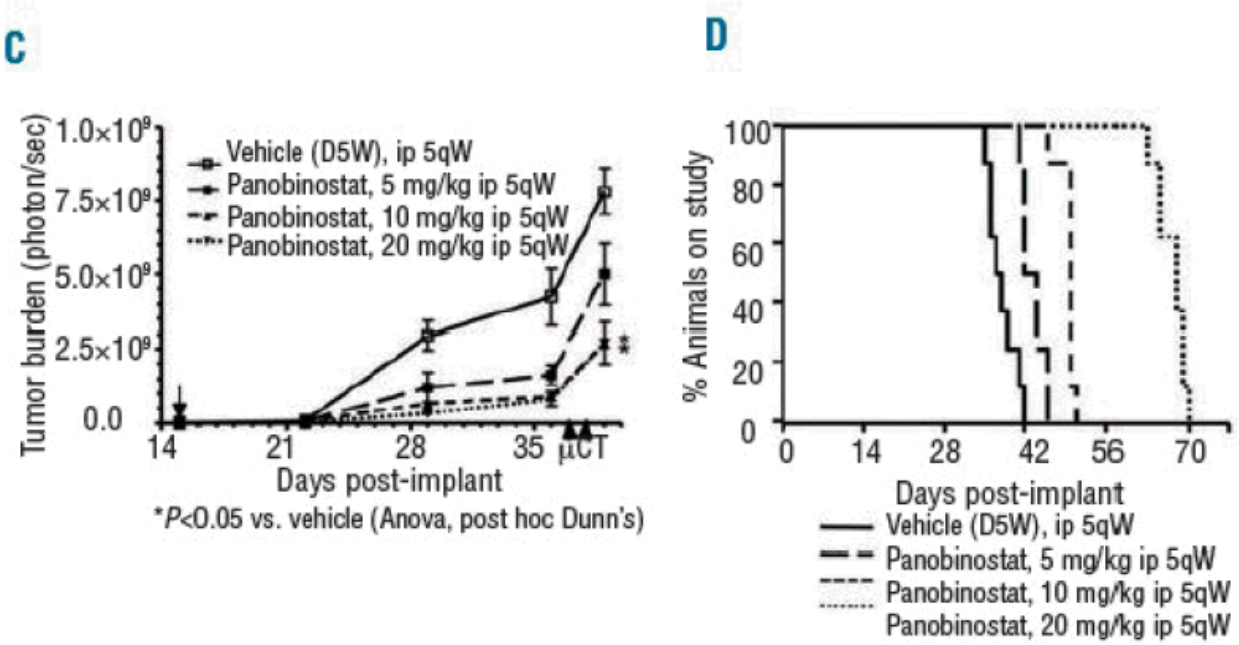
Figure 11. Panobinostat activity in a human xenograft model of subcutaneous plasmacytoma (NOD-SCID)



(Excerpted from the submission)

Treatment of panobinostat on a model of human disseminated myeloma was examined using SCID-beige mice injected i.v. (tail vein) with 2e6 luciferase labeled MM1.S cells. Mice were treated with vehicle control or 5, 10, or 20mg/kg Panobinostat 5qW. Tumor burden was quantified using bioluminescence (photon/sec) of the luciferase labeled MM1.S cells and assessed using clinical endpoints (hand-limb paralysis, spinal curvature). Panobinostat delayed tumor burden at 10 and 20mg/kg doses and increased TTE (~35days vehicle, 50days at 10mg/kg, 65days at 20mg/kg) significantly compared to vehicle control (Number of animals not provided). These effects on the xenograft model correlated to increased levels of acetylated histone H4 and cleavage of caspase 3.

Figure 12. Panobinostat activity in a mouse model of disseminated human myeloma

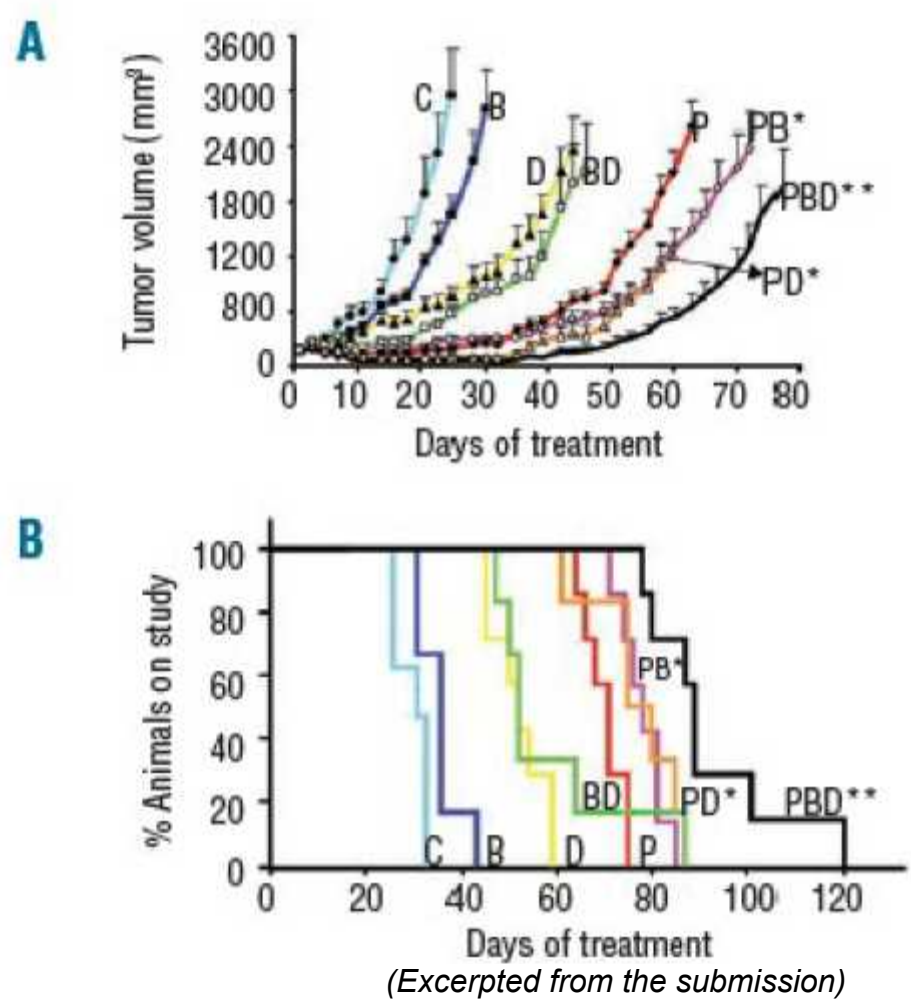


(Excerpted from the submission)

The combination treatment with panobinostat, bortezomib and dexamethasone was evaluated in the subcutaneous human plasmacytoma mouse model. Potential for synergy was examined using suboptimal dosing (0.1mg/kg i.p., 5 days per week bortezomib; 1mg/kg i.p., 5 days per week dexamethasone) and optimal dosing for Panobinostat (10mg/kg i.p. 5 days per week). Panobinostat was lowered to 5mg/kg i.p. 5 days per week after day 21 due to robust reduction in tumor growth. Panobinostat treatment in combination with either bortezomib or dexamethasone significantly reduced tumor volume. The combination of all three (panobinostat, dexamethasone and bortezomib) produced the largest difference in tumor burden compared to the vehicle control and was statistically significant when compared with control or either dual treatment with panobinostat ($p<0.05$). Kaplan Meier analysis was used to determine survival showing a statistically significant survival advantage in mice given panobinostat

combined with either bortezomib (PB) or dexamethasone (PD), or panobinostat with bortezomib and dexamethasone (PBD, triple combination) compared to the vehicle control. The triple combination PBD also showed statistically significant increase in survival time compared to either PB or PD supporting the prosed triple combination.

Figure 13. Activity of panobinostat in combination treatments on human xenograft model of subcutaneous plasmacytoma (NOD-SCID)

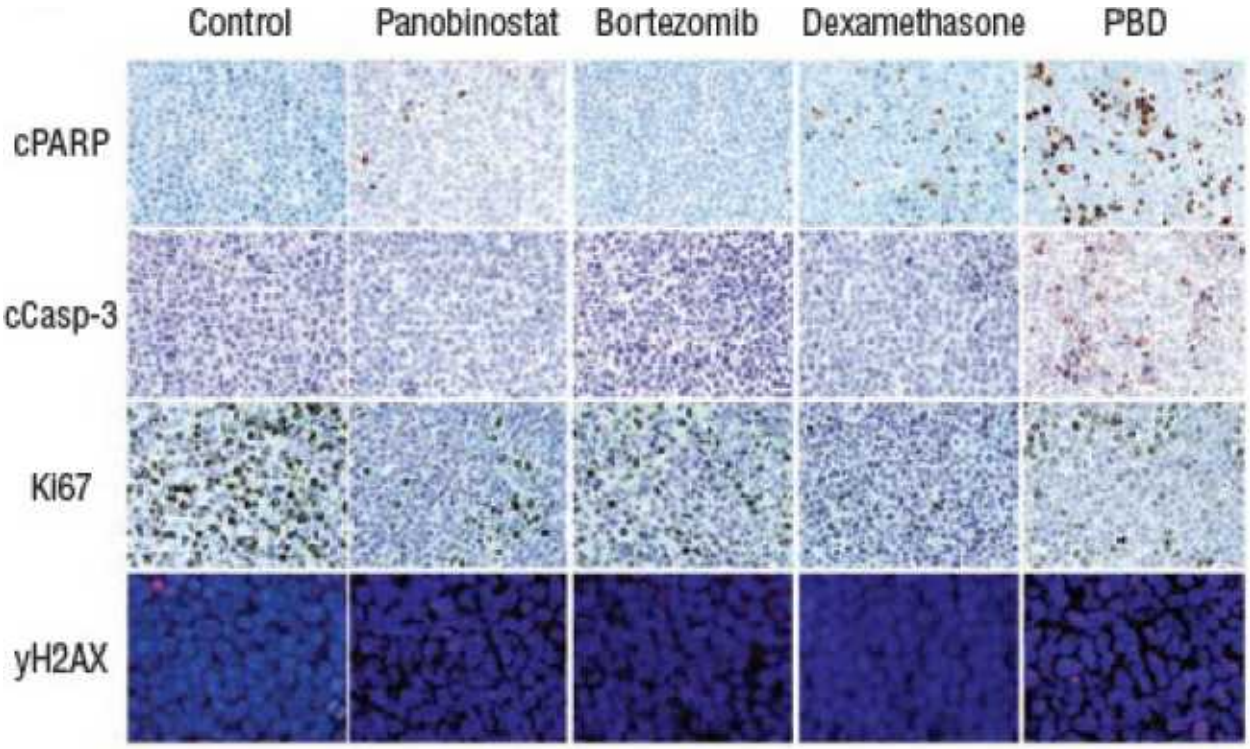


(Excerpted from the submission)

Immunohistochemistry was used to show the effect of the triple combination, PBD on markers of tumor proliferation and apoptosis in the human xenograft model of subcutaneous plasmacytoma (NOD-SCID). Tumor tissue was dissected at endpoints following treatment with the triple combination and individual agents. Proliferation was assessed by staining with Ki67 and apoptosis by staining with cleaved caspase-3 and cleavage of PARP. Results showed that in comparison to the control, tumor tissue from the triple combination showed decreased Ki67 and increased cleaved caspase-3 and cleavage of PARP. Immunofluorescence images show phospho-H2AX, a gene that encodes for histone 2A. The brighter fluorescence in the PBD combination indicates

gene activation most likely due to the inhibition of histone deacetylase activity (acetylation) of the H2AX gene.

Figure 14. Immunohistochemical analysis of single agent and triple agent treatments from human xenograft model of subcutaneous plasmacytoma (NOD-SCID)



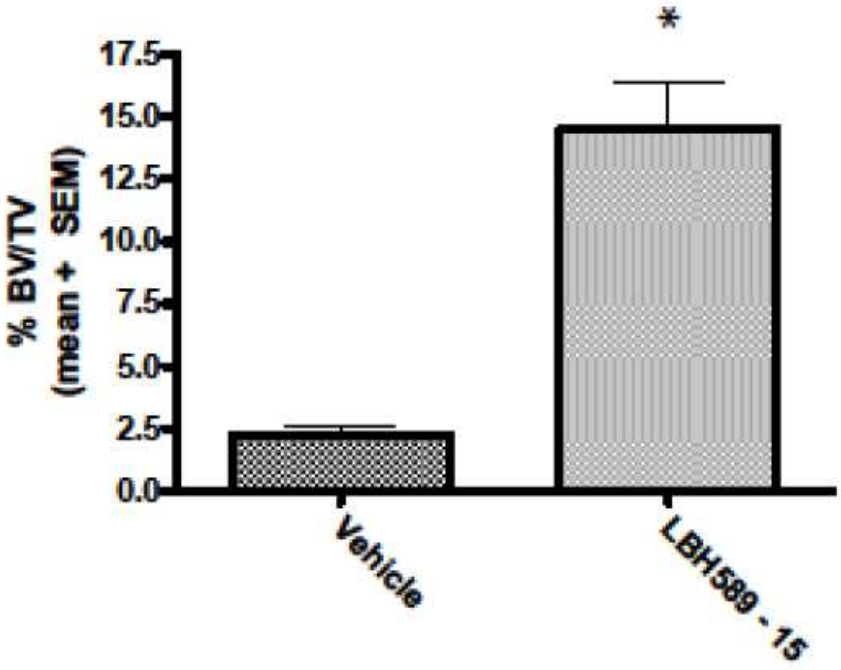
(Excerpted from the submission)

4.2 Secondary Pharmacology

Study title: In vivo activity of NVP-LBH589 and bortezomib in the MM1.S-luciferase human systemic multiple myeloma xenograft model
Study number: RD-2008-51313
Location: 4.2.1.2

In the disseminated human myeloma mouse model, bone disease was monitored in mice treated with panobinostat (15mg/kg i.p. 5 times a week for 28 days) by monitoring luciferase expressing MM1 cells disseminated to the bone. Trabecular bone density (%BV/TV) in the proximal tibia was examined using a high resolution MicroCT scanner following onset of clinical symptoms of bone damage in vehicle treated animals. Panobinostat treatment correlated with decreased trabecular bone damage (%BV/TV) when compared to vehicle treated animals.

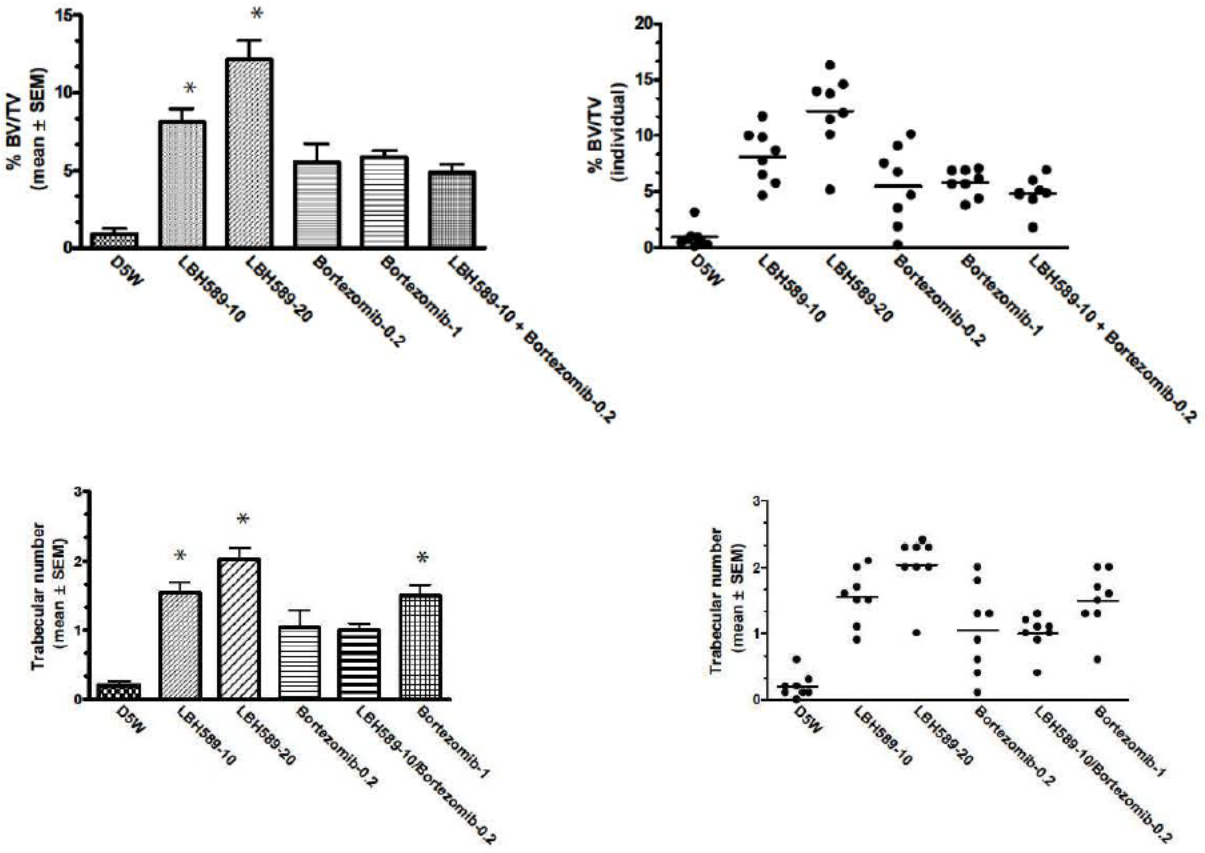
Figure 15. Panobinostat activity on bone loss in mouse model of disseminated human myeloma



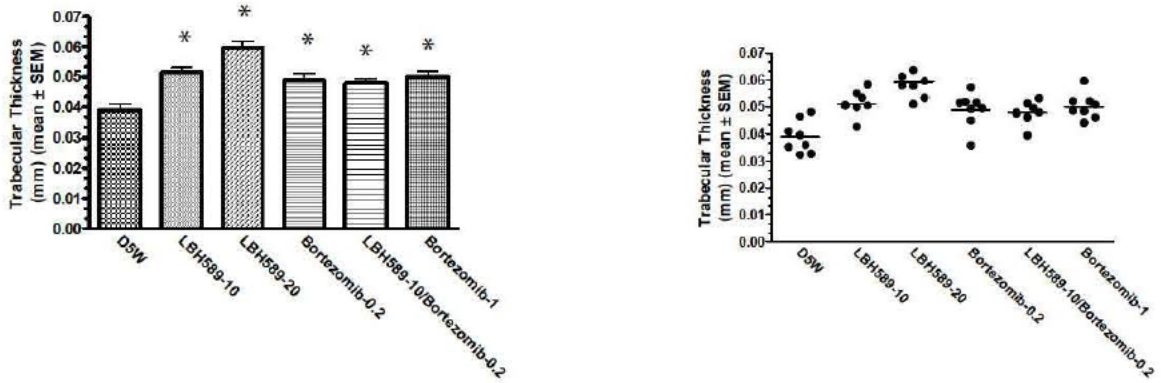
(Excerpted from the submission)

The effect of panobinostat on decreased bone damage was determined to be dose dependent, as assessed by micro-CT, and statistically significant compared to control at 10 and 20 mg/kg i.p. 5 times a week f as measured by bone density, trabecular number and trabecular thickness). Shown below is trabecular bone damage (%BV/TV) with increasing doses of panobinostat (mg/kg) with or without bortezomib (mg/kg).

Figure 16. Panobinostat activity on bone loss in mouse model of disseminated human myeloma



C. Ex. 0942 Trabecular thickness



(Excerpted from the submission)

4.3 Safety Pharmacology

Neurological effects (GLP)
The effects of panobinostat on the central nervous system were determined in study (b) (4) r0280108 by examining three groups of 10 male mice treated i.v. with a single dose of either vehicle or 30 mg/kg (10mL/kg), 60 mg/kg, or 100 mg/kg drug (20mL/kg). The vehicle was 0.07N lactic acid in 5% dextrose. Neurological and toxicology endpoints included parameters of behavior (24 hour observations), temperature (60 minutes following dosing) and gross necropsy (following 24 hour observation).

Behavior assessments consisted of fixed environment (non-home cage), open-field observations at 15, 30, and 45 minutes, and 1, 2, 3, 4 and 24 hours following dosing. Included in the clinical observations were symptoms of seizures or convulsions, awareness reaction, startle response, vocalization, irritability, decreased abdominal tone, increased secretion, tremors, grip strength and mobility, abnormal posture, piloerection, ataxia, stereotypy, excretion, pupil size, impaired righting reflex, pain response, pinnal reflex, corneal reflex, motor activity and decreased respiration. Pathology was examined by gross necropsy.

Results
- There were no adverse findings attributed to vehicle or the 30 mg/kg dose.
- At 60mg/kg all animals displayed immediate decreased motor activity, wobbly gait and convulsions. Nine of 10 animals still showed decreased motor activity at 15 minutes and these symptoms had completely subsided at 30 minutes following dosing. The remaining animal died.
- At 100mg/kg all animals showed immediate decreased motor activity, wobbly gait and convulsions. Five of these 10 animals showed decreased motor activity and decreased grip strength from 15-45 minutes, decreased motor activity at 1 hour and symptoms subsided by 2 hours following dosing. The remaining 5 animals died.
- Gross necropsy findings were normal for all animals in all dose categories following 24 hour observations.

Respiratory (GLP):
The pharmacological effects of panobinostat on the respiratory system were determined in study (b) (4) r0280118 by examining Wistar rats treated i.v. with a single dose of 1 mg/kg, 3 mg/kg, or 10 mg/kg drug or vehicle (2mL/kg). The vehicle was 0.07N lactic acid in 5% dextrose. Measures of respiratory function (tidal volume, respiratory rate and derived minute volume) were captured using plethysmographs over 15 minute periods pre-dose, and at 1, 2 and 6 hours following dosing.

Results:
- No adverse effects in treatment groups or control; all respiratory measures were within limits for healthy animals at doses up to 10 mg/kg, i.v.

Cardiovascular (GLP):
hERG patch-clamp HEK293 cells:
The effect of panobinostat (LBH589) (1, 3, 10 and 30 µM) and a human metabolite, BJB432 (at <1% of the total drug-related material in plasma following a 20 mg dose), on in vitro hERG current was evaluated in study (b) (4)r0870294 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 using a pulse pattern with a voltage step from -80 mV to a test potential of +20 mV repeated at 5 second intervals, designed to roughly mimic a cardiac action potential. Concentrations of 1, 3, 10 and 30 µ panobinostat and 0.3,1,3 and 10 µM BJB432 were chosen to determine concentration response. Peak current was measured during test ramp and until steady state was achieved.

Results

- The IC50 for inhibition due to panobinostat was 3.5 µM (Hill coefficient= 1.2), and for BJB432 was 1.6 µM (Hill coefficient=1).
- The positive control, terfenadine, inhibited hERG potassium current by 83.1 ± 2.8%.

Telemetry study in Dogs/Beagle for oral administration (GLP):
The effect of panobinostat on cardiovascular parameters were assessed in telemetered dogs (n=4 males) in study (b) (4)0680202. Dogs were given 3 doses of 1.5 mg/kg panobinostat orally (capsule) or vehicle control, on Monday, Wednesday, Friday schedule. The vehicle was sterile water for injection, USP. Cardiovascular parameters included arterial blood pressures, pulse pressure, heart rate and electrocardiogram measurements prior to dosing and following dosing at 30 minutes, 1, 2, 3, 4,5 ,6, 7, 8, 9, 10 ,11 hours. Parameters at different timepoints were compared to the animal's own baseline values.

Results

- There were no significant clinical signs.
- QTc prolongation was observed up to 25 msec in panobinostat treated Beagle dogs.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Pharmacokinetics of ^{14}C Panobinostat after single oral or intravenous administration in the rat

Key study findings:

- Panobinostat was rapidly eliminated in the blood following intravenous dosing (half-life = 4h); half-life was not determined for the oral route.
- Hepatic elimination: 80% of radioactivity was detected in feces 96 h after oral or IV dosing; in bile duct cannulated rats, 60% of radioactivity was detected in the bile. However, the amount of the parent drug, panobinostat, in the bile or feces was below the limit of quantification, suggesting high metabolism.
- Absorption of radioactivity was rapid following oral dosing (t_{max} 0.5 h).
- C_{max} values for radioactivity following oral or IV dosing were comparable in the blood and in the plasma.
- $AUC_{(0\text{-}96h)}$ values for radioactivity following an oral or IV dosing were slightly higher in blood compared to plasma.
- Absorption after oral administration was 15% (total radioactivity after oral administration/ total radioactivity after IV administration)
- Bioavailability was 6% (exposure to parent compound/ total radioactivity)

Study no.:	0201550
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	23 April 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{14}C]panobinostat, 0211-058-22, 96%

Methods:	Radioactivity detected by liquid scintillation counting (LOQ=3.4 ng/ml)
Species/strain:	HanWistar rats
N:	3 males/group
Dose:	10mg/kg
Frequency:	single dose
Route:	oral gavage (1mg/ml) or intravenous (5 mg/ml bolus)
Volume:	10 mL/kg (oral) or 2ml/kg (intravenous)
Observations and times:	Oral: Samples collected from jugular cannula at 0, 5, 15, 30 minutes and 1, 2.5, 5, 8, 24 and 48 hr post dose; Intravenous: Samples collected at 0, 5, 15, 30 minutes and 1, 2, 4, 8, 12, 24, 72, and 96 h post dose.

Results:

Table 3. Pharmacokinetic parameters and elimination following 10mg/kg dose of ^{14}C panobinostat in rats

Species/strain/sex	Rat/Han Wistar/Male	
Route/formulation:	Intravenous solution 30 mM lactic acid in aqueous 5% dextrose	Oral solution 30 mM lactic acid
Dose (mg/kg):	10	10
Number of animals:	3	3
Specific activity of [^{14}C](µCi/mg):	20.6	20.6
Plasma LBH589:	I.V.	Oral
C_{max} (ng/mL):	1016*	BLQ
t_{max} (h):	0.083	ND
$AUC_{0\text{-}inf}$ (ng•h/mL):	459 ± 70	ND
Half-life (h)	3.81 ± 1.39	ND
CL (L/h/kg):	22.1 ± 3.49	ND
V_{ss} (L/kg):	40.2 ± 16	ND
Blood [^{14}C]radioactivity		
C_{max} (ngEq/mL):	3340*	108 ± 33.1
t_{max} (h):	0.083	0.5
$AUC_{0\text{-}96\ h}$ (ngEq•h/mL):	8029	1216
Plasma [^{14}C]radioactivity		
C_{max} (ngEq/mL):	3220*	92.6 ± 13.2
t_{max} (h):	NA	ND
$AUC_{0\text{-}96\ h}$ (ngEq•h/mL):	6120	1042
Excretion in urine (%dose): radioactivity		
0-24 h:	10.3 ± 3.7	0.66 ± 0.22
0-96 h:	12.5 ± 4.5	0.73 ± 0.28
Excretion in feces (% dose): radioactivity		
0-24 h:	69.2 ± 8.7	76.2 ± 6.7
0-96 h:	80.9 ± 3.7	83.4 ± 3.2
Cagewash (% dose)	1.4 ± 1	0.43 ± 0.66
Total radioactivity recovery (% dose):	94.7 ± 2.8	84.6 ± 2.9
Absorption (% dose): % Bioavailability		15-17 ~6**
Radioactivity recovery in bile duct cannulated rats following iv dosing (% dose):	Bile Urine Feces	61.7 ± 22.3 31.4 ± 21.4 9.9 ± 2.0
LBH589 recovery in bile duct cannulated rats following iv dosing (% dose):	Bile Urine Feces	BLQ 5.26 BLQ

* n=1, NA = not applicable, ND = not determined, BLQ = below limit of quantification, **estimate is based on low levels of urinary excretion

PK parameters for the parent drug were not calculated following oral dosing due to difficulty in detection of parent compound.

(Excerpted from the submission)

Pharmacokinetics of ^{14}C panobinostat after single oral or intravenous administration in the dog

Key study findings:

- Absorption of panobinostat was rapid (oral t_{max}= 15 min).
- Panobinostat had a long plasma half-life (half-life = 16 h) when dosed IV.
- Volume of distribution was >30L/kg indicating tissue distribution.
- Oral bioavailability was approximately 50%.
- Absorption, based on a comparison of radioactivity AUC for oral vs. intravenous administration was 70%.
- Excretion was mainly fecal, with ≥ 50% of the radioactivity recovered in the feces; approximately 30% was recovered in the urine.

Study no.:	R0300092
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 13 December 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not mentioned

Methods:

Radioactivity:	Detected by liquid scintillation counting (LOQ=1.0-2.5 ng/ml)
Species/strain:	Male beagle dogs
N:	2/ intravenous group, 3/oral group
Dose:	0.5mg/kg intravenous, 1.5mg/kg oral
Frequency:	single dose
Route:	oral gavage (0.5 mg/ml) or intravenous (0.5 mg/ml bolus)
Volume:	3 mL/kg (oral) or 1 ml/kg (intravenous)
Observations and times:	Intravenous: Samples collected at 0, 5, 15, 30 minutes and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and148 h post dose. Oral: same without 0 or 5min samples taken.

Results:

Table 4. Pharmacokinetic parameters and elimination of ^{14}C panobinostat following oral or intravenous dose in dogs

Animal species/strain/gender:		Male Beagle Dog	
Animal number:		2	3
Dose (mg/kg) as free base equivalent:		0.5	1.5
Route and formulation:		Intravenous/bolus Solution in 30 mM lactic acid in an aqueous 5% dextrose solution	Oral/gavage Solution in 30 mM lactic acid
Specific activity of [^{14}C]LBH589 (μCi/mg):		50	16
Samples collected:		Serial blood up to 168 hours, complete urine and feces in 24 h intervals up to 96 h post-dose and as a 96-168 h collection. Cage wash at termination.	
Samples analyzed:		All samples were assayed for radioactivity. Plasma samples were analyzed for LBH589 by LC/MS/MS. Serial pooled plasma samples and excreta samples were assayed for LBH589, metabolite profiles and structural characterization by LC/MS	
Plasma LBH589:			
C_{max} (ng/mL):		76.7	95.2
t_{max} (h):		--	0.25
$AUC_{0-\infty}$ (ng•h/mL):		155	244
CL (L/h/kg):		3.3	--
V_{ss} (L/kg):		42	--
Plasma $t_{1/2}$ (h):		16	--
Plasma [^{14}C]radioactivity:			
C_{max} (ngEg/mL):		161	270
t_{max} (h):		0.083	1
AUC_{0-last} (ngEg•h/mL):		2610	5310
Terminal $t_{1/2}$ (h):		140	--
Excretion in urine (% dose):			
Radioactivity	**0-24 h:**	27.1	30.4
	0-168 h:	32.8	33.7
LBH589	**0-48 h:**	1.16	0.43
Excretion in feces (% dose):			
Radioactivity	**0-24 h:**	29.6	48.9
	0-168 h:	49.1	58
LBH589	**0-48 h:**	1.73	1.89
Cage wash (% dose)		2.36	1.3
Total radioactivity recovery (% dose)		84.3	93
Absorption (%)			**70-100%**
Bioavailability (%)			**~50%**

(Excerpted from the submission)

Distribution

Distribution of ^{14}C panobinostat after oral administration in male and female rats by quantitative whole body radiography

Key study findings:

- Potential for drug-related materials to bind to melanin containing tissues.
  - Tissue half-life for radioactivity was longest in the skin ($t_{1/2}$= 124-601 h) and uveal tract (140 h). In addition, the highest AUC of radioactivity was observed in the uveal tract.
  - Radioactivity (panobinostat and/or its metabolites) was detected at 168 hours postdose in the skin and uveal tract of pigmented animals and not in controls (HansWistar).
- In addition to the uveal tract, exposure based on Cmax and/ or AUC was high in the bile, colon, small intestine and liver, consistent with hepatobiliary route of excretion.
- Relatively high amount of radioactivity in the pituitary gland indicates that drug-related materials can cross the blood-brain barrier.

Study no.:	R0500724
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 29 September 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[14C]panobinostat, Lot # 0263-048, > 97%

Methods:
Tissue levels of [^{14}C] radioactivity detected by whole body radiography (LOQ ranged from 19.5 to 64.2 ngEq/g).

Species/strain:	Han Wistar (n=2) and Long Evans (n=16) pigmented rats
N:	18 total; n=8/sex Long Evans; n=1/sex HanWistar
Dose:	25 mg/kg
Frequency:	single dose
Route:	oral gavage
Volume:	5 ml/kg
Observations and times:	0.5, 1, 2, 4, 8, 24, 48 and 168 h post-dose for Long Evans; 168h for HanWistar

Results:

Table 5. Pharmacokinetic parameters of tissue radioactivity following single oral dose of panobinostat in male Long Evans rats

Tissue	t_{max} (h)	C_{max} (µgEq/g)	C_{max} (µM)	AUC_∞ (µgEq•h/mL)	t_{last} (h)	$t_{1/2}$ (h)	Ratio[a] $C_{max,\ tissue}$	Ratio[a] AUC_∞
Adrenal cortex	1	1.40	4.01	29.2	48	10.6	2.0	9.3
Adrenal medulla	8	1.68	4.81	40.0	48	11.9	2.5	13
Bile	0.5	131	375	286	48	13.3	192	91
Blood	0.5	0.683	1.96	3.15	48	14	1.0	1.0
Bone marrow	0.5	0.66	1.89	7.84	24	8.8	1.0	2.5
Bone mineral	24	0.0759	0.217	0.67[b]	24	N.D.	0.11	N.D.
Brain	4	0.0113	0.03	0.01[b]	4	N.D.	0.02	N.D.
Brown fat	0.5	0.838	2.40	11.6	48	10.7	1.2	3.7
Colon wall	8	23.1	66.2	335	48	6.2	34	106
Epididymis	0.5	0.275	0.788	65.7	24	271	0.40	21
Esophagus	0.5	65.2	187	76.7	48	7.6	95	24
Harderian gland	0.5	1.7	4.87	60.7	168	45.6	2.5	19
Heart	1	0.943	2.70	8.04	24	6.9	1.4	2.6
Kidney cortex	0.5	2.58	7.39	22.6	48	10.7	3.8	7.2
Kidney medulla	0.5	2.51	7.19	39.2	168	35.3	3.7	12
Kidney pelvis	0.5	3.04	8.71	33.2	24	11.6	4.5	11
Lachrymal gland	0.5	0.305	0.874	11.2	24	41.2	0.45	3.6
Liver	0.5	8.97	25.7	96.1	168	32.7	13	31
Lung	0.5	0.811	2.32	13.0	24	9.6	1.2	4.1
Lymph node	1	2.52	7.22	14.0	48	13.9	3.7	4.4
Muscle	0.5	0.171	0.490	2.70	24	10	0.25	0.86
Pancreas	1	1.46	4.18	17.4	48	9.9	2.1	5.5
Pituitary gland	1	1.49	4.27	87.3	48	44.1	2.2	28
Salivary gland	1	1.05	3.01	13.2	48	8.0	1.5	4.2
Seminal vesicles	8	0.217	0.622	4.87	48	21.3	0.32	1.5
Skin	0.5	0.676	1.94	54.8	168	124	1.0	17
Small intestine wall	8	10.6	30.4	189	48	5.9	16	60
Spleen	0.5	1.96	5.62	17.2	48	12.8	2.9	5.5
Stomach cutaneous	1	2.44	6.99	42.2	48	8.3	3.6	13
Stomach glandular	1	2.04	5.85	14.4	48	10	3.0	4.6
Testis	24	0.115	0.330	13.8	168	95.8	0.17	4.4
Thymus	0.5	0.412	1.18	11.0	48	34.1	0.60	3.5
Thyroid gland	1	2.67	7.65	26.1	48	19.4	3.9	8.3
Trachea	4	3.96	11.3	16.9	24	4.3	5.8	5.4
Uveal tract	24	18.4	52.7	2890	168	142	27	917
White fat	4	0.0964	0.276	2.34	24	15.1	0.14	0.74

[a] Calculated as (value of tissue)/(value of blood)
[b] Due to the shortage of enough data, AUC_∞ could not be calculated; AUC_{last} is used to calculate the ratio
N.D. = not determined

(Excerpted from the submission)

Table 6.Pharmacokinetic parameters of tissue radioactivity following single oral dose of panobinostat in female Long Evans rats

Tissue	t_{max}	C_{max}		AUC_∞	t_{last}	$t_{1/2}$	Ratio[a]	
	(h)	(µgEq/g)	(µM)	(µgEq•h/mL)	(h)	(h)	$C_{max,tissue}$	AUC_∞
Adrenal cortex	8	1.42	4.07	30.2	48	7.2	4.4	5.1
Adrenal medulla	8	3.03	8.68	46.3[b]	24	N.D.	9.4	7.9[b]
Bile	0.5	21.3	61.0	199	48	15.5	66	34
Blood	0.5	0.322	0.92	5.89	8	24[c]	1.0	1.0
Bone marrow	0.5	0.49	1.40	9.36	24	10.0	1.5	1.6
Bone mineral	8	0.0101	0.03	0.02[b]	8	N.D.	0.0	0.0034[b]
Brain	4	0.0197	0.06	0.02[b]	4	N.D.	0.06	0.0033[b]
Brown fat	0.5	0.896	2.57	14.0	48	12.6	2.8	2.4
Colon wall	8	21.9	62.8	285[b]	24	N.D.	68	48[b]
Esophagus	0.5	21.8	62.5	43.3	24	4.5	68	7.4
Harderian gland	2	2.58	7.39	85.1	168	58	8.0	14.4
Heart	2	0.563	1.61	8.54	48	11.2	1.7	1.4
Kidney cortex	0.5	2.30	6.59	30.1	48	11.5	7.1	5.1
Kidney medulla	0.5	2.49	7.13	21.6	48	7.5	7.7	3.7
Kidney pelvis	0.5	1.89	5.42	25.4	24	6.3	5.9	4.3
Lachrymal gland	0.5	1.23	3.52	2.96[b]	4	N.D.	3.8	0.50[b]
Liver	0.5	4.67	13.4	80.3	168	32.5	15	14
Lung	4	0.475	1.36	9.09	24	8.0	1.5	1.5
Lymph node	2	0.595	1.70	10.2	24	11.6	1.8	1.7
Mammary gland	4	0.375	1.07	8.65	48	20.7	1.2	1.5
Muscle	8	0.161	0.46	0.82[b]	8	N.D.	0.50	0.14[b]
Ovary	8	0.783	2.24	16.4	48	8.4	2.4	2.8
Pancreas	2	1.01	2.89	13.3	48	7.8	3.1	2.3
Pituitary gland	8	1.56	4.47	88.3	168	141	4.8	15
Salivary gland	0.5	0.612	1.75	11.8	48	8.5	1.9	2.0
Skin	0.5	0.398	1.14	287	168	601	1.2	49
Small intestine wall	8	14.8	42.4	177[b]	24	N.D.	46	30[b]
Spleen	0.5	1.70	4.87	15.8	48	10.5	5.3	2.7
Stomach cutaneous	0.5	1.45	4.15	17.9	24	21.6	4.5	3.0
Stomach glandular	2	1.20	3.44	12.7	48	11.9	3.7	2.2
Thymus	0.5	0.308	0.883	11.9	48	25.6	1.0	2.0
Thyroid gland	2	1.04	2.98	21.7	48	14.6	3.2	3.7
Trachea	4	1.09	3.12	11.7	48	9.50	3.4	2.0
Uterus	8	1.05	3.01	20.5	48	7.30	3.3	3.5
Uveal tract	24	14.0	40.1	1770[b]	168	N.D.	43	301[b]
White fat	8	0.0957	0.274	1.62[b]	24	N.D.	0.30	0.28[b]

[a] Calculated as (value of tissue)/(value of blood).
[b] Due to the shortage of data, AUC_∞ could not be calculated; AUC_{last} presented and used to calculate ratios
[c] $t_{½}$ for the blood was based on only 2 time-points, due to the data variability. N.D. = not determined

(Excerpted from applicant)

Distribution of ^{14}C panobinostat in pregnant rats following a single oral dose (100mg/kg)

Key study findings:

- Panobinostat and/or its metabolites distributed to the fetus, uterus, placenta and mammary gland.
- On gestational day 12, the fetus-to-maternal blood ratio (C_{max} and AUC) was ~1. On gestational day 17, fetal distribution was lower with a fetus-to-maternal blood ratio (C_{max} and AUC) of ~0.5.
- At the last time-point of 24 h post-dose, the highest AUC was in the liver, consistent with the hepatobiliary route of excretion.
- Radioactivity also detected in: kidneys, heart, lung, and spleen

Study no.:	R0700906
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 22 October 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[14C]panobinostat, Lot #0231-068-28, >97%

Methods:
Tissue levels of radioactivity detected by whole body autoradiography in selected tissues (LOQ= 263 to 616 ngEq/g) and direct scintillation counting of blood.
In this study, in addition to the fetus, only the following maternal organs were examined for drug distribution: brain, liver, kidney, spleen, lung, white fat, muscle, blood and plasma, heart, mammary gland, uterus, amnion/amniotic fluid and placenta.

Species/strain:	pregnant Han Wistar, gestational day 11 and 16
N:	8 total; n=4/group dosed at gestational day 12, 17
Dose:	100 mg/kg
Frequency:	single dose
Route:	oral gavage)
Volume:	10 ml/kg

Observations and times: 10 min, 1, 3 and 24h post-dose

Results:

Table 7. Pharmacokinetic parameters following a single oral dose (100mg/kg) of ^{14}C panobinostat in pregnant rats on gestational day 12

Gestational day 12

Tissue	C_{max} (ngEq/mL)	T_{max} (h)	AUC_{last} (ngEq•h/g)	T_{last} (h)	Ratio (tissue/blood) $C_{max, tissue}$	Ratio (tissue/blood) AUC_{last}
Amnion	NA	NA	NA	NA	NA	NA
Amniotic Fluid	NA	NA	NA	NA	NA	NA
Blood	1580	24	24100	24	1.0	1.0
Brain	NA	NA	NA	NA	NA	NA
Fat (white)	344	24	7240	24	0.22	0.30
Fetus	1730	3	26000	24	1.1	1.1
Heart	2200	24	35900	24	1.4	1.5
Kidney Cortex	6550	0.167	121000	24	4.1	5.0
Kidney Medulla	7950	0.167	132000	24	5.0	5.5
Kidney pelvis	9120	0.167	113000	24	5.8	4.7
Liver	12500	24	229000	24	7.9	9.5
Lung	6860	24	86600	24	4.3	3.6
Mammary gland	2600	24	39000	24	1.6	1.6
Muscle	1250	24	17600	24	0.79	0.73
Placenta	1990	24	31600	24	1.3	1.3
Spleen	5770	24	103000	24	3.7	4.3
Uterus	3660	24	51800	24	2.3	2.1

NA = not applicable

(Excerpted from the submission)

Table 8. Pharmacokinetic parameters following a single oral dose (100mg/kg) of ^{14}C panobinostat in pregnant rats on gestational day 17

Gestational day 17

Tissue	C_{max} (ngEq/mL)	t_{max} (h)	AUC_{last} (ngEq•h/g)	t_{last} (h)	Ratio (tissue/blood) $C_{max, tissue}$	Ratio (tissue/blood) AUC_{last}
Amnion	5680	3	108000	24	3.9	32
Amniotic Fluid	NA	NA	NA	NA	NA	NA
Blood	1470	0.167	3410	3	1.0	1.0
Brain	NA	NA	NA	NA	NA	NA
Fat (white)	421	1	NA	1	0.29	NA
Fetus	679	3	1630	3	0.46	0.48
Heart	2220	0.167	29200	24	1.5	8.6
Kidney Cortex	8430	0.167	129000	24	5.7	38
Kidney Medulla	10800	0.167	126000	24	7.3	37
Kidney pelvis	8000	0.167	83900	24	5.4	25
Liver	18700	0.167	180000	24	13	53
Lung	3860	24	85700	24	2.6	25
Mammary gland	2590	1	52000	24	1.8	15
Muscle	1030	1	2630	3	0.70	0.77
Placenta	2210	0.167	28800	24	1.5	8.4
Spleen	7360	3	156000	24	5.0	46
Uterus	2130	24	41000	24	1.4	12

NA = not applicable

(Excerpted from the submission)

Binding of ^{14}C panobinostat to plasma protein in the mouse, rat, dog and human

Study no: r02000414
Location: 4.2.2.3
Conducting laboratory: Novartis Pharmaceutical Corporation

Key study findings:

- Plasma stability data at 37º C indicates that panobinostat is converted to the carboxylic acid metabolite in rat and mouse plasma. This metabolite is not found in the dog and human plasma. At 4º C, no conversion took place in rat or mouse plasma.
- Protein binding of panobinostat (and its carboxylic metabolite for mouse and rat data) was independent of concentration when 0.1 to 100 µg of drug was used.
- Protein binding was ~60% in mouse plasma, ~80% in rat and dog plasma, and ~90% in human plasma.
- Heparin, an anticoagulant and binding partner to plasma proteins, had no effect on protein binding of panobinostat

Methods:
Binding of radiolabel to protein was determined using liquid scintillation counting to determine radioactivity and protein concentrations were measured using a commercially available Bradford assay and spectrophotometer.

Table 9. Fraction of panobinostat bound to protein in plasma of mouse, rat, dog and human at 37ºC

[^{14}C]LBH589	Mouse[a]			Rat[a]			Dog			Human		
(µg/mL)	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
0.1	0.623	[b]		0.823	±	0.019	0.791	±	0.032	0.907	±	0.018
0.5	0.628	±	0.026	0.835	±	0.019	0.827	±	0.008	0.897	±	0.038
1	0.556	±	0.094	0.782	±	0.069	0.753	±	0.060	0.925	[a]	
10	0.537	±	0.073	0.794	±	0.072	0.788	±	0.009	0.895	±	0.022
100	0.660	±	0.055	0.723	±	0.125	0.776	±	0.025	0.868	±	0.024
Overall average	0.599	±	0.074	0.791	±	0.074	0.787	±	0.037	0.896	±	0.028

[a]data includes carboxycylic acid metabolite of LBH589
[b]n=2

(Excerpted from the submission)

Metabolism

Identification and quantification of metabolites in male rats administered a single oral dose of ^{14}C panobinostat

Key study findings:

- The metabolites in male rats account for 67% of the total radioactivity.
- Parent drug accounted for only 4% of the total radiolabel in plasma, indicating extensive metabolism.

Study no.:	R0900383
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 10 August 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{14}C]panobinostat, Lot # 0321-31, >98%

Methods: Levels of radioactive panobinostat was detected by direct scintillation counting of feces, urine, or plasma. Metabolites were structurally characterized by HPLC coupled to mass spectrometry.

Species/strain:	Han Wistar rats
N:	6 total; n=3/group collecting either plasma or urine and feces
Dose:	10 mg/kg
Frequency:	single dose
Route:	oral gavage
Volume:	10 ml/kg

Observations and times: 15, 30 min, 1, 2, 4, 8, 24, 48h post-dose (blood collection)

Table 10. Plasma exposure of metabolites following a single oral dose in rats

Metabolite	$AUC_{(0\text{-}8\ h)}$ (ngEq•h/mL)	% AUC
T23f	30.5	6.24
M26.8 / BJC319	49.2	10.1
M24.3	9.35	1.92
[M34.4 / BJB875] / T27d	165	33.8
M37.8 / BJB432	34.0	6.95
M36.9	19.9	4.08
M40.8	10.6	2.16
M43.5 / AFN835	9.35	1.92
Other	161	32.9
Total	489	100

(Excerpted from the submission)

Identification and quantification of metabolites in male dogs administered a single oral dose of ^{14}C panobinostat

Key study findings:

- Parent compound and identified metabolites account for 87% of the radiolabeled material in plasma.
- Parent drug accounted for only 8.5% of the total radiolabel in plasma, indicating extensive metabolism.

Study no.:	R0900382
Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 06 August 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{14}C]panobinostat, Lot # 0321-31, >98%

Methods: Levels of radioactive panobinostat was detected by direct scintillation counting of feces, urine, or plasma. Metabolites were structurally characterized by HPLC coupled to mass spectrometry.

Species/strain:	Beagle dogs
N:	3
Dose:	1.5 mg/kg
Frequency:	single dose

Route: oral gavage
Volume: 3 ml/kg
Observations and times: 15, 30 min, 1, 2, 4, 8, 24, 48h post-dose

Results:

Table 11. Plasma exposure of panobinostat and metabolites following a single oral dose in dogs

Metabolite	$AUC_{(0\text{-}8\ h)}$ ngEq•h/mL	% AUC
M24.2 / BJC330	43.3	4.20
T23c	21.9	2.12
M26.8 / BJC319	18.0	1.75
M24.3	54.0	5.24
T24.0	22.7	2.20
T25e	4.7	0.46
LBH589	88.4	8.57
M37.8 / BJB432	8.6	0.83
M36.9	537.2	52.1
M40.8	15.9	1.54
[M43.5 / AFN835] / M44.6	84.0	8.15
Other	132.0	12.8
Total	1031	100

(Excerpted from the submission)

Excretion
Excretion of ^{14}C panobinostat after single intravenous administration in the rat

Key study findings:

- The feces was the primary route of elimination of panobinostat following both intravenous and oral administration.
  - Recovery in the urine was 12.5% and 0.73% following intravenous and oral dosing respectively.
  - Recovery in the feces was 80% and 83% following intravenous and oral dosing respectively.
- Unchanged parent drug was detected in the feces at 7- 8% after IV or oral dosing, indicating high metabolism.

Study no.: 0201550
Study report location: eCTD 4.2.2.2
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation: 23 April 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [^{14}C]panobinostat, 0211-058-22, 96%

Methods: Radioactivity detected by liquid scintillation counting (LOQ=3.4 ng/ml)

Species/strain: HanWistar rats
N: 3 males/group
Dose: 10mg/kg
Frequency: single dose
Route: oral gavage or intravenous bolus
Volume: 10 mL/kg (oral) or 2ml/kg (intravenous)
Observations and times: 24, 48, 72 and 96 hours post-dose

Table 12. Excretion of radioactivity (0-96h) following single intravenous dose in urine and feces in rats (% dose)

Sample	Time (h)	Rat 1	Rat 2	Rat 3	Mean	±SD
Feces	0-24	67.80	61.30	78.50	69.20	8.72
Feces	24-48	9.18	6.60	5.17	6.98	2.03
Feces	48-72	0.53	11.20	0.97	4.25	6.06
Feces	72-96	0.42	0.45	0.37	0.41	0.04
	Subtotal	78.00	79.50	85.00	80.90	3.71
Urine	0-24	14.40	8.91	7.51	10.30	3.67
Urine	24-48	1.92	0.44	1.63	1.33	0.78
Urine	48-72	0.65	0.15	0.63	0.48	0.28
Urine	72-96	0.68	0.05	0.46	0.40	0.32
	Subtotal	17.70	9.54	10.20	12.50	4.52
Cage Wash	96	0.89	2.44	0.71	1.35	0.95
Carcass	96	N.S.	N.S.	N.S.	N.A.	N.A.
	Subtotal	0.89	2.44	0.71	1.35	0.95
	Total	96.60	91.50	96.00	94.70	2.75

N.S.= no sample, N.A.= not applicable

(Excerpted from the submission)

Table 13. Excretion of radioactivity (0-96h) following single oral dose in urine and feces in rats (% dose)

Sample	Time (h)	Rat 1	Rat 2	Rat 3	Mean	SD
Feces	0-24	83.90	72.00	72.60	76.20	6.69
Feces	24-48	2.81	10.40	7.67	6.96	3.85
Feces	48-72	0.19	0.21	0.23	0.21	0.02
Feces	72-96	0.03	0.05	0.08	0.05	0.02
Subtotal		86.90	82.60	80.60	83.40	3.21
Urine	0-24	0.54	0.52	0.91	0.66	0.22
Urine	24-48	0.05	N.S.	0.10	0.07	0.04
Urine	48-72	0.01	0.00	0.03	0.01	0.01
Urine	72-96	0.01	0.00	0.01	0.00	0.00
Subtotal		0.61	0.52	1.05	0.73	0.28
Cage Wash	96	0.03	1.19	0.08	0.43	0.66
Carcass	96	0.08	0.08	0.14	0.10	0.04
Subtotal		0.10	1.27	0.23	0.53	0.64
Total		87.60	84.40	81.90	84.60	2.87

N.S.= no sample

(Excerpted from the submission)

Table 14. Parent drug and metabolites in excreta of rats

Table 6-11 Metabolite profiles in excreta following a 10 mg/kg intravenous or oral dose of [^{14}C]LBH589 in rats

(Amounts expressed as % of dose; See Table 6-8 and Figure 7-18 for metabolite structures)

	IV			PO		
Time (h)	0-48 h					
Matrix/ Metabolite	Urine	Feces	Total excreta	Urine	Feces	Total excreta
P13.4[a]	1.34	1.07	2.41	0.09	3.7	3.80
M26.8	0.25	19.6	19.9	0.02	17.7	17.7
M36.9	1.25	3.50	4.75	0.08	1.29	1.38
M34.4	0.09	0.69	0.78	0.01	0.28	0.29
LBH589	3.20	6.58	9.78	0.21	8.42	8.63
M40.8/ M37.8[b]	0.98	28.1	29.1	0.06	49.4	49.5
M43.5	1.31	1.50	2.81	0.07	0.64	0.71
M44.6	0.46	1.45	1.90	0.03	1.04	1.07
Other	2.72	13.7	16.4	0.16	10.4	10.6
Total	11.6	76.2	87.8	0.73	92.9	93.7

[a] Structure of this metabolite undetermined

[b] Partially co-eluting peaks

(Excerpted from the submission)

Excretion of ^{14}C panobinostat after single oral or intravenous administration in the dog

Key study findings:

- The main route of elimination was the feces after intravenous or oral administration, accounting for ~50% of radioactivity after oral dosing and up to 70% of radioactivity after IV dosing.
- Approximately 30% of the radioactivity was recovered in the urine after oral or IV dosing.
- Unchanged parent drug was detected in the feces or the urine at < 2% following intravenous or oral dosing.

Study no.: R0300092

Study report location:	eCTD 4.2.2.2
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA
Date of study initiation:	N/A release date 13 December 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not mentioned

Methods: Radioactivity detected by liquid scintillation counting (LOQ=1.0-2.5 ng/ml)

Species/strain:	Male beagle dogs
N:	2/ intravenous group, 3/oral group
Dose:	0.5mg/kg (intravenous), 1.5mg/kg (oral)
Frequency:	single dose
Route:	intravenous bolus, oral gavage
Volume:	1 ml/kg (intravenous), 3 mL/kg (oral)

Observations and times: Samples collected at 0, 24, 48, 72, 96 and148 h post dose.

Results:

Table 15. Excretion of radioactivity (0-148h) as % of dose in urine and feces in dogs

Two dogs were dose intravenously at 0.5 mg/kg. Three dogs were dosed orally at 1.5 mg/kg

	Intravenous dosing						Oral dosing					
	Time	1	2	Mean	SD		Time	3	4	5	Mean	SD
Feces	0-24 h	34.8	24.5	29.6	7.27	Feces	0-24 h	47.6	42.8	56.4	48.9	6.94
	24-48 h	9.37	15.7	12.5	4.5		24-48 h	6.09	3.13	7.89	5.7	2.4
	48-72 h	2.08	3.93	3	1.31		48-72 h	1.25	1.94	1.52	1.57	0.347
	72-96 h	1.68	2.56	2.12	0.626		72-96 h	1.14	0.792	0.835	0.923	0.19
	96-168 h	1.81	1.91	1.86	0.071		96-168 h	0.802	0.619	1.13	0.852	0.261
	Subtotal	49.7	48.6	49.1	0.764		Subtotal	56.8	49.2	67.8	58	9.34
Urine	0-24 h	29.2	24.9	27.1	3.01	Urine	0-24 h	31.4	33	27	30.4	3.09
	24-48 h	2.19	3.65	2.92	1.03		24-48 h	2.61	1.11	1.93	1.88	0.754
	48-72 h	0.888	1.34	1.11	0.321		48-72 h	0.675	0.786	0.661	0.708	0.0687
	72-96 h	0.651	0.763	0.707	0.0789		72-96 h	0.279	0.275	0.351	0.302	0.0426
	96-168 h	0.982	0.999	0.991	0.0119		96-168 h	0.334	0.295	0.476	0.368	0.0955
	Subtotal	33.9	31.7	32.8	1.57		Subtotal	35.3	35.4	30.4	33.7	2.85
Cage Wash	Day 7	1.66	3.07	2.36	0.997	Cage Wash	Day 7	2.73	0.586	0.585	1.3	1.24
	Total	85.3	83.4	84.3	1.34		Total	94.8	85.3	98.8	93	6.97

(Excerpted from the submission)

Table 16. Parent drug and metabolites in excreta of beagle dogs (expressed as ng-Eq/mL)

	i.v.			p.o.		
Time (h)	**0-48 h**					
Matrix/ Metabolite	**Urine**	**Feces**	**Total excreta**	**Urine**	**Feces**	**Total excreta**
M36.9	16.6	12.2	28.8	22.6	22.2	44.8
LBH589	1.16	1.73	2.89	0.43	1.89	2.32
M40.8/ M37.8[a]	1.75	7.52	9.27	0.89	9.60	10.5
M43.5	1.87	1.22	3.10	0.81	1.41	2.22
M44.6	2.92	0.59	3.51	1.69	0.69	2.38
Other	5.70	16.8	22.5	5.82	18.8	24.7
Total	30	40.1	70.1	32.3	54.6	86.9

[a] Partially co-eluting peaks

(Excerpted from the submission)

6 General Toxicology

6.1 Overall Toxicology Summary

Nonclinical findings in the rat and dog show that panobinostat targets the bone marrow, lymphatic systems, liver, lung, kidney, thyroid, mammary gland (rat only), GI tract, skin (dog only), and male reproductive organs (dog only). Panobinostat was mutagenic in the AMES assay, caused endo-reduplication (increased number of chromosomes) in human peripheral blood lymphocytes and was positive for DNA damage in the COMET assay in mouse lymphoma cells. In the combined male and female fertility study, reduced fertility index and conception rate was seen in rats. Panobinostat was teratogenic in the rat and rabbit.

6.2 Single-dose Toxicity

Studies not reviewed.

6.3 Repeat-dose Toxicity

The 4-week study in rats was reviewed by Shwu-Luan Lee, Ph.D., under IND 69862. The review has been slightly modified to fit the format of the NDA review.

Study title: 4-week (3 doses/week) oral (gavage) toxicity study in rats with a 4-week recovery period.

Key study findings:

- The target organs in this study were thymus, thyroid and spleen.

Study no.: 0370121
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, One Health Plaza East, Hanover, NJ 07936-1080.
Date of study initiation: July 14, 2003
GLP compliance: yes.
QA report: yes (x) no ( ).
Drug, lot #, and % purity: LBH589 lactate salt, lot #0351001, purity: 95.2%.
Formulation/vehicle: purified water, USP. The concentration was calculated to give a constant dose volume of 10 mL/kg for each dose level (see table below).

Table 3-1 Study design, animal allocation and test article doses

Group	Number/sex	Animal Numbers males	Animal Numbers females	Dose[a] (mg/kg) Base/Salt	Concentration (mg/mL) Salt
1 Control	10 +6 recovery	1001-10 1011-16	1501-10 1511-16	0	0
2 Low	10	2001-10	2501-10	3/3.78	0.38
3 Mid	10	3001-10	3501-10	10/12.6	1.26
4 High	10 +6 recovery	4001-10 4011-16	4501-10 4511-16	30/37.8	3.78

[a] Doses were not corrected for active moiety. Salt/base ratio for LBH589 is 1.26.

Methods:

Species: IGS Wistar Hannover rats; Crl: WI (Glx/BRL/Han)IGS BR
n: 10/sex/group, plus 6 to the control and 30 mg/kg group as recovery animals.
Age/weight: 8 weeks/169.2 to 285.7 g.
Doses: 0 (control), 3, 10, 30 mg/kg (groups 1, 2, 3 and 4, respectively).
Schedule: Once daily on Study days 1, 3, 5, 8, 10, 12, 15, 17 etc. (Days 1, 3, 5/week), through the last day of end of dosing (EOD). The recovery animals (Groups 1 and 4) were dosed as mentioned and followed by a 28-day recovery phase (no treatment).
Route: Oral by gavage

Observations and times:

Clinical signs: Twice daily for mortality, moribundity and gross abnormality. Detailed physical examination: twice daily on each day of dosing days and at least once on non-dosing days.

Body weights: Once prior to treatment and weekly thereafter.
Food consumption: Weekly pre-study and throughout the remainder of the study.
Ophthalmoscopy: On all animals during pretest and on all control and Group 4 animals during week 3 with an indirect ophthalmoscope.
Electrocardiography: Not performed.
Hematology: In Week 4 and on Day 57.
Clinical chemistry: In Week 4 and Day 57.
Urinalysis: Up to 5 hour urine samples (approximately 1 ml) were collected in Week 4 and Day 57.
Gross pathology (necropsy): Scheduled sacrifice: Day 29 or after recovery period (D57).
Organ weights: At scheduled sacrifice. Adrenal, brain, heart, kidney, liver, ovary, pituitary gland, prostate, spleen, testis (including epididymides), thymus, thyroid and uterus.
Histopathology: Day 29 or Day 57. All tissues collected from all animals were subjected to histopathology examination (inventory: see results).

Results:

Mortality /Moribundity	No mortality or moribundity related to LBH589. A male #4007 was dead due to the blood collection procedure.
Clinical signs	Unremarkable, except Group 4 female #4501: thin appearance, unkempt coat, slightly decreased. locomotor activity, opacity eye, absent feces, perineal and fur staining (no histopathological findings to support the clinical signs).
Body weights	Unremarkable in group mean body weight. The mean weight gain was reduced significantly in treated Group 3 males on Days 15 and 22 (19% and 18% decrease to the control, respectively) and Group 2 females on Day 29 (14% decrease to the control). Some Group 4 females (#4501, 4506, 4508, 4510, 4513, and 4514) showed transient reduction in body weight gains. The finding was not dose-related and resolved in the recovery period.
Food consumption	Unremarkable

Ophthalmoscopy: Based on the summary presented, there were no ocular changes attributable to treatment with LBH589.

Electrocardiography: not performed

Hematology:

Hematology: % changes from the control (Group 1)

	Males			Females		
	Week 4			Week 4		
	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
WBC ↓			23			24
Lymph ↓			26			25
MCV ↓						2
MCH						3
Platelets ↓		9	16			20
Retic % ↑	↓ 17					25

Most hematology findings were minor and resolved during the recovery period.

Bone marrow smears: No data were provided. The following statements were included in the submission:

- No article-related changes in bone marrow smears. The M:E ratios of male control and Group 4 were 1.03 and 1.38, respectively. The M:E ratios of female control and Group 4 (excluding #4501) were 1.21 and 1.38, respectively.
- Female #4501 showed an M:E ratio of 7.53 in which the numbers of erythroid precursors were decreased and myeloid precursors were increased.

Clinical chemistry: Unremarkable

Urinalysis: Unremarkable

Organ weights: Drug-related absolute and relative organ weight changes expressed as % change of control (Groups 2, 3 and 4 referred to 3, 10 and 30 mg/kg, respectively):

	Males							Females						
	Day 29						Day 57 (recovery)	Day 29						Day 57 (recovery)
	Group 2		Group 3		Group 4			Group 2		Group 3		Group 4		
	Abs (gm)	% BW	Abs (gm)	% BW	Abs (gm)	% BW		Abs (gm)	% BW	Abs (gm)	% BW	Abs (gm)	% BW	
Ovary ↑									16	15	19	18	39	% BW ↓ 11%
Pituitary ↓	10	10	10		20	13				13		20	10	
Spleen ↓	12	13	10		12		% BW ↓ 11%	11		16	14	27	20	
Thymus ↓			11		45	43	% BW ↓ 13%					53	49	
Thyroid ↓	33	33	23	19	23	11		22	18	15	11	30	20	
Uterus ↑										10	12	↓ **7**	↓ **10**	

Most of changes in organ weight were reversible during the recovery period.

Macroscopic findings:
The findings included: small thymus (in Group 4: 2M and 2F), findings in female Group 4 rat #4501 (kidney, small and large bowels).

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Spleen: Accessory spleen			1					
Thymus: small Discoloration[a]				2				2
Eye: Enlarged, rough, red Discoloration, retrobulbar		1						1
Kidney: Enlarged Hydronephrosis[b] Multiple yellow focus			1					*1 *1
Lung: Discoloration, red, multifocal Failure to collapse				1		1		
Small and large bowels: Distension with gas, serosa white discoloration								*1
Uterus: Dilatation						1	1	

Note: *: findings in #4501 only. a: found in one recovery control male #1012. b: also found in one male recovery animal #4011.

The changes in the kidney in animal #4 501 included tubular dilatation, hyaline cast, mineralization, and pyelonephritis. Changes in eyes were related to hemorrhage. Small thymus found in Group 4 females was supported by the finding of lymphocyte depletion and thinned cortex.

Histopathology findings:

Histopathology Gr	Group 2		Group 3		Group 4		Recovery (G4)	
Sex	M	F	M	F	M	F	M	F
Number of animals	10	10	10	10	10	10	6	6
Eyes	[1]	[0]	[0]	[0]	[10]	[10]		
moderate neutrophilic panophthalmitis								
marked mixed cell inflammation	1							
moderate to marked hemorrhage	1					1		
Harderian gland	[1]	[1]	[0]	[0]	[10]	[10]		
minimal to moderate chronic inflammation*	1				1	2		
minimal to moderate hemorrhage*	1	1			1	1		
slight to minimal acini dilatation					3			
minimal congestion		1						
minimal necrosis						1#		
moderate mixed cell inflammation						1		
hemoglobin crystal						1		
Lacrimal gland	[0]	[0]	[0]	[0]	[10]	[10]		
slight acini atrophy						1#		
Lung	[0]	[1]	[0]	[0]	[10]	[10]		
minimal chronic subpleural inflammation						1		
slight interstitial mineralization						1#		
minimal chronic interstitial inflammation						1#		
moderate congestion					1			
Salivary gland	[0]	[10]	[0]	[10]	[10]	[9]		
minimal diffuse edema						1#		
minimal to slight diffuse mucous cell atrophy						2#		
Stomach	[0]	[0]	[0]	[0]	[10]	[10]		
minimal epithelial necrosis						1#		
severe serosa mineralization						1#		
marked mucosal mineralization						1#		
slight mixed cell inflammation						1#		
slight glandular dilatation						1#		
Cecum	[0]	[0]	[0]	[0]	[10]	[10]		
minimal leukocytic infiltrate						1#		
Colon	[0]	[0]	[0]	[0]	[10]	[10]		
slight serosa mineralization						1#		
minimal chronic serosa inflammation						1#		
Rectum	[0]	[0]	[0]	[0]	[10]	[10]		
slight serosa mineralization						1#		
minimal chronic inflammation						1#		
Bone: femur/tibia								
moderate marrow cavity new bone formation						1#		
minimal hypercellular periosteum					1			
minimal marrow cavity fibrosis						1#		
slight cortex depletion						1#		
minimal macrophage brown pigment	1							
Bone marrow	[0]	[0]	[1]	[0]	[10]	[10]		
minimal to slight increased granulopoiesis			1			2#		
minimal to slight decreased erythropoiesis			1			2#		
minimal fibrosis					1			

Note: The number in the parenthesis indicates the number of animal examined.

*: findings also in the control animals.
#: findings in animal #4501 (female Group 4)
a: findings in animal #4005 (male Group 4)

Summary:

- The most prominent toxicity was spleen (in females, decreased extramedullary hematopoiesis, lymphoid depletion), thymus (in females, thinned cortex due to a decrease in the lymphocyte population), thyroid (decreased colloid, cytoplasmic vacuolation of follicular epithelium), kidney (in males, hydronephrosis), and Harderian gland (inflammation, hemorrhage, acini dilatation). Lesions in thyroid, kidney and Harderian gland occurred in all dose levels. Hydronephrosis was found in 2/6 recovery male Group 4 animals.
- One non-recovery animal, #4501, exhibited microscopic lesions different from those found in other females. These lesions included severe pyelonephritis and other renal tubular lesions, multisystemic vascular and tissue mineralization and inflammation (liver, heart, aorta, GI tracts, lung, muscle and fat), and other unique changes in mesenteric lymph node, salivary gland, pituitary gland, urinary bladder, ovary and vagina. These findings were unique to the animal and may not be LBH589-related.

Study title: 13 Week Oral Toxicity Study in rats followed by a 4 week recovery period

This study was reviewed to note toxicities in the thyroid that were not observed in the 26 week toxicity studies. Limited endpoints have been reviewed.

Study no.:	0680019
Study report location:	eCTD 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03 April 2006
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0622003, 98.1 %

Key Study Findings

- Thyroid toxicity was observed and supported by decreased levels of triodothyronine (T_3), tetraiodothyronine (T_4) and thyroid stimulating hormone (TSH).
- Histopathology findings in the thyroid included follicular cell hypertrophy at 100 mg/kg/day that was reversible.
- Additional toxicities were observed in the bone marrow, spleen and thymus.
- At 100 mg/kg/day toxicities in the liver, gastrointestinal tract, lung, and bone were also observed.

Methods

Doses:	0, 10, 30, and 100 mg/kg
Frequency of dosing:	3 doses/week (M, W, F)
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% w/v hydroxylpropylcellulose (Klucel)
Species/Strain:	Rat/Han Wistar
Number/Sex/Group:	Main: 10/sex/group; Recovery: 5/sex/group(control and HD groups)
Age:	8 weeks at the start of dosing
Weight:	Males: 230-296 g; Females: 173-208 g
Satellite groups:	8/sex/group
Unique study design:	None
Deviation from study protocol:	None that affected study outcome

Observations and Results
Mortality

All animals were checked twice daily for viability, early morning and late as possible each day.

Table 17. Cause of death in repeat dose toxicity study in rats

Animal #	Dose (mg/kg)	Sex	Week of Death	Observations	
				Reason	General (include pathology)
43	0	F	5	Sacrificed	Right eye damage post orbital sinus bleed damage

Clinical Signs
Animals were checked for treatment reactions at pre-dose, immediately after dose and 1-hr post-dose on each day of dosing. All animals received detailed evaluations once each week. Agitation was noted in all groups, including controls, but was higher in the treated animals, and highest in the high dose group (100 mg/kg/day). No other drug-related clinical signs were observed.

Body Weights
Recorded once the week before treatment started and on days of dosing until week 13, then once each week until the end of the recovery period. Results below show body weight gain over 13 weeks (13 week) and from 13 to 17 weeks (17 week).

Table 18. Body weight % change in repeat dose toxicity study in rats

	males			females		
Body weight gain	10	30	100	10	30	100
T	-15.5	-10.1	-46.4	-8.8	-3.5	-29.8
R			130.8			-1400.0**

T: terminal (0 – 13 weeks), R: recovery (14-17 weeks); Significantly different from the Control:
** P<0.01

Thyroid

Thyroid function tests were performed on main study animals during week 11.

Table 19.Thyroid function tests in repeat dose toxicity study in rats

	% change from control					
sex	males			females		
dose (mg/kg/day)	10	30	100	10	30	100
thyroid function test						
TSH	-4.3	-12.8	-4.3	-16.2	-8.1	-27.0
T3	0.0	-13.9	-16.7	-9.8	-12.2	-19.5
T4	-7.7	-9.1	-26.9***	5.7	4.3	-10.0

Significantly different from the Control: *** P<0.001

Gross Pathology

Examined in main animals at completion of week 13 and week 17 (recovery).

Table 20. Gross pathology findings in repeat dose toxicity study in rats- main study

Macroscopic findings-terminal		**No. of animals affected**							
		males				**females**			
dose (mg/kg/day)		0	10	30	100	0	10	30	100
Number of animals examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Organ	**Finding**								
lung	adhesion				1				
	raised focus				1				
lymph node, bronchial	enlarged				1				
lymph node, mesneteric	redened				4				
thymus	small				8				7
ovary	Cyst								1
stomach	masses				1				
	raised focus								1

Table 21. Gross pathology findings in repeat dose toxicity study in rats- recovery

Macroscopic findings-recovery		**No. of animals affected**							
		males				**females**			
dose (mg/kg/day)		0	10	30	100	0	10	30	100
Number of animals examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Organ	**Finding**								
Lung	dark focus				1				
	spongy								1
	redened				1				
seminal vesicle	small, left				1				

Histopathology
Examined in main animals at completion of week 13 and week 17 (recovery).
Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 22. Histological findings in repeat dose toxicity study in rats- main study

Treatment related microscopic findings			No. of animals affected							
			males				females			
dose (mg/kg/day)			0	10	30	100	0	10	30	100
Number of animals examined			10	10	10	10	10	10	10	10
Organ	Finding	Grade								
lung	abscess, multiple					1				
Bone marrow	Aplasia, granulocytic					3				2
	Hypoplasia, granulocytic					4				6
	Erythrocytes, present									3
	Inadequate, smear					1			2	3
lymph node, bronchial	plasmocytosis					1				
lymph node, mandibular	plasmocytosis									
	lymphoid depletion					4				1
lymph node, mesenteric	erythrophagocytosis					6				1
lymph node, lumbar	lymphoid depletion					1				
spleen	lymphoid atrophy					9				5
	pigment increased	1				4				5
		2				2				3
	increased haemopoiesis	1				1				1
		2								2
		3				1				1
	mineralization, multifocal					1				
thymus	atrophy	1			2				3	
		2							3	
		3				1				2
		4				3				3
		5				6				5
	lymphoid foci, medulla					1				
Heart	pericarditis					1				
thyroid	follicular cell hypertrophy	1				2				
pituitary gland	cyst					1				
testis	seminiferous epithelial degeneration	3				1				

(Cont..)

Treatment related microscopic findings			No. of animals affected							
			males				females			
dose (mg/kg/day)			0	10	30	100	0	10	30	100
Number of animals examined			10	10	10	10	10	10	10	10
Organ	Finding	Grade								
epididymis	oligospermia					1				
vagina	Oestrous cycle: oestrus						2	2	2	8
jejenum	lymphoid atrophy, peyers patch	2								1
		3				1				1
illeum	lymphoid atrophy, peyers patch	2				4				2
		3				1				1
caecum	lymphoid atrophy, peyers patch	1				1				
		2				2				3
		3				1				1
colon	lymphoid atrophy, peyers patch	2				1				
rectum	lymphoid atrophy, peyers patch	2				2				
		3								3
liver	Haemopoiesis, extramedullary					1				1
	inflammatory cell foci		1			3	1			2
salivary gland	acinar cell atrophy					1				
hardarian gland	atrophy, unilateral diffuse					3				
sternum	atrophy, marrow	1		1	1	2		1	3	
		2				1				1
		3				2				2
		4								2
	atrophy, marrow, focal			4	2					
femur	atrophy, marrow	1			1	2			2	1
		2				3				2
		3								2
	hyperostosis	1				1				1
		2								1
		3				1				

Recovery

Table 23. Histological findings in repeat dose toxicity study in rats- recovery

Microscopic findings-recovery			No. of animals affected							
			males				females			
dose (mg/kg/day)			0	10	30	100	0	10	30	100
Number of animals examined			10	10	10	10	10	10	10	10
Organ	Finding	Grade								
lung	Polypoid hyperplasia, bronchiolar, focal									1
	Agonal congestion/haemorrhage					1				1
Bone marrow	Erythrocytes, present					1				1
spleen	pigment increased	1				2				2
		2								2
thyroid	follicular cell hypertrophy	1				1				
kidney	Cystic tubules, focal					1				
	Focal transitional cell hyperplasia					1				
	inflammatory cell foci									1
	mineralization pelvic, focal					1				2
pancreas	inflammatory cell foci					1				

Study title: 26 Week (3 doses/week) Oral (gavage) Toxicity Study in Rats Followed by a 4-Week Recovery Period

Study no.:	060134
Study report location:	eCTD 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	19 June 2006
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0623003, 99.4 %

Key Study Findings

- 6 mortalities observed (1 at 0 mg/kg/day, 2 at 30 mg/kg/day & 3 at 300 mg/kg/day).
- All 3 animals sacrificed early due to bleeding had reduced platelets at 13 weeks.
- Toxicities in the hematopoietic/lymphocytic systems: ↓ WBCs, differentials, and platelets, microscopic findings of bone marrow plasmacytosis and erythrophagocytosis as well as fatty atrophy, lymph node erythrophagocytosis, increased hemosiderin in the spleen, thymic atrophy.
- Abnormalities in bone marrow smears, e.g.
  - Increased number of granulocytic cells; decrease in the proportion of late stage granulocytic precursors and mature cells; presence of abnormal cytoplasmic granulation, irregular nuclear shaped and enlarged cell size.
  - an increased proportion of eosinophilic cells; presence of abnormal cytoplasmic granulation, irregular nuclear shaped and enlarged cell size;
- Other toxicities: Inflammatory cell foci in lung and liver; hemorrhage (lacrimal gland, brain, spinal cord); thyroid follicular cell hypertrophy; atrophy of mammary gland.
- Thyroid follicular cell adenoma observed in 1/10 (10%) of male animals at the end of the recovery period. Other findings in the thyroid in the recovery animals included focal C cell hyperplasia (10% of females) and follicular cell hypertrophy (50% of males and females)

Methods

Doses:	0, 10, 30, and 75 mg/kg
Frequency of dosing:	3 doses/week
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% w/v hydroxypropylcellulose (Klucel) or 0.5% w/v hydroxyethylcellulose (Natrosol)
Species/Strain:	Rat/Han Wistar
Number/Sex/Group:	Main: 20/sex/group; Recovery: 10/sex/group (control and HD groups)
Age:	8 weeks at the start of dosing

Weight: Males: 208-276 g; Females: 158-209g
Satellite groups: 8/sex/group
Unique study design: None
Deviation from study protocol: None that affected study outcome

Observations and Results

Mortality

All animals were checked twice daily for viability, early morning and late as possible each day.

Table 24. Cause of death in repeat dose toxicity study in rats

Animal #	Dose (mg/kg)	Sex	Week of Death	Observations	
				Reason	General (include pathology)
87	0	F	24	Sacrificed	Right eye post orbital sinus bleed and protruding, kidneys dark. Thymus, exorbital lacrimal glands mandibular lymph nodes reddened. Uterus dilated with clear fluid
131	30	F	7	Found dead	Lungs dark, trachea froth filled, liver prominent lobulation
252	30	F	3	Sacrificed	Pregnant
68	75	M	23	Sacrificed	Right eye post oribital sinus bleed. Mandibular lymph nodes reddened. Right eye bulging and dark. Exorbital lacrimal glands dark focus
72	75	M	24	Sacrificed	Right eye post orbital sinus bleed. Harderian gland and right eye enlarged. Right eye green color
198	75	F	6	Sacrificed	Locomotion difficulties. Right hind foot subcutaneous muscles swollen

Clinical Signs

Animals were checked for treatment reactions at pre-dose, immediately after dose and 1-hr post-dose on each day of dosing. All animals received detailed evaluations once each week. No drug-related clinical signs were observed in addition to those listed under the mortality section.

Body Weights

Recorded once the week before treatment started and on days of dosing until week 26, then once each week until the end of the recovery period.

Table 25. Body weight changes in repeat dose toxicity study in rats

Body Weight Gain	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg/day)	0	10	30	75	0	10	30	75
T	247	↓10*	↓19***	↓29***	69	-	-	-
R	19	NA	NA	↑58	5	NA	NA	↑1.4-FOLD**

T: terminal (0 – 26 weeks), R: recovery (26 – 30 weeks);
Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Food Consumption

Recorded once prior to the start of treatment then weekly up until the end of the recovery period.

Table 26. Food consumption in repeat dose toxicity study in rats

Food Consumption	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	10	30	75	0	10	30	75
Week 13 *T*	21.5	-	-	↓12***	15	-	-	-
Week 26 *T*	22	-	↓13***	↓14***	14.9	-	-	-
Week 30 *R*	21.1	NA	NA	-	15.6	NA	NA	↓10

T: terminal, R: recovery; significantly different from the Control: *** P<0.001
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Water Consumption

Changes monitored by visual inspection on a weekly basis throughout the study. No drug-related changes were observed.

Ophthalmoscopy

Examinations performed pre-treatment and at week 26 of treatment. No drug-related changes were observed.

Hematology

Blood samples were taken at week 13, week 27, and week 31 (recovery).

Table 27. Hematology parameters in repeat dose toxicity study in rats (% change from control)

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	10	30	75	0	10	30	75
WBC								
Week 13	5.59	↓10	↓53	↓80	3.40	↓11	↓51	↓77
Week 27	4.99	↓20	↓51	↓69	3.40	↓20	↓31	↓69
Week 31	4.17	NA	NA	↓31	3.00	↓NA	↓NA	↓31
NEU								
Week 13	0.85	-	↓48	↓74	0.60	↓18	↓43	↓77
Week 27	1.04	-	↓33	↓63	0.75	↓11	↓25	↓76
Week 31	0.74	NA	NA	↑19	0.67	NA	NA	↓16
LYM								
Week 13	4.51	↓11	↓54	↓82	2.61	-	↓53	↓50
Week 27	3.65	↓25	↓58	↓74	2.46	↓25	↓57	↓69
Week 31	3.21	NA	NA	↓44	2.15	NA	NA	↓37
MON								
Week 13	0.10	↓10	↓30	↓50	0.08	↓13	↓25	↓50
Week 27	0.14	-	-	-	0.09	-	-	-
Week 31	0.07	NA	NA	↑14	0.06	NA	NA	-
EOS								
Week 13	0.01	↓10	↓30	↓70	0.09	↓22	↓56	↓78
Week 27	0.12	-	↓25	↓50	0.07	-	-	-
Week 31	0.10	NA	NA	↓10	0.08	NA	NA	↓25
BAS								
Week 13	0.01	-	↓100	↓100	0.01	↓100	↓100	↓100
Week 27	0.01	-	-	-	0.01	-	-	-
Week 31	0.02	NA	NA	↓50	0.01	NA	NA	-
LEU								
Week 13	0.03	-	↓33	↓100	0.01	-	-	↓100
Week 27	0.03	↓33	↓67	↓67	0.02	-	-	-
Week 31	0.03	NA	NA	↓33	0.02	NA	NA	↓50
PLT								
Week 13	692	-	↓17	↓24	684	-	↓11	↓32
Week 27	718	↓16	↓26	↓25	608	-	↓14	↓36
Week 31	555	NA	NA	-	566	NA	NA	-

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Clinical Chemistry

Blood samples were taken at week 13, week 27, and week 31 (recovery). No drug-related changes observed.

Urinalysis

Samples were taken at week 13, week 26, and week 30 (recovery) over a 4-hr period of food and water deprivation.

Table 28. Urinalysis parameters in repeat dose toxicity study in rats (% change from control)

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	10	30	75	0	10	30	75
VOLUME								
Week 13	1.5	-	-	-	0.6	↑33	↑67	↑1.5-fold
Week 27	1.7	-	-	-	0.5	↑20	↑80	↑2-fold
Week 31	1.4	NA	NA	-	0.5	NA	NA	-

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Troponin

Samples were taken at day 5, week 13, week 26, and week 30 (recovery). No drug-related changes observed.

Gross Pathology

Animals were necropsied on completion of treatment with recovery animals necropsied 4-weeks after completion of treatment.

Table 29. Gross pathology findings in repeat dose toxicity study in rats

Macroscopic findings - Terminal		Male				Female			
No. animals		20	20	20	20	20	20	20	20
Dose (mg/kg)		0	10	30	75	0	10	30	75
Head	Masses	-	-	-	-	-	-	-	1
Lacrimal gland	Dark focus, (R)	-	-	-	1	-	-	-	-
LN, mediastinal	Reddened	-	-	-	1	-	-	-	1
Thymus	Small	-	-	1	2	-	-	-	7

Macroscopic findings - Recovery		Male				Female			
No. animals		20	20	20	20	20	20	20	20
Dose (mg/kg)		0	10	30	75	0	10	30	75
Leg	Swollen	-	NA	NA	-	-	NA	NA	1
Lung	Reddened	-	NA	NA	2	-	NA	NA	-
Mass	Subcutaneous mass	-	NA	NA	-	-	NA	NA	1

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Organ Weights

Animals were necropsied on completion of treatment with recovery animals necropsied 4-weeks after completion of treatment.

Table 30. Organ weights in repeat dose toxicity study in rats (% change relative to body weight)

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	10	30	75	0	10	30	75
Adrenal								
T	0.01077	-	-	↓14	0.02813	-	↓25	↓26
R	0.01083	NA	NA	↓15	0.02619	NA	NA	↓15
Brain								
T	0.436	-	↑10	↑17	-	-	-	-
R	0.434	NA	NA	-				
Pituitary								
T	-	-	-	-	0.005	-	↓12	↓17
R					0.005	NA	NA	-
Prostate								
T	0.82	↓15	↓15	↓26	NA	NA	NA	NA
R	0.81	NA	NA	-				
Testes								
T	0.1288	-	-	↑18	NA	NA	NA	NA
R	0.1255	NA	NA	↑18				
Salivary gland								
T	0.1345	↓10	↓33	↓53	0.1771	-	↓31	↓63
R	0.1337	NA	NA	-	0.1741	NA	NA	↓16
Spleen								
T	0.137	-	-	↓11	0.180	-	↓15	↓24
R	0.136	NA	NA	-	0.184	NA	NA	NA
Thymus								
T	0.057	↓10	↓33	↓53	-	-	-	-
R	0.051	NA	NA	-				

T: terminal necropsy; R: recovery necropsy;
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Histopathology

Animals were necropsied on completion of treatment with recovery animals necropsied 4-weeks after completion of treatment.

Adequate Battery (yes)
Peer Review (yes)
Histological Findings:

Table 31. Histopathology findings in repeat dose toxicity study in rats

Microscopic findings - Terminal	Male				Female			
No. animals	20	20	20	20	20	20	20	20

Dose (mg/kg)		0	10	30	75	0	10	30	75
Lung	Inflammatory cell foci, minimal	-	-	-	5	-	-	-	-
Bone Marrow	Fatty atrophy, Minimal	-	-	-	16	-	-	-	16
	Plasmacytosis -minimal -mild -moderate	 3 - -	 - - -	 - - -	 2 1 1	 4 2 -	 - - -	 - - -	 5 3 0
	Erythrophagocytosis, mild	-	-	-	1	-	-	-	-
LN, mandibular	Erythrophagocytosis, mild	-	-	-	1	-	-	-	-
	Germinal cenre atrophy -minimal -mild	 - -	 - -	 - -	 5 6	 - -	 - -	 - -	 7 4
LN, mesenteric	Erythrophagocytosis -minimal -mild -moderate	 1 - -	 - - -	 - - -	 2 8 -	 - - -	 - - -	 - - -	 3 4 1
LN, mediastinal	Erythrophagocytosis, mild	-	-	-	1	-	-	-	1
Spleen	Increased hemosiderin -minimal -mild -moderate -marked	 - - - -	 2 - - -	 10 - - -	 6 5 7 -	 - - - -	 8 4 2 -	 2 6 11 -	 - 2 12 6
	Atrophy, periarteriolar lymphoid sheaths, minimal	-	-	-	15	-	-	-	13
Thymus	Tubular cystic hyperplasia, mild	-	-	-	-	-	-	-	1
	Atrophy -minimal -mild -moderate -marked	 - - - -	 - - - -	 2 2 - -	 1 4 2 -	 - - - -	 - - - -	 3 4 - -	 1 4 7 1
Thyroid	Follicular cell hypertrophy, minimal	4	7	12	15	1	2	4	12
Kidney	Mineralization, focal, minimal	-	-	-	-	11	-	-	18
Liver	Inflammatory cell foci, minimal	5	-	-	14	13	-	-	18
Mammary gland	Atrophy, acinar	-	-	4	16	-	-	-	-
Eye	Hemorrhage, retrobulbar, minimal	-	-	-	2	-	-	-	-
	Retinal fold	-	-	-	1	-	-	-	-
	Abscess, retrobulbar, marked	-	-	-	1	-	-	-	-
Lacrimal gland, exorbital	Hemorrhage	-	-	-	1	-	-	-	-
Brain	Hemorrhage, meningeal	-	-	-	1	-	-	-	-
Spinal cord	Hemorrhage, meningeal	-	-	-	1	-	-	-	-

Microscopic findings - Terminal		Male				Female			
No. animals		20	20	20	20	20	20	20	20
Dose (mg/kg)		0	10	30	75	0	10	30	75
Sternum	Fatty atrophy, marrow -minimal -mild	 - -	 - -	 2 -	 5 1	 1 -	 1 -	 7 -	 11 1

Microscopic findings - Recovery		Male				Female			
No. animals		10	0	0	10	10	0	0	10
Dose (mg/kg)		0	10	30	75	0	10	30	75
Lung	Agonal congestion, hemorrhage	-	NA	NA	3	-	NA	NA	-
	Inflammatory cell infiltration, focal	-			-	-	NA	NA	1
Bone Marrow	Fatty atrophy, Minimal	-	NA	NA	3	-	NA	NA	-
LN, mandibular	Plasmacytosis, mild -minimal -moderate	 - -	NA	NA	 1 2	-	NA	NA	-
	Germinal centre atrophy -minimal	-	NA	NA	-	-	NA	NA	1
	Erythrophagocytosis, minimal	-	NA	NA	-	-	NA	NA	1
LN, mesenteric	Erythrophagocytosis -minimal -mild	 - -	NA	NA	 3 -	-	NA	NA	 4 2
	Sinus histiocytosis,	2	NA	NA	6	-	NA	NA	-
Spleen	Increased hemosiderin -minimal -mild -moderate -marked	 - - - -	NA	NA	 7 3 - -	 - - - -	NA	NA	 1 5 1 1
Thymus	Atrophy, mild	-	NA	NA	-	-	NA	NA	1
Thyroid	Follicular cell hypertrophy, minimal	4	NA	NA	5	-	NA	NA	5
	Focal C-cell hyperplasia	-	NA	NA	-	-	NA	NA	1
	Follicular cell adenoma	-	NA	NA	1	-	NA	NA	-
Heart	Fibrosis, local	-	NA	NA	1	-	NA	NA	-
Kidney	Mineralization	-	NA	NA	3	-	NA	NA	-
Stomach	Inflammatory cell infiltration, serosa	-	NA	NA	-	-	NA	NA	1
Sternum	Fatty atrophy, marrow -minimal -mild	 - -	NA	NA	 - 1	 - -	NA	NA	 1 3

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Bone Marrow Smears

Duplicate femoral smears were taken at necropsy. No individual sample data was given. Summary data excerpted from the submission.

Samples from other animals were not available due to mortality or poor quality of slide preparation.

In total, 33/40 smears from control animals, 31/40 smears from animals at 10 mg/kg, 23/40 smears from animals at 30 mg/kg and 25/40 smears from animals at 75 mg/kg were available for bone marrow examination, as well as 17/20 smears from Control recovery and 12/20 smears from recovery animals at 75 mg/kg.

There were no abnormalities with regards to number, maturation and morphology of hematopoietic cell lines in any of the control animals.

In 5/31 animals (3 males and 2 females) receiving 10 mg/kg and in 3/23 animals (2 males and 1 female) receiving 30 mg/kg, a minimal to mild decrease in the number of cells from the erythroid series was observed, associated subsequently with a mild increase in the M:E ratio. Similar findings associated with increased proportion of cells from the eosinophilic series were seen in 1 female at 10 mg/kg and associated with an increased number of cells from the granulocytic series were seen in 1 male at 10 mg/kg.

In 5/31 animals (1 male and 4 females) at 10 mg/kg, and in 4/23 animals (2 males and 2 females) at 30 mg/kg, there was an increased proportion of cells from the eosinophilic series, with or without abnormal morphology characterized by the presence of abnormal cytoplasmic granulation, irregular nuclear shaped and enlarged cell size.

In 2/31 animals (2 females) at 10 mg/kg, 6/23 animals (4 males and 2 females) at 30 mg/kg and in 22/25 animals at 75 mg/kg (12 males and 10 females), the granulocytic series was characterized by maturation arrest, consisting of a mild to marked decrease in the proportion of late stage granulocytic precursors and mature cells, with or without abnormal morphology, including abnormal cytoplasmic granulation, irregular nuclear shaped and enlarged cell size. Often associated with the abnormal granulocytic maturation were an increased proportion of cells from the eosinophilic series, as described above.

The findings in the granulocytic series were associated with decreased number of cells from the erythroid series in the affected animals at 10 and at 30 mg/kg as well as in 6 males and 7 females at 75 mg/kg. In addition, in 8 animals at 75 mg/kg (5 males and 3 females), there were increases in the number of active macrophages and/or plasma cells.

After 4 weeks of recovery, the findings in granulocytic cell lines showed reversibility. Decreased number of erythroid cells were still observed in 5/12 recovery treated animals (4 males and 1 female) but were also seen in 1 recovery control male. Persistence of an increased proportion of cells from the eosinophilic series was seen in 1 female of the recovery treated group. All other findings showed complete reversibility.

Toxicokinetics

Methods
Blood samples collection: on day 1 and on the Friday dose cycle during weeks 11 and 25.

Results

Table 32. Toxicokinetic parameters in repeat dose toxicity study in rats

Toxicokinetic Parameters of panobinostat in Male Rat Plasma						
Dose (mg/kg)	Week	AUC $_{(0\text{-}24hr)}$	AUC/dose	C_{max}	C_{max}/dose	t_{max}
10	0	5	0.5	2	0.2	0.5
	11	54	5	12	1	1
	25	61	6	18	2	0.5
30	0	42	1	9	.5	0.5
	11	296	10	96	3	0.5
	25	266	9	90	3	0.5
75	0	101	1	26	0.5	0.5
	11	391	5	107	1	0.5
	25	555	7	129	2	1.0
Toxicokinetic Parameters of panobinostat in Female Rat Plasma						
Dose (mg/kg)	Week	AUC $_{(0\text{-}24hr)}$	AUC/dose	C_{max}	C_{max}/dose	t_{max}
10	0	4	0.5	2	0.5	0.5
	11	50	5	19	2	0.5
	25	95	9	39	4	0.5
30	0	22	1	9	0.5	1.0
	11	212	7	73	2	0.5
	25	313	10	172	6	0.5
75	0	148	2	58	1	0.5
	11	872	12	278	4	0.5
	25	662	9	279	4	0.5

Summary
- Panobinostat Cmax and AUC values in rats increased proportionally between doses.
- Exposure was higher at week 25, which suggests some drug accumulation.
- Female exposure appeared higher than males at the 75 mg/kg/day.

Stability and Homogeneity

Samples were taken during weeks 1 and 6 at the top, middle, and bottom from each formulation and also during weeks 11, 18, and 25 at the middle position prior to the commencement of dosing.

The panobinostat concentrations measured in the samples taken from the test item formulations at the beginning of dosing, during week 6, 11, 18, and 25 were found to be

within the accepted range of variation of ± 15%. In addition, the test item was demonstrated to be homogeneously distributed in the dose formulations. panobinostat was demonstrated to be stable in hydroxypropyl cellulose when kept during 24 hours at room temperature under ambient dark conditions or during 8 days in the refrigerator. In addition, the stability of the test item was demonstrated for 3 days when prepared as suspensions in hydroxyethylcellulose. The test item was not found in the vehicle samples.

The results of the additional sampling taken during Week 5 for stability from the lowest and highest concentrations after 24 hours at room temperature and 8 days refrigerated were found to be outside the acceptable criteria of ±15% (-18.8% after 8 days refrigerated at 2-8°C). The results of the repeat analysis taken during Week 11 were within the acceptable criteria.

Study title: 4 week (3 dose/week) oral (gavage) toxicity study in dogs with a 4 week recovery period

This GLP-compliant study was reviewed under IND 69862. The summary results are tabulated below.

Table 33. Results from repeat dose toxicity study in dogs under IND 69862

Results:

Animals	Beagle dogs, n= 3/sex/group. Recovery animals: n=2/sex for Groups 1 and 4.
Dose schedule	0 (control), 0.15, 0.5 and 1.5 mg/kg/day (groups 1, 2, 3, and 4, respectively). Three days/week x 4 with 4 week recovery period.
Mortality /Moribundity	No mortality or moribundity related to LBH589.
Clinical signs	Unremarkable.
Body weights	Unremarkable in group mean body weight or mean weight gain in males. The mean weight gain was reduced significantly in Group 4 females. The finding resolved in the recovery period.
Food consumption	Unremarkable
Ophthalmoscopy	Unremarkable
Electrocardiography	Unremarkable
Hematology	↓ ab. lymphocyte (♂ G4: #4001, 4002 and 4003), ↑ total WBC (1.6 fold) and ab. neutrophil (2.2 fold) in ♂ G3 #3001, slight ↓ red blood cell parameters (RBC, HGB, Hct) in all LBH589 treated animals (♂ and ♀). ↑ APTT in G4 animals: 5/5 ♂ and 4/5 ♀ (18% and 22%, respectively). The findings all resolved. Bone marrow smears results: low cellularity of bone marrow was noted in all animals, especially #1501, 2002, 3503 and 4501, may not be LBH589 related.
Clinical chemistry	Unremarkable, except ↓ cholesterol (G4 ♀), ↑ creatinine in all LBH589 treated animals (especially ♂ #3003, ♀ #4502 and 4504). All findings resolved
Urinalysis	Unremarkable
Organ weight	↓ thyroid (♂ and ♀ at all doses), and prostate, spleen, kidney and testes (♂ G4). All findings resolved.
Macroscopic findings	Unremarkable, except small prostate in G4 ♂ #4003.
Histopathological findings	Target organs included thyroid, bone marrow, prostate, testes, epididymides, stomach, small intestine and lymphoid tissue (thymus, spleen, mandibular, mesenteric lymph nodes and ileal lymphoid tissue). • Thyroid: decreased colloid and vacuolar changes in epithelium, all LBH589 treated animals, still see in one ♀ recovery animal. • Bone marrow: reduced cellularity (1/5 ♂and ♀ G4), ↓ erythropoiesis (all LBH589 treated males), still found reduced cellularity in recovery femles. • Prostate: attenuation of epithelium with reduced secretory granules (G4), resolved. • Male reproductive organs: epididymides (↑ luminal debris: 2/5 G4), testis (degeneration of seminiferous tubular epithelium with increased necrotic debris: 1/5 G4). 1/2 G4 recovery animal still noted oligospermia, ↑ epididymal debris and testicular degeneration. • Stomach: atrophy of gastric glands with increased fibrous tissue within the lamina propria (cardia and pyloric regions): all LBH589 treated animals, resolved. • Small intestine: dilated crypts containing necrotic debris or inflammatory cells (all G4), resolved. • Lymphoid tissue: thymus (atrophy: G4 ♂ and G 3, 4 ♀), spleen (depletion of splenic lymphoid tissues (G2, 3, and 4 ♀), other lymphoid tissues (mandibular & mesenteric lymph nodes and ileal lymphoid tisse: depletion, G4 ♂and ♀), partially resolved: splenic lymphoid depletion in females and mandibular lymph node depletion in 1/2 male.

Summary

- Male reproductive effects including attenuation of prostatic epithelium with reduced secretory granules, testicular degeneration, degeneration of seminiferous tubules, oligospermia and increased epididymal debris were observed at 1.5mg/kg/day (Group 4 or “G4” above).

- Thyroid toxicity included decreased colloid and vacuolation in epithelium.
- Other target organs include bone marrow, stomach, small intestine and other lymphoid tissues (thymus, spleen, mandibular and mesenteric lymph nodes and ileal lymphoid tissue).

Study title: 13 week (3 doses/week) Oral (Gavage) Toxicity Study in Dogs with a 4 Week Recovery Period

This study was not reviewed; it is summarized below to capture findings in the thyroid and reproductive organs.

Study no.:	510395
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
GLP compliance:	Statement included and signed

Key study findings

- Low triiodothyronine (T_3) was observed in many animals at 1 to1.5 mg/kg/dose. No microscopic changes were observed in these animals.
- Effects on male reproductive organs include prostatic atrophy, segmental hypoplasia in the testes and oligospermia in the epididymis.
- Other target organs for toxicities included the thymus, submandibular and mesenteric lymph nodes.
- All histological changes had resolved during the recovery period.

Study title: 39 Week (3 doses/week) Oral (Gavage) Toxicity Study in Dogs with a 4 Week Recovery Period

Study no.:	510746
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12 June 2006
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0623003, 99.4 %

Key Study Findings

- Enlarged vulva with bloody discharge in females at ≥ 0.15 mg/kg/day.
- Toxicities in the hematopietic/lymphocytic system: ↓ RBCs and lineages, ↓ WBCs and most differentials, ↑monocytes, immature myeloid series and ↓ segmented neutrophils in bone marrow, thymic atrophy, hemosiderin pigment in the spleen, lymphoid depletion or plasmacytosis in the lymph nodes.

- Other findings: liver (pigment), kidney (inflammatory cells, pigment, mineralization), lung (inflammation/ bronchopneumonia/ granuloma, osseous metaplasia), GI tract (inflammation, edema, microabcess, hemorrhage), and skin.
- Skin: wart-like growth (clinical observation), scab/abnormal shape (gross pathology), and papilloma and hyperplasia (histopathology).

Methods

Doses:	0, 0.15, 0.5, and 1.0 mg/kg
Dose justification:	Rising dose dog study (No. 0270176) & 4-week toxicity dog study, & 13-week toxicity study (No. 510395)
Frequency of dosing:	3 doses/week for 39 weeks with 4 week recovery
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	Sterile water
Species/Strain:	Beagle Dog
Number/Sex/Group:	Main: 6/sex/group for control & HD; 4/sex/group for LD & MD; Recovery: 2/sex/group (control and HD groups only)
Age:	8 months on arrival
Weight:	7.0 – 8.2 kg
Satellite groups:	Main study animals used
Unique study design:	None
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

Animals were checked early morning and as late as possible each day. No drug-related mortalities were observed.

Clinical observations

Animals were checked at regular intervals during the day for signs of ill health or reaction to treatment.

Table 34. Clinical observations in repeat dose toxicity study in dogs (39 weeks with 4 week recovery)

Clinical signs - Terminal	Male				Female			
No. animals	4	4	4	4	4	4	4	4
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
Loose feces	-	NA	2	4	-	-	-	-
Hair loss	-	-	-	2	-	-	-	1
Enlarged vulva, bloody discharge	-	-	-	-	-	2	4	4
Wart-like growth (interdigit, on pinna)	-	-	-	2	-	-	-	1
Cyst on left hind foot	-	-	-		-	-	-	1

Clinical signs - Recovery	Male				Female			
No. animals	4	4	4	4	4	4	4	4
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
Enlarged vulva, bloody discharge	-	-	-	-	-	-	-	1

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Bodyweight

Animals were weighed at least once weekly during acclimation, treatment periods and before recovery. No drug-related changes observed.

Food consumption

Food was measured and recorded daily. No drug-related changes observed.

Ophthalmoscopy

Animals were examined at pre-dose and during weeks 13 and 39 of treatment and week 4 of recovery. No drug-related changes were observed.

Electrocardiography

ECG tracings were recorded from all animals at pre-dose, and during weeks 1 and 39 of treatment; and during week 4 of the recovery period. Slight reductions (↓26% change control) in heart rate were observed in females given 75 mg/kg/day after 1-hr of dosing.

Hematology

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery) during week 4 of recovery

Table 35. Hematology parameters in repeat dose toxicity study in dogs (% change from control)

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
HB								
Week 13	15.9	-	↓14	↓23	15.7	-	-	↓25
Week 27	16.1	-	↓15	↓27	15.9	-	-	↓23
Week 39	16.6	-	-	↓25	16.9	-	-	↓24
Week 43	17	NA	NA	↓16	16.2	NA	NA	-
RBC								
Week 13	6.71	-	↓11	↓18	6.75	-	-	↓21
Week 27	6.93	-	-	↓18	6.83	-	-	↓15
Week 31	6.91	-	-	↓15	7.13	-	-	↓16
Week 43	7.31	NA	NA	↓12	6.94	NA	NA	-
HCT								
Week 13	0.463	-	↓15	↓21	0.454	-	-	↓23
Week 27	0.476	-	↓12	↓23	0.460	-	-	↓20
Week 31	0.488	-	-	↓21	0.496	-	-	↓20
Week 43	0.504	NA	NA	↓15	0.474	NA	NA	-
RET								
Week 13	1.1	-	-	↑36	0.8	-	↑50	
Week 27	1.3	-	-	-	0.7	-	↑57	↑188
Week 31	1.3	-	-	-	1.1	↑27	↑36	↑129
Week 43	1.4	NA	NA	↑43	1.2	NA	NA	↑45
LYM								
Week 13	2.67	-	↓21	↓35	2.37	↓16	↓7	↓25
Week 27	2.65	↓14	↓19	↓35	2.69	↓24	↓26	↓39
Week 31	2.57	-	↓13	↓37	2.85	↓17	↓18	↓37
Week 43	2.26	NA	NA	↓25	2.73	NA	NA	↓46
MON								
Week 13	0.45	↑13	↑87	↑136	0.42	↑19	↑64	↑140
Week 27	0.43	-	↑40	↑88	0.40	-	-	↑75
Week 31	0.42	-	↑144	↑69	0.44	-	↑102	↑189
Week 43	0.33	NA	NA	↑48	0.43	NA	NA	↓49
EOS								
Week 13	0.52	↓37	↓40	↓67	0.36	↓25	-	↓72
Week 27	0.49	↓35	↓31	↓59	0.51	↓47	-	↓67
Week 31	0.59	↓54	↓47	↓78	0.53	↓36	↓58	↓75
Week 43	0.60	NA	NA	↓12	1.11	NA	NA	↓74
BAS								
Week 13	0.11	↓18	↓36	↓64	0.12	↓33	↓33	↓75
Week 27	0.12	↓42	↓42	↓67	0.12	↓17	↓25	↓67
Week 31	0.14	↓43	↓36	↓71	0.19	↓26	↓58	↓79
Week 43	0.14	NA	NA	↓29	0.10	NA	NA	-
LEU								
Week 13	0.07	↓43	↓43	↓43	0.08	↓50	↓63	↓63
Week 27	0.02	-	↓50	↓100	0.03	↓33	↓67	↓100
Week 31	0.04	↓25	↓25	↓50	0.07	↓29	↓43	↓71
Week 43	0.05	NA	NA	↓40	0.02	NA	NA	↑50

T: Terminal necropsy; R: Recovery necropsy;
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Coagulation

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, and 39, and during week 4 of recovery. No drug-related changes were observed.

Clinical chemistry

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, and 39, and during week 4 of recovery.

Table 36. Clinical chemistry parameters in repeat dose toxicity study in dogs

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
AST								
Week 13	32	-	-	↑19	28	-	-	↑25
Week 27	39	-	-	↑26	29	↑14	-	↑23
Week 39	33	-	↑15	↑52	27	↑15	-	↑24
Week 43	38	NA	NA	-	27	NA	NA	-

T: Terminal necropsy; R: Recovery necropsy;
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Thyroid function

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, and 39, and during week 4 of recovery. No drug-related changes observed.

Troponin

Serum samples were obtained from clinical pathology blood samples as mentioned above. No drug-related changes were observed.

Urinalysis

Urine samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes observed.

Gross Pathology

Animals were necropsied up to 30-hr after their last dose or at the end of the 4-week recovery. Except for the finding on the skin, no drug-related changes observed.

NECROPSY FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4
DOSE	0 mg/kg	0.15 mg/kg	0.5 mg/kg	1.0 mg/kg	0 mg/kg	0.15 mg/kg	0.5 mg/kg	1.0 mg/kg
SKIN AND SUBCUTIS								
Scab				1				
Abnormal shape				1				

Organ Weights

Organ samples were taken at scheduled necropsy.

Table 37. Organ weights in repeat dose toxicity study in dogs

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
Spleen								
Week 39	0.78	-	-	↓13	1.053	↓24	↓28	↓60
Week 43	1.145	NA	NA	-	0.811	NA	NA	↑56
Thyroid								
Week 39	0.081	↓17	↓35	↓35	-	-	-	-
Week 43	0.007	NA	NA	↓40				

T: Terminal necropsy; R: Recovery necropsy;
NA: not applicable: no values given
(-): no change/ toxicologically significant change

Histopathology

Necropsied on completion of treatment with recovery animals necropsied 4-weeks after completion of treatment.

Adequate Battery (yes)
Peer Review (yes)
Histological Findings:

Table 38. Histopathology findings in repeat dose toxicity study in dogs

Microscopic findings - Terminal	Male				Female			
No. animals	4	4	4	4	4	4	4	4

Dose (mg/kg)		0	0.15	0.5	1	0	0.15	0.5	1
Lung	Inflammation, interstitial	1	1	1	2	-	-	-	4
	bronchopneumonia	-	-	-	1	-	-	-	2
	Granuloma, foreign body	-	-	1	2	-	-	-	1
	Pigmented macrophages	-		-	2	-	-	-	-
	Alveolar proteinosis, localized	-	-	-	1	-	-	-	-
	Osseous metaplasia, focal	-	-	-	1	-	-	-	1
	Bronchiolitis, fibrotic, chronic	-	-	-	-	-	-	-	1
LN, mesenteric	Lymphoid depletion								
	-minimal	-	-	-	-	-	-	-	3
	-mild	-	-	-	3	-	-	-	0
	-moderate	-	-	-	-	-	-	-	1
	Sinus histiocytosis	-	-	-	1	-	-	-	-
LN, axillary	Plasmacytosis	-	-	-	1	-	-	-	1
Spleen	Increased hemosiderin								
	-minimal	-	-	-	1	-	-	-	1
	-mild	-	-	-	1	-	-	-	1
	-moderate	-	-	-	1	-	-	-	0
	Increased hematopoeisis, extramedullary	-	-	-	-	-	-	-	1
Thymus	Atrophy								
	-minimal	-	-	-	1	-	1	1	-
	-mild	-	1	1	-	-	-	1	1
	-moderate	-	-	1	-	-	-	-	1
	-marked	-	-	-	1	-	-	-	1
Adrenal gland	Diffuse cortical cell vacuolation								
	-minimal	-	-	-	-	-	1	-	-
	-mild	-	-	-	-	-	-	1	-
	-moderate	-	-	-	-	-	-	-	2
Kidney	Inflammatory cell foci	-	1	1	1	-	-	-	-
	Pigment, epithelium	1	1	3	3	-	-	1	2
	Mineralization, medullary	-	-	-	2	-	-	-	-
Ileum	Inflammatory cell infiltration, lamina proporia	-	-	-	1	-	-	-	-
Cecum	Edema, submucosal	-	-	-	1	-	-	-	-
Colon	Dilated glands	-	-	-	-	-	-	-	1
Rectum	Crypt microabscess	-	-	-	-	-	-	-	1
Intestines	Agonal congestion, hemorrhage	-	-	2	2	-	-	-	-
	Typhlitis, hemorrhagic, localized	-	-	-	-	-	-	-	1
Liver	Hepatocyte pigment, centrilobular	-	-	-	-	-	-	-	2
	Pigmented Kupffer cells	-	-	-	2	-	-	-	3
Salivary gland	Inflammatory cell foci	-	-	-	-	-	-	-	1

(-): no change/ toxicologically significant change

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP	Grp 1	Grp 2	Grp 3	Grp 4	Grp 1	Grp 2	Grp 3	Grp 4
DOSE	0 mg/kg	0.15 mg/kg	0.5 mg/kg	1.0 mg/kg	0 mg/kg	0.15 mg/kg	0.5 mg/kg	1.0 mg/kg
SKIN AND SUBCUTIS	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
No abnormality detected	3	4	4	2	4	4	4	4
PAPILLOMA [B]	0	0	0	1	0	0	0	0
Inflammation, adnexal	0	0	0	1	0	0	0	0
Parasite granuloma, focal	1	0	0	0	0	0	0	0
Reactive hyperplasia, sebaceous glands, adnexal	0	0	0	1	0	0	0	0
Scab, healed	0	0	0	1	0	0	0	0

Microscopic findings - Recovery		Male				Female			
No. animals		2	0	0	2	2	0	0	2
Dose (mg/kg)		0	0.15	0.5	1	0	0.15	0.5	1
Lung	Alveolar foamy macrophage accumulation	-	NA	-	1	-	-	-	-
	Inflammatory cell foci, scattered	-	-	-	1	-	-	-	2
	Osseous metaplasia, focal	-	-	-	-	-	-	-	1
	Bronchiolitis, fibrotic, chronic	-	-	-	-	-	-	-	1
Thymus	Involution	1	-	-	2	1	-	-	2

NA: not applicable: no values given
(-): no change/ toxicologically significant change

Bone Marrow Smears

Smears and specimens were collected from each animal at necropsy.

Table 39. Bone marrow findings in repeat dose toxicity study in dogs

Bone Marrow Smears - T	Male				Female			
No. animals	4	4	4	4	4	4	4	4
Dose (mg/kg)	0	0.15	0.5	1	0	0.15	0.5	1
Increased in immature myeloid series, minimal	-	-	-	1	-	-	-	1
Decrease in mature segmented neutrophils	-	-	-	1	-	-	-	1

T: Terminal necropsy
(-): no change/ toxicologically significant change

Toxicokinetics

Methods

Blood samples collection: on day 1 and during weeks 13 and 39 at 0.5, 1, 2, 6, and 24-hr after dosing.

Results

Table 40. Toxicokinetic parameters in repeat dose toxicity study in dogs

Toxicokinetic Parameters of panobinostat in Male Rat Plasma						
Dose (mg/kg)	Week	AUC $_{(0\text{-}24hr)}$	AUC/dose	C_{max}	C_{max}/dose	t_{max}
0.15	0	10	64	3	23	0
	13	23	153	5	33	0
	39	17	113	3	19	0.25
0.5	0	36	71.8	9	19	0.25
	13	63	125	17	34	0
	39	61	122	14	28	0
1.0	0	63	63	25	25	0.25
	13	96	96	34	34	0
	39	71	71	16	16	0.25
Toxicokinetic Parameters of panobinostat in Female Rat Plasma						
Dose (mg/kg)	Week	AUC $_{(0\text{-}24hr)}$	AUC/dose	C_{max}	C_{max}/dose	t_{max}
0.15	0	12	78	4	25	0
	13	20	131	4	30	0.25
	39	14	90	3	23	0
0.5	0	31	61	12	24	0
	13	67	134	15	30	0.25
	39	60	121	14	27	0.25
1.0	0	86	86	38	38	0
	13	96	96	32	32	0
	39	92	92	24	24	0.25

Summary

- Panobinostat Cmax and AUC values in dogs increased proportionally between doses.
- There was no difference in exposure between males and females.
- Exposure at 13 and 39 weeks was similar.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity test using *Salmonella typhimurium*

Study no.:	0212012
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	Novartis Pharma AG; Basel, Switzerland
Date of study initiation:	24 July 02
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0251001, 98.8%

Key Study Findings

- Panobinostat was positive in this mutagenicity test using TA97a and TA1535 strains.

Methods

Strains: *Salmonella typhimurium* TA1535, TA97a, TA98, TA100, TA102
Concentrations in definitive study:
1st study: 8, 40, 200, 1000, 5000, μg/plate;
2nd study: 312.5, 625, 1250, 2500, 5000 μg/plate
Basis of concentration selection: Preliminary experiment was performed (data not shown)
Negative control: Vehicle control
Positive control:
With S9: 2-aminoanthracene
Without S9: benzo(a)pyrene, sodium azide, 9-aminoacridine, 2-nitrofluorene, mitomycin
Formulation/Vehicle: DMSO
Incubation & sampling time:
Pre-incubation: 37°C at 20 minutes
Plate incorporation: 37°C for 3 days
Analysis: After incubation, the plates were counted and were visually checked for the presence of a light background lawn of growth. Counting was done using an image analyzer

Study Validity

This study is considered valid due to the following reasons:

- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2).
- The positive control values were within the laboratory historical ranges.
- The appropriate positive control compounds (± S9 mix) induced increases in revertant colony numbers to at least twice the respective vehicle control levels with the appropriate tester strain.
- The mean mutant number of negative control plates lay within the range of acceptable values

Criteria for positive

- If the test item produces a response equal to twice (or more) of the negative control incidence to the test item in the test plate.
    - Exception: strain TA102: Increase by a factor of 1.5 over the negative control is considered an indication of mutagenic effect.
- Negative and positive findings are reproducible in at least two independent experiments

  - If results are not clear after two independent experiments, additional experiments using only the critical strains and concentrations of the test item close to the critical range in question.
- Results have greater significance if there is a concentration-related increase in the number of reverent colonies
- If there are borderline effects, inter-plate variability is taken into consideration

Study Outcome

Precipitation: No precipitation was observed at any concentration in the plate incorporation assay. In the pre-incubation assay, precipitation was observed 2500 μg/plate for the TA97a +S9 and all other plates at 5000 μg/plate except for the TA102 plate -S9 activation

Bacteriotoxicity: Changes were observed at 5000 μg/plate for all strains in the plate incorporation assay. Changes were also observed at all concentrations in the pre-incubation assay.

Mutagenicity: *(figures excerpted from the submission)*

Figure 17. Colony growth following treatment with panobinostat (Ames assay)

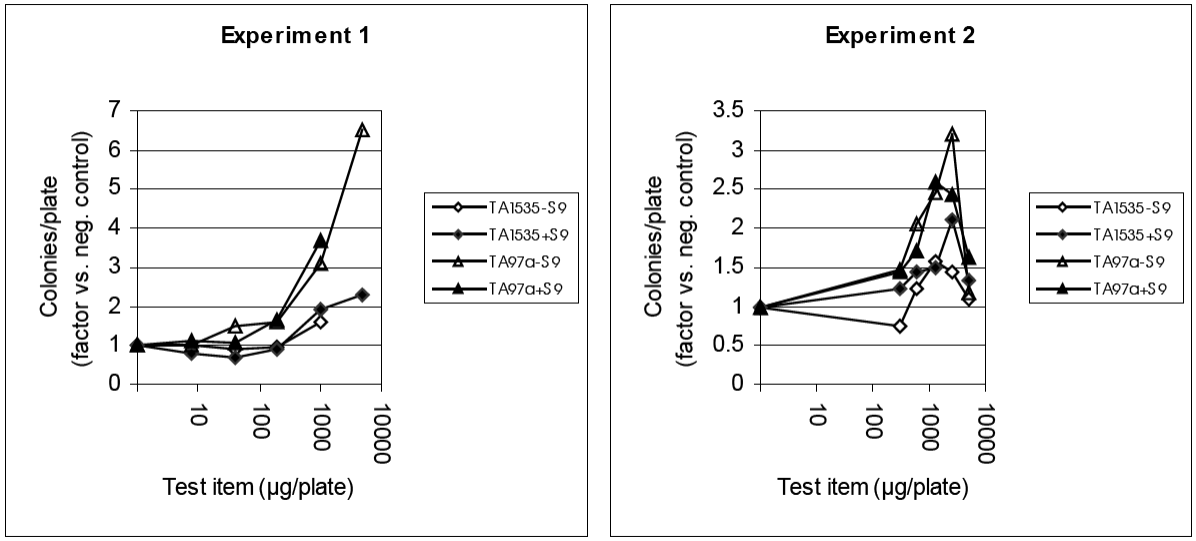


Table 41. Revertant colony count following treatment with panobinostat (Ames assay)

Plate Incorporation Assay -S9						
Treatment	Concentrations (μg/plate)	Revertant Colonies Per Plate (mean ± SD, n = 3)				
		TA1535	TA97a	TA98	TA100	TA102
DMSO	0	23±8	154±10	34±3	134±8	300±28
panobinostat	8	24±8	152±14	28±9	125±12	330±28
	40	21±1	226±13	29±2	128±12	310±12
	200	22±1	243±11	34±3	136±12	300±16
	1000	37±13	499±27	48±5	188±18	292±29
	5000	BGO	BGO	BGO	BGO	113±26 BGR
Positive Control	-	979±24	1916±267	515±7	731±47	521±78
Plate Incorporation Assay +S9						
Treatment	Concentrations (μg/plate)	Revertant Colonies Per Plate (mean ± SD, n = 3)				
		TA1535	TA97a	TA98	TA100	TA102
DMSO	0	18±4	136±7	39±2	127±8	262±58
panobinostat	8	14±1	153±14	49±3	123±8	306±20
	40	13±2	148±10	44±4	124±10	316±24
	200	16±3	222±17	41±5	148±8	313±19
	1000	35±9	499±27	56±10	206±7	312±35
	5000	BGO	BGO	BGO	BGO	220±19 BGR
Positive Control	-	479±24	1828±189	3215±616	972±199	470±20

BGO- No background growth
BGR- Background growth reduced

Pre-incubation Assay -S9						
Treatment	Concentrations (μg/plate)	Revertant Colonies Per Plate (mean ± SD, n = 3)				
		TA1535	TA97a	TA98	TA100	TA102
DMSO	0	21±3	173±11	33±5	130±3	134±23
panobinostat	312.5	16±1	256±3	29±4	127±17	128±39
	625	26±6	358±42	29±6	145±8	87±20
	1250	33±3	426±31	33±7	138±4	75±1
	2500	30±6	553±49	39±8	190±27	180
	5000	23±4	203	38±2	140±6	184
Positive Control	-	875±19	1872±326	364±51	643±23	304.55
Pre-incubation Assay +S9						
Treatment	Concentrations (μg/plate)	Revertant Colonies Per Plate (mean ± SD, n = 3)				
		TA1535	TA97a	TA98	TA100	TA102
DMSO	0	18±5	164±8	37±7	129±11	283±5
panobinostat	312.5	22±5	237±5	45±9	153±7	280±7
	625	26±5	280±29	37±3	149±11	279±24
	1250	27±5	424±28	40±5	181±22	306±37
	2500	38±9	400±191	45±2	161±9	302±4
	5000	24±7	265±14	39±8	151±12	290±22
Positive Control	-	332±25	1962±279	2122±398 124±5	1613±109	369±29

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Chromosome aberration test in cultured human peripheral blood lymphocytes

Study no.:	0212210
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	Novartis Pharma AG; Basel, Switzerland
Date of study initiation:	5 July 02
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0251001, 98.8%

Key Study Findings

- Panobinostat tested negative for chromosome aberrations; however, increased frequency of endoreduplication was observed indicating increased number of chromosomes.

Methods: *In vitro* clastogenicity test
Cell line: Human peripheral blood lymphocytes
Concentrations in definitive study:
Experiment C
-S9: 0.4, 0.5, 0.6, 0.7, 0.9, 1.1, 1.3, 1.6, 1.9, 2.4 μg/mL
+S9: 0.6, 0.9, 1.3, 1.9, 2.8, 4.2, 6.3, 9.4, 14, 24 μg/mL
Experiment D
-S9: 0.01, 0.02, 0.04, 0.10, 0.21, 0.44, 0.95, 2.05, 4.42, 9.52 μg/mL
+S9: 0.1, 0.2, 0.3, 0.9, 1.5, 2.7, 4.7, 8.2, 14.3 μg/mL
Experiment E
-S9: 0.01, 0.02, 0.04, 0.10, 0.21, 0.44, 0.95, 2.05, 4.42, 9.52 μg/mL

In experiments A, B and D (3-hour treatment, 17 hours recovery, in the absence of S9) panobinostat were toxic at all tested concentrations.
Basis of concentration selection: based on the examination of the depression of the mitotic index during the chromosomal aberrations test. panobinostat was proven to be stable for 24 hours at room temperature in DMSO and phosphate buffer
Negative control: Vehicle control
Positive control:
-S9: ethyl methanesulfonate (EMS)
+S9: cyclophosphamide (CP)
Formulation/Vehicle: DMSO

Incubation & sampling time: The cells were incubated with or without metabolic activation in a cell incubator at 37°C, 5% CO_2
Treatment: Rat liver S9 was used as the metabolic activation system. The concentration the S9 mix was 10%, which is within the acceptable range described in the FDA/CFSAN Redbook.

Table 42. Summary of treatment conditions in chromosomal abberation assay (human peripheral blood lymphocytes)

Summary of treatment conditions

Treatment		S9	Duration of treatment (hours)	Harvest time (hours after start of treatment)
Continuous	20+0	-	20	20
Pulse	3+17	-	3	20
	3+17	+	3	20

Cultures receiving continuous treatments retained treatment medium until harvest. Pulse treatments were for 3 hours only. Cells were then pelleted (200g, 10 minutes), washed twice with sterile saline (prewarmed to 37°C), and resuspended in fresh medium containing fetal calf serum and gentamycin.

(Excerpted from the submission)

Study Validity
This study is considered valid, due to the following reasons:

- The positive controls were appropriate and produced expected results.
- The appropriate numbers of cells were evaluated (100 metaphase spreads per duplicate treatment) and duplicate cultures were treated.

Criteria for positive genotoxicity

- Panobinostat was considered to be positive for clastogenicity if there are statistically significant increases in the frequency of metaphases with aberrant chromosomes observed at one or more concentrations. The increases exceed the historical negative control ranges. The increases are reproducible between replicates and tests. The increases are not associated with large changes in pH or osmolarity of the treatment medium or extreme toxicity. There is evidence of a dose-dependent relationship.

Study Outcome
Precipitation: observed at concentrations of $\geq$ 918.7μg/mL

Mitotic Index: Dose-dependent decrease in the mitotic index in experiment C, D, and E in the presence and absence of S9.

Concentration analysis (based on mitotic index):

-S9: 0.6, 1.1, 2.4 μg/mL (20-hr continuous treatment, experiment C); 0.04, 0.10, 0.21, 0.44μg/mL (3-hr treatment with 17-hr recovery, experiment E)
+S9: 0.6, 1.3, 9.4 μg/mL (3-hr treatment with 17-hr recovery, experiment C); 0.1, 0.2, 0.3, 0.5 μg/mL (3-hr treatment with 17-hr recovery, experiment D)

Chromosome aberration test: aberration frequents were all within the historical range controls in the presence and absence of metabolic activation. There was in increase in frequencies of endoreduplicated cells.

Table 43. Results of chromosomal aberration assay

Experiment C	-S9: after continuous treatment for 20 hours				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells	% Endo-reduplicated Cells
Control					
DMSO/RPMI	3.6*	0.5	0.5	0.5	0.0
LBH589 [μg/ml]					
2.4	32.4 **	0.6	0.0	2.0	62.4
1.9	31.0 **	ND	ND	ND	ND
1.6	47.9 **	ND	ND	ND	ND
1.3	56.3 **	ND	ND	ND	ND
1.1	60.6 **	0.0	0.0	2.2	58.1
0.9	46.5 **	ND	ND	ND	ND
0.7	43.7 **	ND	ND	ND	ND
0.6	63.4 **	0.6	0.0	2.4	64.1
0.5	60.6 **	ND	ND	ND	ND
0.4	59.2 **	ND	ND	ND	ND
Positive Control, EMS (mM)					
8.0	0.8 *	18.0	3.0	1.0	0.0

Experiment E	-S9: after treatment for 3-hr and recovery for 17-hr				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells	% Endo-reduplicated Cells
Control					
DMSO/RPMI	5.4 *	0.5	0.0	1.5	0.0
LBH589 [μg/ml]					
9.52	16.8 **	ND	ND	ND	ND
4.42	23.4 **	ND	ND	ND	ND
2.05	21.5 **	ND	ND	ND	ND
0.95	18.7 **	ND	ND	ND	ND
0.44	40.2 **	1.5	0.0	2.2	23.7
0.21	47.7 **	1.5	0.0	1.8	8.5
0.10	55.1 **	0.5	0.0	1.9	1.9
0.04	71.0 **	0.5	0.0	2.4	1.4
0.02	65.4 **	ND	ND	ND	ND
0.01	73.8 **	ND	ND	ND	ND
Positive Control, EMS (mM)					
10.0	1.8 *	32.0	8.0	5.7	0.0

ND: not determined; *: mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells;
**: mitotic indices as % controls; % Cells Exch: % cells with exchanges; % Ab. Cells: % aberrant cells (exclusive gaps)

Experiment C	+S9: after treatment for 3-hr and recovery for 17-hr				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells	% Endo-reduplicated Cells
Control					
S9/DMSO	4.9 *	1.0	0.0	2.0	0.0
LBH589 [µg/ml]					
21.0	9.3 **	ND	ND	ND	ND
14.0	22.7 **	ND	ND	ND	ND
9.4	46.4 **	0.6	0.0	1.2	60.9
6.3	20.6 **	ND	ND	ND	ND
4.2	21.6 **	ND	ND	ND	ND
2.8	18.6 **	ND	ND	ND	ND
1.9	24.7 **	ND	ND	ND	ND
1.3	29.9 **	1.2	0.0	1.1	52.6
0.9	29.9 **	ND	ND	ND	ND
0.6	63.9 **	1.1	0.0	1.5	52.2
Positive Control, CP (µM)					
65.0	3.0 *	7.0	1.0	1.0	1.0

Experiment D	+S9: after treatment for 3-hr and recovery for 17-hr				
	Mitotic Index %	% Ab. Cells	% Cells Exch.	% Poly-ploid Cells	% Endo-reduplicated Cells
Control					
S9/DMSO	7.6 *	2.0	0.0	0.0	0.0
LBH589 [µg/ml]					
14.3	7.9 **	ND	ND	ND	ND
8.2	13.2 **	ND	ND	ND	ND
4.7	3.3 **	ND	ND	ND	ND
2.7	7.9 **	ND	ND	ND	ND
1.5	14.6 **	ND	ND	ND	ND
0.9	6.0 **	ND	ND	ND	ND
0.5	24.5 **	3.0	0.0	2.1	12.8
0.3	55.0 **	1.5	0.0	1.9	4.2
0.2	60.9 **	1.0	0.0	1.0	2.9
0.1	82.1 **	0.5	0.0	1.5	1.0
Positive Control, CP (µM)					
65.0	2.4 *	22.0	3.0	1.0	0.0

ND: not determined; *: mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells;
**: mitotic indices as % controls; % Cells Exch: % cells with exchanges; % Ab. Cells: % aberrant cells (exclusive gaps)

(Excerpted from the submission)

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

The rodent micronucleus assay was not performed.

7.4 Other Genetic Toxicity Studies

Study title: Comet assay in vitro with L5178Y mouse lymphoma cells

Study no.: 0115032
Study report location: eCTD 4.2.3.3.1

Key Study Findings: panobinostat was positive in vitro for DNA damage.

Methods:
In comet assay panobinostat (55 nM-225 nM) was incubated in vitro for 3 hours with L5178Y mouse lymphoma cells that had been pre-treated with/without S9-liver homogenate from Aroclor 1254-treated male rats. DNA damaged was assessed by tail moment.

Results:

Table 44. Results from in vitro Comet assay in L5178Y mouse lymphoma cells

Experiment 1	DNA damage	Viability (ATP content)	Cytotoxicity
Treatment (3 hr, -S9)	Tail Moment	% of control	% cells with non-detectable nuclei
RPMI/DMSO	0.32	100	0.0
55.5 µg/ml	0.55	100	0.0
83.5 µg/ml	0.61	100	1.0
111.9 µg/ml	0.75	100	7.5
166.9 µg/ml	0.95*	100	12.0
222.2 µg/ml	2.06*	100	18.5
MMS, 25 µg/ml	3.47*	100	0.0

Experiment 2

Treatment (3 hr, +S9)	Tail Moment	ATP content % of control	% cells with non-detectable nuclei
S9/DMSO	0.13	100	0.0
20.9 µg/ml	0.19	100	0.0
27.8 µg/ml	0.32	97	1.0
41.7 µg/ml	0.30	100	1.0
55.5 µg/ml	0.50*	91	3.0
83.5 µg/ml	0.38	76	8.0
111.0 µg/ml	0.37	59	16.0
166.0 µg/ml	Toxic	27	n.d.
2-AA, 19 µM	1.51*	24	n.d.

MMS: methyl methanesulfonate
RPMI: medium
2-AA: 2-amino anthracene
DMSO: dimethyl sulphoxide
* indicates positive effect

Summary

Panobinostat caused DNA damage in a concentration-dependent manner without S9 mix. There was no toxicologically significant effect observed in the presence of S9. Under the assay condition, panobinostat was considered positive in vitro for DNA damage.

8 Carcinogenicity

Carcinogenicity studies were not conducted and are not necessary to support the safety of panobinostat for the proposed refractory multiple myeloma indication.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: panobinostat: An oral (gavage) fertility and early embryonic development study in the rat

Study no.:	0670759
Study report location:	eCTD 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	18 July 07
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0623006, 99.4%

Key Study Findings

- 2 mortalities observed (1/25 at 0 mg/kg/day & 1/25 at 30 mg/kg/day). No death at the high dose of 100 mg/kg/day.
- ↓ body weights throughout treatment at all doses with > 10% reduction at 100 mg/kg/day.
- Gross pathology indicated small prostate, dark red ovary at MD and HD, and dark material (suggests bleeding) in vagina.
- No drug-related effect on estrous cycle.
- Mating Index, Fertility Index and Conception Rate were decreased in female rats by 8%, 20%, and 13%, respectively, at 100 mg/kg/day.
- ↑ early resorptions ≥ 10 mg/kg/day.
- ↑ post-implantation loss at doses ≥ 10 mg/kg/day.
- ↓ live embryos ≥ 30 mg/kg/day.
- ↓ sperm motility at 100 mg/kg/day dose.

Methods

Doses*:	0, 10/12.6, 30/30.7, and 100/125.8 mg/kg/day * *Dose levels indicated as base/salt - conversion factor base to salt for panobinostat is 1.258
Dose justification:	13-week oral toxicity study (No. 457803)
Frequency of dosing:	Males: 3 doses/week for 4 weeks prior to mating and during the 2 week mating period until terminal necropsy. Females: 3 doses/week 2 weeks prior to mating,

	during the 2 week mating period or until mated. During gestation, females were dosed on days 0, 3 and 6 of gestation.
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) hydroxypropylcellulose (grade HF) NF (Klucel)
Species/Strain:	Rat, Crl:WI (Han)
Number/Sex/Group:	25/sex/group
Age:	12 weeks (males); 9 weeks (females)
Weight:	311 – 370 (males); 170 – 215 (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

Animals were checked twice daily (am/pm).

Table 45. Cause of death in fertility and early embryonic development toxicity study in rats

Animal #	Dose (mg/kg/day)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
1021	0	M	19	Sacrificed	Decreased activity, teeth grinding, black/red fur staining, abnormal breathing, weak, hunched posture, cranial mass, right kidney: dilatation pelvis
3024	30	M	20	Found dead	No clinical signs

Clinical Signs

Animals were checked within 3-hrs after dosing. Detailed examinations were performed weekly.

Table 46. Clinical signs in fertility and early embryonic development toxicity study in rats

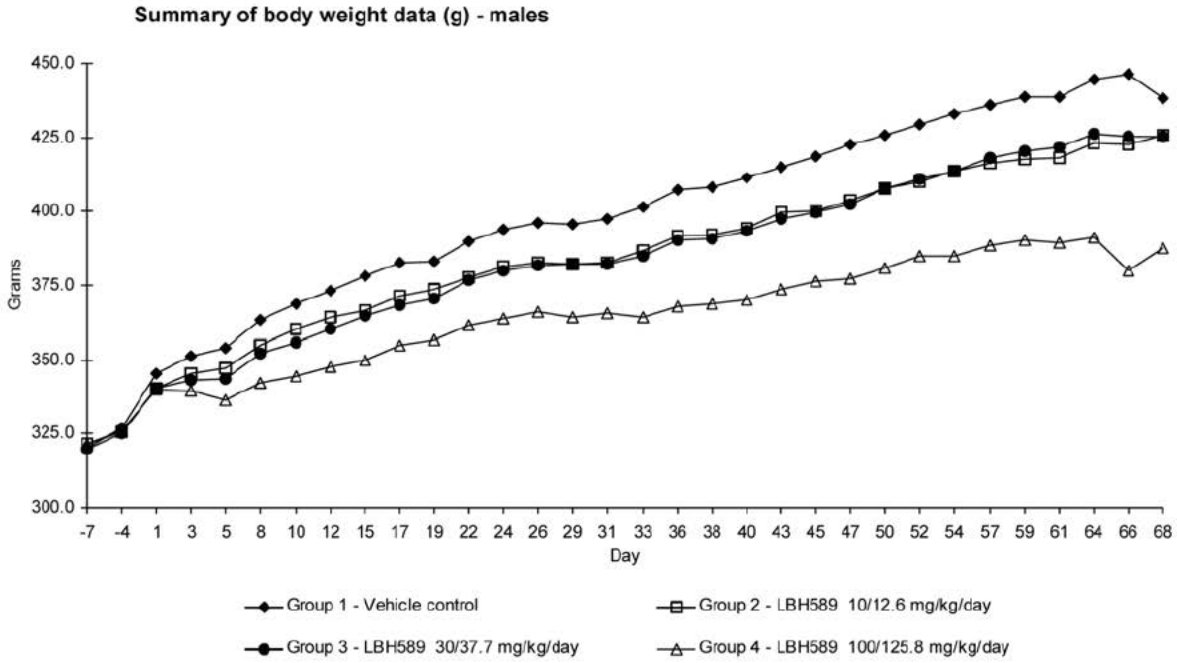
Dose (mg/kg/day)	0	10	30	100
No. animals (M & F)	25	25	25	25
Decreased activity	1	-	-	2
Labored breathing	-	-	-	1
Suspected dehydration	1	-	-	2
Hunched posture	-	-	-	2
Salivation	-	-	-	4
Thin	-	-	-	2

M: males; F: females
(-): no change/ toxicologically significant change

Body Weight

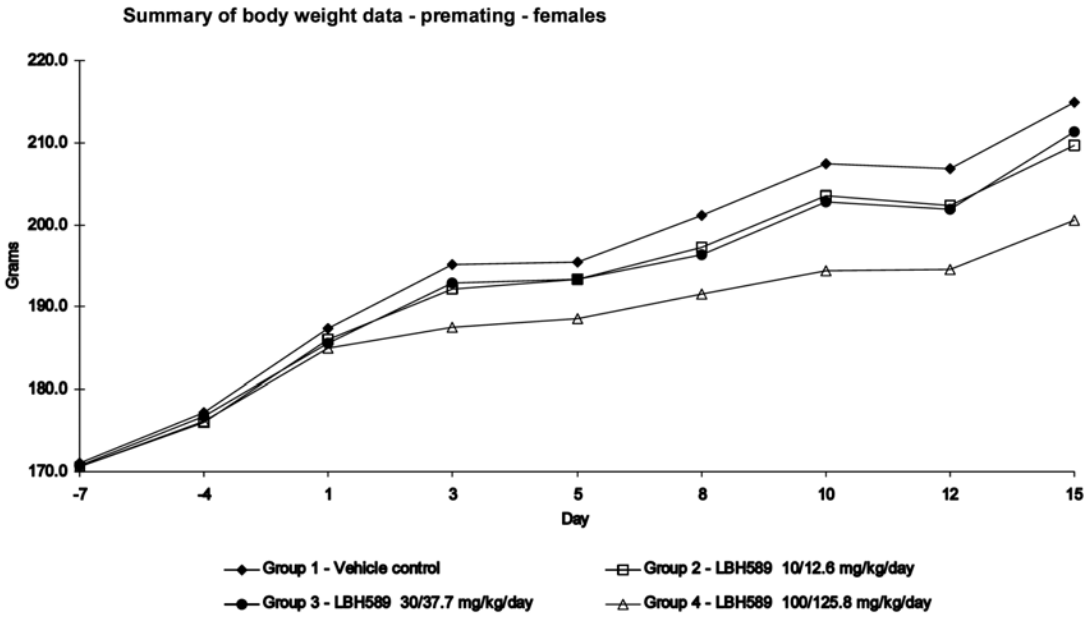
Animals were measured 3 times per week during dosing. Mated females were weighed on days 0, 3, 6, 10, and 13 of gestation. Decreased body weights were observed at all doses throughout treatment.

Figure 18. Body weight change in fertility and early embryonic development toxicity study in rats (males)



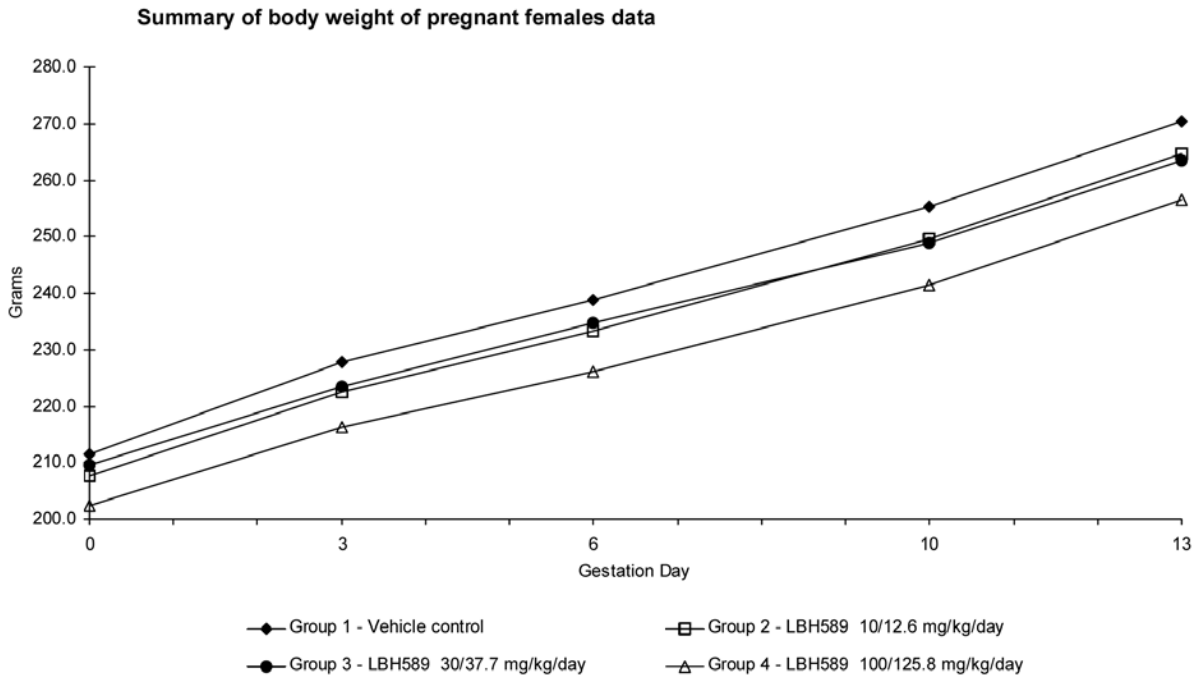
(Excerpted from the submission)

Figure 19. Body weight change in in fertility and early embryonic development toxicity study in rats (females)



(Excerpted from the submission)

Figure 20. Body weight change in fertility and early embryonic development toxicity study in rats (pregnant females)



(Excerpted from the submission)

Food Consumption

Food was measured weekly except during mating. Mated females' food consumption was measured on days 0 – 3, 3 – 6, 6 – 10, and 10 – 13 of gestation. No drug-related changes observed.

Estrous cycles

Cycles were determined for 14 days prior to mating and during mating until the day of positive identification of mating by vaginal lavage. Animals were observed for the number of days in estrus, the number of cycles seen, and the average cycle length. No drug-related changes observed.

Necropsy

Animals were necropsied at the end of treatment.

Table 47. Gross pathology findings in fertility and early embryonic development toxicity study in rats

Macroscopic findings		Male				Female			
Dose (mg/kg/day)		0	10	30	100	0	10	30	100
No. animals		25	25	25	25	25	25	25	25
Kidney	Dark area	-	-	-	1	-	-	-	-
Liver	Mass	-	-	-	1	-	-	-	-
Lung	Dark area	-	-	-	2	-	-	-	-
LN, mesenteric	Discolored, dark	-	-	2	3	-	-	-	-
Prostate	Small	-	-	1	4	-	-	-	-
Thymus	Small	-	-	-	1	-	-	-	-
Ovary	Dark area	-	-	-	-	-	-	1	1
Uterus	Dilatation	-	-	-	-	-	-	-	1
	Fluid pale opaque	-	-	-	-	-	-	-	1
Vagina	Dark material	-	-	-	-	-	-	1	1

(-): no change/ toxicologically significant change

Organ Weights

Organs were taken from animals at the completion of treatment. Paired epididymides and testes were weighed separately and compared relative to bodyweight. No drug-related changes were observed between epididymides and testes weight compared to controls.

Fertility Parameters

Mating Index = (# of females mated)/ (# of females placed for mating)
Fertility Index= (# of pregnant females)/ (# of females placed for mating)
Conception rate= (# of pregnant females)/ (# of mated females)

Table 48. Fertility parameters in fertility and early embryonic development toxicity study in rats

Dose (mg/kg/day)	0	10	30	100
No. Animals (M/F)	24/25	25/25	25/25	25/25
Mating Index (%)	100	100	100	92
Days to Mating (mean)	2.2	2	2.2	2.6
Fertility Index (%)	100	100	96	80
Conception Rate (%)	100	100	96	87

Pregnancy Parameters

Table 49. Pregnancy parameters in fertility and early embryonic development toxicity study in rats

Dose (mg/kg/day)	0	10	30	100
No. Pregnant Animals	25	25	24	20
Number Corpora Lutea	13.8	13.1	13.4	13.1
Number Implantation Sites	11.6	11.9	10.8	10.9
Number Live Embryos	11.2	11.1	9.9*	9.4*
Number Dead Fetuses	0	0	0	0.1
Early Resorptions	0.3	0.8	0.9*	1.5*
Pre-implantation Loss	17.62	10.07	19.10	18.07
Post-implantation Loss	2.74	6.99	8.62	13.86**

*: statistically significant compared to controls $p \leq 0.05$; **: statistically significant compared to controls $p \leq 0.01$

Male Sperm Parameters

Table 50. Male sperm parameters in fertility and early embryonic development toxicity study in rats

Dose (mg/kg/day)	0	10	30	100
No. Animals	23	25	24	25
Spermatozoa Count	362	455	373	394
Motility (%)	80.8	81	80	76

Stability and Homogeneity

(Excerpted from the submission)

Samples were taken from the top, middle and bottom of the dose formulations prepared for Week 1 of the study. Samples were also taken from the middle of the dose formulations for Week 10 of the study. Each was diluted with diluent to give concentrations within the range of the calibration curve. The control samples were diluted equivalent to the low dose study samples. Mean sample concentrations within ±15% of their nominal concentrations (individual values within ±20%) were considered acceptable. Homogeneity was considered acceptable if the difference between the individual recoveries from the top, middle and bottom samples, and the overall average recovery for each group, was within ±15%.

The dose formulations were within specifications (the mean % target values were within 95.0 to 104% of target). Homogeneity testing showed that the formulation technique used produces homogenous preparations.

TK
Not done.

9.2 Embryonic Fetal Development

Study title: An oral embryo-fetal development study of panobinostat in the rat

Study no.:	0670511
Study report location:	eCTD 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08 Oct 06
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0623006, 99.4%

Key Study Findings

- 31 maternal mortalities observed (9/22 at 100 mg/kg/day & 22/22 at 300 mg/kg/day). Therefore, maternal data discussed are mainly for the low dose (30 mg/kg/day) and the mid dose (100 mg/kg/day). In addition, there was no live fetus at the mid dose of 100 mg/kg/day; hence, no fetal finding is available at this dose.
- Clinical signs of ↓ activity, partly closed eyes, weakness, thin observed.
- Maternal pathologic findings include hemorrhage in adrenals, GI tract, and lung as well as erosion in GI tract and skin..
- ↑ resorptions and post implantation loss at 30 and 100 mg/kg/day.
- No live fetus at 100 mg/kg/day; reduced live fetuses at 30 mg/kg/day.
- ↓ fetal weights at 30 mg/kg/day.
- Fetal malformations at 30 mg/kg/day include: cleft palate and short tail (microcaudia).
- Skeletal anomalies and variations at 30mg/kg/day include: incomplete ossification, extra presacral vertebrae, and extra ribs.

Methods

Doses*:	0, 30/30.7, 100/125.8, and 300/377.4 mg/kg/day * Dose levels indicated as base/salt - conversion factor base to salt for panobinostat is 1.258
Dose justification:	13-week oral toxicity study (No. 457803)
Frequency of dosing:	Groups 1, 2, and 3 were treated from gestation days (GD) 6 – 17 of gestation. Group 4 animals were dosed from GD 6 – 10 (replicate 1), GD 6 – 9 (replicate 2), GD 6 – 8 (replicate 3) and 6 – 7 (replicate 4)
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) hydroxypropylcellulose (grade HF) NF (Klucel)
Species/Strain:	Wistar Hannover Rat, Crl:WI

Number/Sex/Group:	22/group
Age:	74 – 80 days old
Weight:	222 – 265 g
Satellite groups:	See table
Unique study design:	None
Deviation from study protocol:	None that affected study outcome

Table 51. Study design for embryonic fetal development toxicity study in rats

Study design, animal allocation and test article doses

Group number identification	Dose level* (mg/kg/day)	Dose volume (mL/kg/day)	Animal number females	
			Main study	Toxicokinetic
1/ Vehicle control	0	10	1501-1522	1523-1526
2/ LBH589	30/37.7	10	2501-2522	2523-2530
3/ LBH589	100/125.8	10	3501-3522	3523-3530
4/ LBH589	300/377.4	10	4501-4522	4523-4530**

* Dose levels indicated as mg/kg/day base/salt

** No blood was collected from group 4 toxicokinetic animals as this dose level was terminated early.

(Excerpted from the submission)

Observations and Results

Mortality

Animals were checked twice daily for signs of mortality, ill health, or reaction to treatment.

Table 52. Cause of maternal death in embryonic fetal development toxicity study in rats

Animal #	Dose (mg/kg/day)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
3503	100	F	12	Sacrificed	↓ activity, cold to touch, weak, ↓ muscle tone
3504	100	F	11	Found dead	↓ activity, ↓muscle tone, ↓, clear eye discharge, eyes partially closed,
3506	100	F	12	Sacrificed	↓ activity, ↑ vocalization, thin, weak
3511	100	F	16	Sacrificed	Decrease activity, thin, weak, pale skin, red vaginal discharge, eyes partially closed
3513	100	F	14	Sacrificed	↓ activity, backbone prominent, thin, weak, decrease feces output
3514	100	F	14	Sacrificed	↓ activity, pale skin, thin, weak, abnormal, reduced feces size, eyes partially closed
3517	100	F	16	Sacrificed	Clear eye discharge, red fur staining, ↓ activity, thin, warm to touch, weak, pale skin, eyes partially closed
3520	100	F	11	Found dead	No clinical signs
3522	100	F	14	Sacrificed	↓ activity, thin, weak, hunched posture,

Animal #	Dose (mg/kg/day)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
					eyes partially closed, clear eye discharge, abnormal breathing sound, black vaginal discharge
4501	300	F	10	Sacrificed	↓ activity, eyes partially closed
4502	300	F	9	Sacrificed	↓ activity, weak, red fur staining, tongue swollen (4 days after dosing)
4503	300	F	9	Sacrificed	No clinical signs
4504	300		9	Sacrificed	↓ activity, weak, eyes partially closed
4505	300	F	9	Found dead	Partially closed eyes, weak
4506 - 4517	300	F	8	Sacrificed	No clinical signs
4518 - 4522	300	F	7	Sacrificed	No clinical signs

Clinical Signs

Detailed examinations were performed on the gestation days of body weight assessment. No other drug-related changes observed in addition to those listed in the mortality section.

Body Weight

Individual bodyweights were measured on GD 0, 3, 6, 9, 12, 15, 18, and 21. Changes in bodyweights were observed in all treatment groups compared to controls with body weight loss at ≥ 100 mg/kg/day.

Reduced BW at 100 mg/kg/day is partially due to the reduced uterine weight; 12 out 20 pregnant rats at this dose (or 60%) had total resorption. There was no live fetus at this dose level. Due to mortality, no data is available for the high dose of 300 mg/kg/day.

Group 1 - Vehicle Control Group 2 - LBH589 30 mg/kg/day

Group	Summary Information	Corrected Body Weights Day 21 of Gestation		Corrected Body Weight Gains Day 6-21 of Gestation	
1	Mean	273.7		28.3	
	SD	12.5		7.4	
	N	22		22	
2	Mean	260.3	+++	16.4	+++
	SD	11.4		6.8	
	N	20		20	

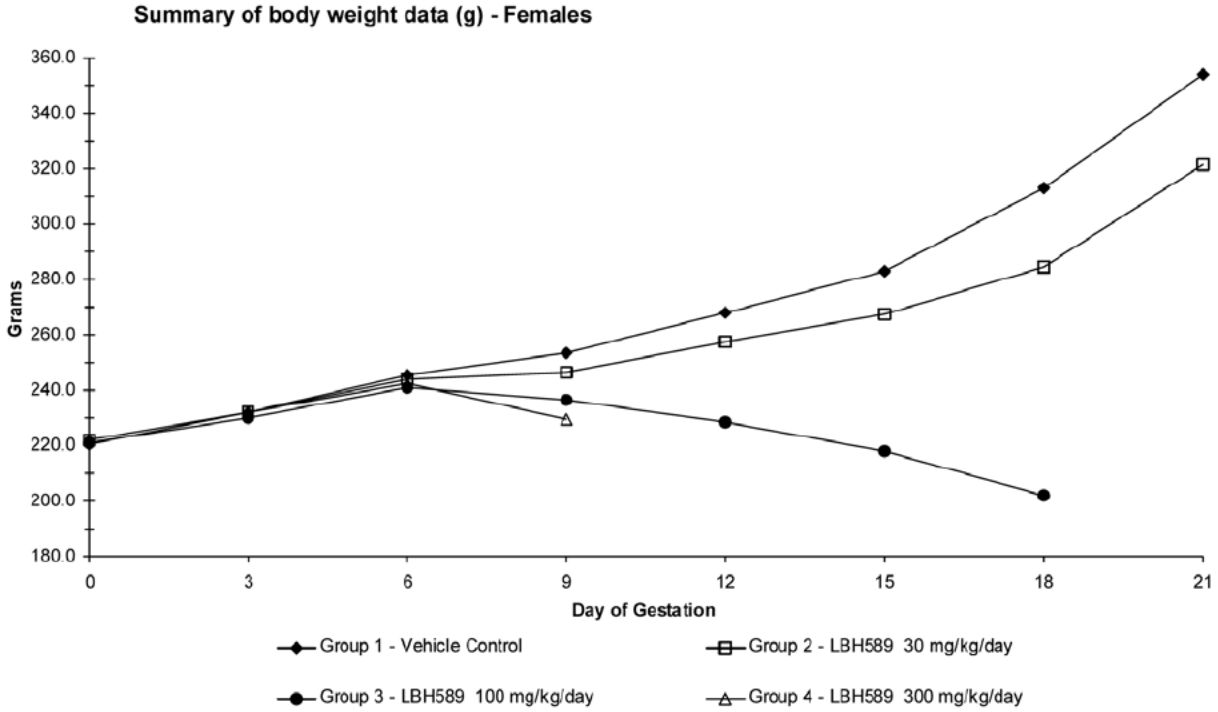
Significantly different from control group (group 1) value: + - P ≤ 0.05 ++ - P ≤ 0.01 +++ - P ≤ 0.001 (t-test)
Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

Table 53. Gravid body weight and uterine weights (GD18)

Dose (mg/kg/day)	0	30	100
No. Dams	22	21	20
Gravid body weight (g)	354.1	321.6**	NA
Gravid uterine weight (g)	80.5	61.3	NA
Net body weight	273.6	260.3	NA

NA: not applicable to due early termination or death/ animals with total resorptions were excluded from the mean
**P≤0.001

Figure 21. Maternal body weight change



(Table and figure excerpted from the submission)

Food Consumption

Individual food consumption was measured on GD 3 – 6, 6 – 9, 9 – 12, 12 – 15, 15 – 18, and 18 – 21.

Table 54. Food consumption

	Dams			
	Control	% change		
Dose (mg/kg/day)	0	30	100	300
GD 3-6	21	-	-	-
GD 6-9	24	↓17	↓25	↓42
GD 9-12	25	↓16	↓40	NA
GD 12-15	25	↓12	↓52	NA
GD 15-18	26	↓15	85	NA
GD 18-21	26	-	NA	NA

NA: not applicable to due early termination or death

Gross Necropsy

Gross observations were conducted on all animals, including animals found dead, and those with unscheduled sacrifices (end of treatment GD 21).

Table 55. Maternal gross pathology findings

Macroscopic findings		Dams			
Dose (mg/kg/day)		0	30	100	300
No. animals		22	22	22	22
Adrenal	Dark discoloration	-	-	-	1
Duodenum	Dark discoloration	-	-	1	1
	Thickening	-	-	2	6
Jejunem	Pale material	-	-	-	1
Lung	Dark area	-	-	2	3
	Spongy	-	-	-	1
LN, mesenteric	Dark area	-	-	2	2
	Dark foci	-	-	-	2
	Mottled	-	-	2	4
Spleen	Small	-	-	-	4
Stomach	Dark discoloration	-	-	1	1
	Dark foci	-	-	3	3
	Pale material	-	-	-	4
	Thickening	-	-	4	13
Subcutaneous tissue	Swelling	-	-	-	1
Thymus	Dark area	-	1	1	3
	Discoloration	-	-	-	1
	Pale foci	-	-	-	1
	Small	-	1	2	2
Tongue	Thickening	-	-	-	1

(-): no change/no toxicologically significant change

Histopathology

Table 56. Maternal histopathology findings

Microscopic findings		Dams			
Dose (mg/kg/day)		0	30	100	300
No. animals		22	22	22	22
Adrenal	Hemorrhage	-	-	10	11
Duodenum	Erosion	-	-	-	1
	Inflammation	-	-	1	2
Lung	Hemorrhage	-	-	-	2
LN, mesenteric	Erythrophagocytosis/ hemorrhage	-	-	7	8
Spleen	Atrophy/necrosis: lymphoid	-	-	-	1
Stomach	Erosion	-	-	1	7
	Edema	-	-	-	8
	Hemorrhage	-	-	1	4
Skin	Erosion	-	-	-	1
	Bacteria	-	-	-	1
Tongue	Inflammation	-	-	-	1
	Edema	-	-	-	1
	Bacteria	-	-	-	1

(-): no change/ no toxicologically significant change

Uterine Parameters

Table 57. Uterine parameters

Dose (mg/kg/day)	0	30	100	300
Mated Rats	22	22	22	22
Pregnant rats	22	21	20	21
Dying/euthanized on study	0	0	9	22
Implantation sites	11.8	11.5	12	12.5
Rats with total resorptions	0	1	12	0

Pregnancy Parameters

Table 58. Pregnancy parameters

Dose (mg/kg/day)	0	30	100	300
No. Dams	22	21	20	12
Corpora lutea	13.4	13.4	15.3	NA
Implantation sites	11.8	12	12.5	NA
Live fetuses	11.1	9.2	0***	NA
Dead fetuses	0	0	0	NA
Total resorptions	0.6	2.2***	12.5***	NA
Pre-implantation loss	11.61	13.88	17.73	NA
Post-implantation loss	5.35	23.84***	100***	NA
Gravid uterine weight (g)	80.5	61.3	NA	NA

*: statistically significant compared to controls $p \leq 0.05$; ***: statistically significant compared to controls $p \leq 0.001$; NA: not applicable due to resorptions at the 100 mg/kg/day and early deaths at 300 mg/kg/day

Embryo-Fetal Examinations

Table 59. Embryo-fetal weights

Dose (mg/kg/day)	0	30	100	300
No. Animals	22	21	20	12
Fetal weight (total)	5.396	4.342**	NA	NA
Male fetal weight	5.554	4.430**	NA	NA
Female fetal weight	5.243	4.249***	NA	NA

: statistically significant compared to controls $p \leq 0.01$; *: statistically significant compared to controls $p \leq 0.001$; NA: not applicable due to resorptions at the 100 mg/kg/day and early deaths at 300 mg/kg/day

Fetal Malformations

Table 60. Fetal malformations, anomalies, and variations

		Fetus				Litter			
Dose (mg/kg)		0	30	100	300	0	30	100	300
Major Malformations									
Numbers Examined *-External* *-Technique of Wilson*		245 121	194 96	NA	NA	22 22	20 20	NA	NA
Head	Cleft palate	-	1	NA	NA	-	1	NA	NA
Tail	Shortened	-	1	NA	NA	-	1	NA	NA
Minor Visceral Anomalies									
Numbers Examined		121	96	NA	NA	22	20	NA	NA
Liver	lobe supernumerary	1	2	NA	NA	1	2	NA	NA
Colon	constricted	-	1	NA	NA	-	1	NA	NA
Minor Skeletal Variations and Anomalies									
Numbers Examined		124	98	NA	NA	22	20	NA	NA
Frontal bone	incomplete ossification	1	5	NA	NA	1	5	NA	NA
Vertebral column	Extra presacral vertebrae	4	12	NA	NA	4	28	NA	NA
	Lumbar centrum semi-bipartite	-	1	NA	NA	-	1	NA	NA
	Thoracic column displaced	-	1	NA	NA	-	1	NA	NA
Ribs	Rudimentary 14^{th} ribs	6	7	NA	NA	11	13	NA	NA
	Extra 14^{th} ribs	3	10	NA	NA	4	21	NA	NA
	Extra 14^{th} ribs with contralateral ossification center	1	5	NA	NA	1	5	NA	NA
	Rudimentary 14^{th} rib with contralateral ossification center	3	4	NA	NA	3	4	NA	NA

NA: not applicable due to resorptions at the 100 mg/kg/day and early deaths at 300 mg/kg/day
(-): no change/no toxicologically significant change

Toxicokinetics

Methods

Blood samples collection: 0.5, 1, 2, 4, 6, 12, and 24 hours post dosing on day 17.
Due to mortalities, no 300 mg/kg/day TK data is available.

Results

Increases in the C_{max} and AUC of panobinostat were proportional to the increases in the doses between 30-100 mg/kg/day.

Table 61. Toxicokinetic parameters in embryonic fetal development toxicity study in rat

(Mean) toxicokinetic parameters of LBH589 in rat plasma

Dose (mg/kg/day)	$AUC_{(0-24h)}$ (ng*h/mL)	AUC/Dose (ng*h/mL) /(mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/ (mg/kg/day)	t_{max} (h)	SE of Mean $AUC_{(0-24h)}$ (ng*h/mL)	SE of Mean AUC /Dose(ng*h/mL) /(mg/kg/day)
30 (n= 3 to 4)	289	9.63	56.0	1.87	2.0	21.7	0.723
100 (n =1 to 2)	816	8.16	277	2.77	0.5	NA	NA

(Excerpted from the submission)

Stability and Homogeneity

(Excerpted from the submission)

All study samples analyzed were within the acceptance criteria of ±15% of their nominal concentrations, except for group 2 top and middle samples prepared on 12-Oct-2006. The group 2 retention samples (top, middle, and bottom) were analyzed in triplicate and all the results met acceptance criteria. An investigation was conducted and it was concluded that the initial out of specification results were likely due to an experimental error during analysis and that the formulation was within specification.

Study title: An oral embryo-fetal development study of panobinostat in the rabbit

Study no.:	0670512
Study report location:	eCTD 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03 Jan 07
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	panobinostat, 0623006, 99.4%

Key Study Findings

- 3 mortalities observed at 80 mg/kg/day.
- ↑pre- and/or post implantation loss at ≥ 10 mg/kg/day.
- ↓ fetal weights ≥ 40 mg/kg/day.
- Fetal malformations observed mainly at 80 mg/kg/day and included: absent digits, cardiac interventricular septal defects and aortic arch interruption, missing gall bladder.
- Other anomalies included: and extra rib and incomplete ossification of hyoid bone and sternebrae.

Methods

Doses*: 0, 10/12.58, 40/50.32 and 80/100.64 mg/kg/day

* Dose levels indicated as base/salt - conversion factor

	base to salt for panobinostat is 1.258
Dose justification:	Dose-finding study in rabbits (No. 0670018)
Frequency of dosing:	Daily oral gavage from gestation day (GD) 7 – 19
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) hydroxypropylcellulose (grade HF) NF (Klucel)
Species/Strain:	New Zealand White rabbit
Number/Group:	22/group
Age:	5 months
Weight:	2.8 – 3.9 kg
Satellite groups:	See table
Unique study design:	None
Deviation from study protocol:	None that affected study outcome

Table 62. Study design for embryonic fetal development toxicity study in rabbit

Study design, animal allocation and test article doses

Group number/ identification	Dose level (mg/kg/day) Base*	Dose concentration (mg/mL)	Female animal numbers	
			Main study	Toxicokinetics
1/ Vehicle control	0	0	1501-1507, 1509-1522, 1527	1523-1525
2/ LBH 589	10/12.58	1	2501-2522	2523-2526
3/ LBH 589	40/50.32	4	3501-3522	3524-3527
4/ LBH 589	80/100.64	8	4501-4522	4523-4526

* dose levels indicated as mg/kg/day base/salt

(Excerpted from the submission)

Observations and Results

Mortality

Animals were checked twice daily for signs of mortality, ill health, or reaction to treatment.

Table 63. Cause of death in embryonic fetal development toxicity study in rabbit

Animal #	Dose (mg/kg/day)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
4518	80	F	18	Sacrificed	↓ activity, cold to touch, weak, ↓ muscle tone , abnormal gait, tremors, GI effects seen macroscopically, dark areas on mesenteric lymph nodes
4519	80	F	11	Found dead	Decreased feces output, dark foci and thickening on stomach
4520	80	F	19	Found dead	Sustained convulsion, panting, labored breathing, dark area on lung, thickening and dark foci on stomach, dark fluid on vagina

Clinical Signs

Detailed examinations were performed on the gestation days of body weight assessment. No other drug-related changes observed in addition to those listed in the mortality section.

Body Weight

Individual bodyweights were measured on GD 0, 4, 7, 10, 13, 16, 20, 23, 26, and 29. No drug-related changes observed in body weights and corrected body weights. Gravid uterine weight was not affected (animals with total resorption were excluded).

Table 64. Gravid body weight and uterine weights (GD29)

Dose (mg/kg/day)	0	10	40	80
No. Pregnant Dams	22	21	21	21
Gravid body weight (kg)	3.54	3.59	3.6	3.6
Gravid uterine weight	454.6	491.4	486.5	464.3
Net body weight	3.09	3.10	3.11	3.12

NA: not applicable to due early termination or death/ animals with total resorptions were excluded from the mean
**P≤0.001

Food consumption

Individual food consumption was measured daily during gestation from day 4 onwards. No drug-related changes observed.

Gross Necropsy

Animals were necropsied at time of death or the end of treatment (GD 29). No drug-related changes observed in addition to those listed under the early death animals.

Pregnancy Parameters

Table 65. Pregnancy parameters

Dose (mg/kg/day)	0	10	40	80
No. Pregnant Dams	22	21	21	21
Corpora lutea	9.2	9.4	9.8	9.9
Implantation sites	7.7	8.2	8.9	8.5
Live fetuses	7.4	7.9	8.4	7.4
Dead fetuses	0	0	0.05	0.18
Total resorptions	0	0	0	1
Pre-implantation loss	17.07	11.78	9.54	14.38
Post-implantation loss	5.34	4.04	4.84	13.50
Spontaneous abortion	5.34	4.07	4.84	$13.5^{A}/8.1^{B*}$
Litter size	7.4	7.9	8.4	$7.5^{A}/7.9^{B*}$
Gravid uterine weight	454.6	491.4	486.5	464.3^{B*}

*A - Including animal(s) with total resorption; B - Excluding animal(s) with total resorption

Embryo-Fetal Examinations

Table 66. Embryo-fetal weights

	Fetal weight (g)				% change from control		
Dose (mg/kg/day)	0	10	40	80	10	40	80
Male	45.11	45.38	41.20	41.10	-	-	-
Female	44.13	43.43	40.06*	39.61*	-	↓9	↓10
Male + female	44.88	44.27	40.57*	40.44*	-	↓10	↓10

*: statistically significant compared to controls $p \leq 0.05$

Fetal Malformations

Table 67. Fetal malformations, anomalies and variations

		Fetus				Litter			
Dose (mg/kg)		0	10	40	80	0	10	40	80
Major External Malformations									
Numbers Examined		162	165	176	127	22	21	21	16
Limbs	Digits of forepaw absent	-	-	-	1	-	-	-	1
Major Visceral Malformations									
No. Examined		162	165	176	127	22	21	21	16
Heart	Interventricular septal defect	-	-	-	1	-	-	-	1
	Major vessels: aortic arch interrupted	-	-	-	2	-	-	-	2
Gall bladder	Absent	-	-	-	1	-	-	-	1
Minor Skeletal Variations and Anomalies									
No. Examined		162	165	176	127	22	21	21	16
Skull	Incomplete ossification	-	-	1	4	-	-	1	2
	Hyoid bone: incomplete ossification	20	32	41	40	9	14	12	13
Vertebral column	Thoracic centrum fused	-	-	1	1	-	-	1	1
	Caudal vertebra(e) bipartite	-	-	-	1	-	-	-	1
Sternebrae	Extra	3	-	5	17	2	-	4	8
Pelvic	Pubic bone: Incomplete ossification	3	2	3	10	3	2	2	5
	Pubic bone: unossified	-	-	-	1	-	-	1	1

Common skeletal variants	Affected fetuses/litters (mean %)				% change from control		
Dose (mg/kg/day)	0	10	40	80	10	40	80
Sternebrae: incomplete/unossified	49.00	54.24	61.28	87.57 ***	↑11	↑25	↑79 ***
Ribs: total 13	52.33	55.23	50.55	80.59 **	-	-	↑55 **

: statistically significant compared to controls $p \leq 0.01$, *: statistically significant compared to controls $p \leq 0.001$

Toxicokinetics

Methods

#/sex/group: Control (n = 3), 10 – 80 mg/kg/day (n = 4)
Schedule: animals were treated from GD 7 - 19
Blood samples collection: 0.5, 1, 3, 6, and 24-hr post dose on day 19

Results

Table 68. Toxicokinetic parameters in embryonic fetal development toxicity study in rabbit

Mean toxicokinetic parameters of LBH589 in rabbit plasma

Dose (mg/kg/day)	Gestation Day	n	$AUC_{(0-24h)}$ (ng*h/mL)		$AUC_{(0-24h)}$ /Dose (ng*h/mL)/ (mg/kg/day)		C_{max} (ng/mL)		C_{max}/Dose (ng/mL)/ (mg/kg/day)		t_{max} (h)	
			Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
10	19	4	49.6	29.8	4.96	2.98	15.1	6.39	1.51	0.639	0.8	0.300
40	19	4	305	197	7.62	4.93	112	35.8	2.81	0.897	0.5	0.00
80	19	4	585	99.7	7.32	1.24	410	44.5	5.13	0.557	0.5	0.00

(Excerpted from the submission)

Stability and Homogeneity

(Excerpted from the submission)

The dose formulations were prepared once prior to study start to confirm the suitability of the preparation method. Homogeneity and stability of the dose formulations were determined by (b) (4) prior to dosing. Homogeneity was considered acceptable if the difference between the mean recoveries from the top, middle and bottom samples and the overall average recovery for each group, was within ±15%.

All study samples analyzed were within the acceptance criteria of ±15% of their nominal concentrations except for week 1, group 2. An investigation was conducted and it was concluded that the out of specification result was likely due to an experimental error during analysis and that the formulation was within specification.

9.3 Prenatal and Postnatal Development

Studies not conducted.

11 Integrated Summary and Safety Evaluation

The nonclinical studies submitted to this NDA provide sufficient information to support the use of panobinostat (FARYDAK) for the treatment of patients with multiple myeloma who have received at least one prior therapy.

Panobinostat is a histone deacetylase inhibitor with activity to HDAC isoforms in class I, II and IV at low nanomolar concentrations in vitro. Panobinostat promoted the accumulation of acetylated histones and non-histone proteins from cells treated with

panobinostat. Panobinostat promoted cell death and cell cycle arrest of cells in vitro, including human multiple myeloma cells. Panobinostat also promoted cell death in multiple myeloma cells from patients ex vivo and in both xenograft and disseminated mouse models of myeloma. Tumor tissues dissected from mice xenografts that were treated with panobinostat showed increased elevated levels of acetylated histones. Panobinostat in combination with bortezomib and dexamethasone had higher activity in reducing tumor burden and increasing survival compared to controls, the agents used individually and in dual combination. Secondary pharmacology studies showed that panobinostat increased trabecular bone density. Safety pharmacology studies showed no adverse respiratory findings. However, neurological findings were evident at the mid dose of 60 mg/kg and high dose of 100 mg/kg in mice. Neurological effects presented as decreased motor activity, wobbly gait, convulsion, and decreased grip strength 15-60 min post-dose. In the hERG assay, the IC_{50}s for panobinostat and a human metabolite BJB432 (at <1% of the total drug-related material in plasma following a 20 mg dose) were 3.5 µM and 1.6 µM, respectively. QTc prolongation was observed in cardiovascular study of telemetry in dogs administered oral panobinostat.

Nonclinical findings show toxicities of panobinostat consistently in the bone marrow and lymphatic systems. Additionally toxicities were noted in the liver, lung, kidney, thyroid, mammary gland and gastrointestinal system. Thyroid findings from the 4, 13 and 26 week study in rats and the 4 week study in dogs included decreases in blood levels of triodothyronine (T_3), decreases in tetraiodothyronine (T_4) and decreases in thyroid stimulating hormone (TSH). Histopathology findings in the thyroid included decreased in follicular colloid and epithelial vacuolation, and increased follicular hypertrophy. One rat in the 26-week study had a benign thyroid follicular cell adenoma. There were no changes in thyroid function tests or histopathology findings in the 26 week study in dogs. The 13 week study in dogs was not reviewed within but was summarized to show the thyroid toxicities.

Panobinostat was mutagenic in the Ames assay, positive for DNA damage in COMET study, and tested negative for chromosome aberrations in human peripheral blood lymphocytes; however, caused endo-reduplication (increased number of chromosomes).

In a combined male and female fertility study, mating index, fertility index, and conception rate were reduced in female rats at the high dose of 100 mg/kg panobinostat. Increased resorption and post-implantation loss were seen at $\geq$ 10 mg/kg dose and reduced number of live embryos was observed at doses $\geq$ 30 mg/kg. Reduced fertility and maternal toxicity occurred in rats. Maternal toxicity occurred in rabbits also. Panobinostat elicited toxicity towards male reproductive organs in the dog in both 4 week and 13 week oral (gavage) repeat dose toxicity studies. The 4 week study is reviewed; the 13 week study has not been reviewed but was summarized to show the male reproductive findings. Toxicity findings included prostatic atrophy accompanied by reduced secretory granules, testicular degeneration and oligospermia and epididymal debri. These effects were not completely reversed following the recovery period. Panobinostat was teratogenic in the rat and rabbit. In the rat,

embryo-fetal toxicity at 30/mg/kg included decreased fetal weights and major malformations (including cleft palate and short tail) and minor skeletal variations or anomalies (including incomplete ossifications, extra presacral vertebrae, and extra ribs). The dose of 30 mg/kg resulted in exposures (AUCs) approximately 3 fold the human exposure at the human dose of 20 mg. There was no live fetus at the mid dose of 100 mg/kg. In the rabbit, developmental toxicities included decreased fetal weight at doses ≥ 40 mg/kg; major malformations at 80 mg/kg (included absent digits, interventricular septal defects and aortic arch interruption of the heart, and missing gall bladder); and minor skeletal variations and anomalies (including incomplete ossification and extra ribs). The dose of 40 mg/kg in rabbits results in systemic exposure approximately 4 fold of the human exposure and the dose of 80 mg/kg results in exposure 7 fold the human exposure, at the human dose of 20 mg.

Thus, panobinostat may impair male and female fertility and administration of panobinostat during pregnancy may pose a risk to the human fetus.

12 Appendix/Attachments: None

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

/s/

EMILY J PLACE
08/28/2014

HALEH SABER
08/28/2014