CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206256Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Beleodaq (belinostat)

Date: May 22, 2014
To: File for NDA 206256
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 Beleodaq conducted by Drs. Del Valle and Ricci, and secondary memorandum and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Beleodaq may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
05/22/2014

MEMORANDUM

Date: May 12, 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: 206256
Drug: BELEODAQ (belinostat)
Indications: Treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL)
Applicant: Spectrum Pharmaceuticals, Inc.

Belinostat is a small molecule histone deacetylase (HDAC) inhibitor. Belinostat inhibited deacetylation of histones and some non-histone proteins in *in vitro* and/or *in vivo* studies. In cell culture studies, belinostat caused cell cycle arrest and/or apoptosis of cancer cells; however, cytotoxic activity was also seen in normal cells. *In vivo*, the HDAC inhibitory activity of belinostat was demonstrated in a mouse xenograft study when mice were injected intraperitoneally with mouse leukemic cells. Belinostat was detected in the ascites and resulted in increased acetalytion of H4. In a separate xenograft study, anticancer activity of belinostat was demonstrated in a solid tumor model.

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. Belinostat-related toxicities in rats and dogs included findings in the following organs: heart (increased heart rate, cardiomyopathy, increased weight), hematopoietic/ lymphocytic system (reduced RBCs and WBCs, atrophy of lymphoid tissues), GI tract (vomiting, liquid feces), male reproductive system (reduced weight of testes and epididymides, immature testes), and injection site reactions.

Hematologic and GI toxicities have been reported in patients treated with BELEODAQ in clinical trials. Hepatotoxicity seen in patients did not occur in animals. Analysis of clinical ECG and belinostat plasma concentration data demonstrated no meaningful effect of BELEODAQ on cardiac repolarization.

Belinostat was genotoxic in the battery of genetic toxicology studies conducted and targeted rapidly dividing cells in animals in general toxicology studies. According to ICHS9 the embryofetal studies could be waived for this class of drugs. This is based on the expected teratogenicity or embryo-fetal lethality when pregnant animals are treated with this class of drugs. Therefore, pregnancy Category D has been assigned to BELEODAQ.

No fertility study has been conducted with belinostat; however, based on the results of the general toxicology study in the dog, belinostat may impair fertility in male subjects. In the 24-week study in the dog, drug-related reduction in the weight of testes and epididymides and reduced testicular maturation were observed.

The nonclinical studies were reviewed by Drs. Pedro Del Valle and Stacey Ricci. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

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

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/s/

HALEH SABER
05/12/2014

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:	206256
Supporting document/s:	1
Applicant's letter date:	December 6, 2013
CDER stamp date:	December 9, 2013
Product:	Belinostat
Indication:	Treatment of Patients with Relapsed or Refractory Peripheral T-Cell Lymphoma
Applicant:	Spectrum Pharmaceuticals, Inc.
Review Division:	Hematology and Oncology Toxicology on behalf of the Division of Hematology Products
Reviewer:	Pedro Del Valle, Ph.D. M. Stacey Ricci, M.Eng., Sc.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John K. Leighton, Ph.D., DABT
Project Manager:	Jessica L. Boehmer, M.B.A.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206256 are owned by Spectrum Pharmaceuticals or are data for which Spectrum Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 206256 that Spectrum Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206256.

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1 Executive Summary

1.1 Introduction

Belinostat (PDX101) is an intravenously administered histone deacetylase (HDAC) inhibitor that can alter acetylation levels of histone and non-histone proteins. HDAC enzymes catalyze the removal of acetyl groups from the lysine residues of histones leading to condensed chromatin and prevention of binding of transcription factors that can result in gene silencing.

Figure 1 HDAC targets and function outcomes[1]

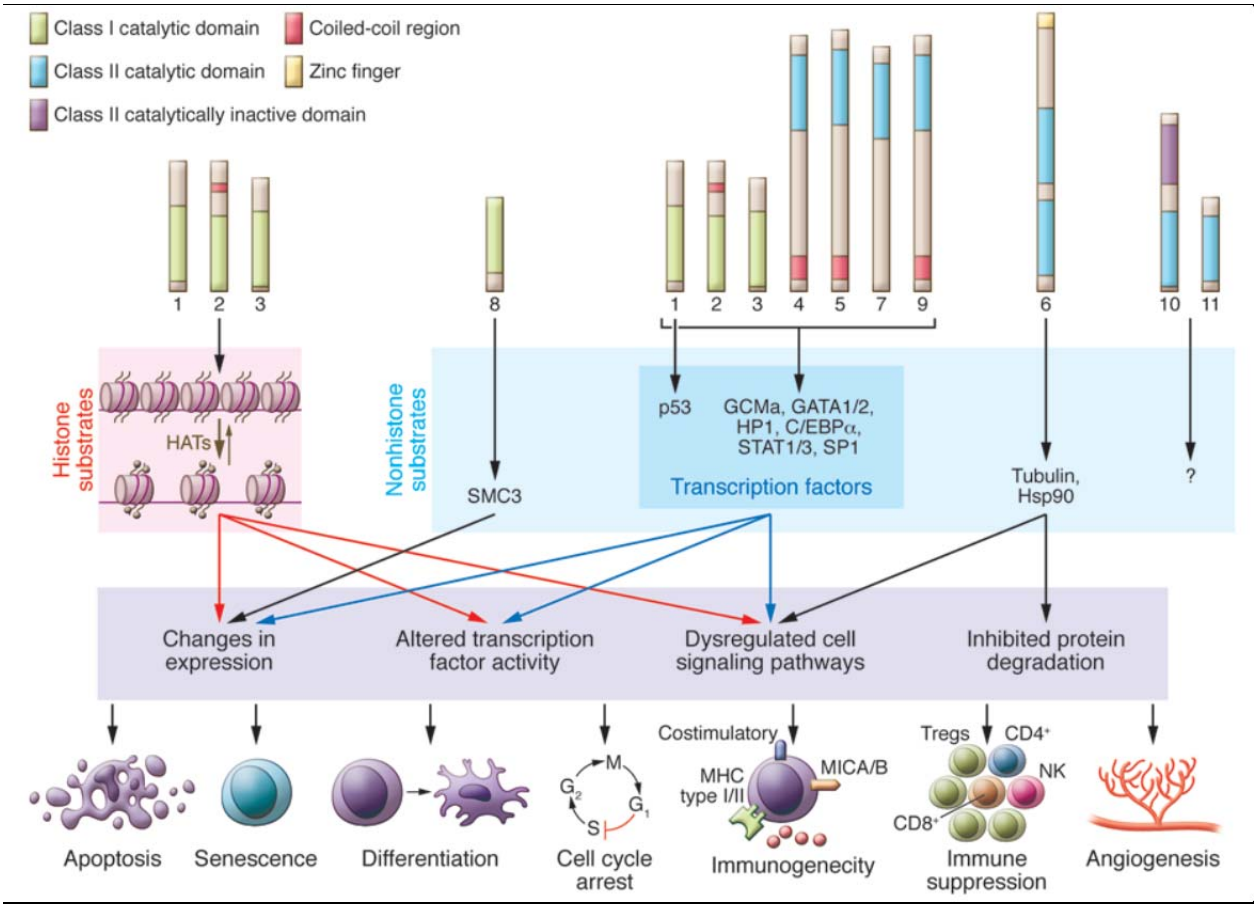


Figure 1 illustrates some of the identified molecular targets of HDACs, downstream cellular pathways, and mechanistic outcomes of HDAC inhibition. Histones are the primary substrates for class I and class II HDACs, while other proteins, including transcription factors, are also targeted. Functional outcomes of HDAC inhibition considered important to their anti-cancer mechanism include apoptosis, cell cycle arrest, and activation of differentiation. .

[1] West, A.C. and Johnstone, R.W. (2014), New and emerging HDAC inhibitors for Cancer Treatment, *J. Clin. Invest*. 124:30-39.

1.2 Brief Discussion of Nonclinical Findings

The proposed mechanism of action of belinostat is that upon inhibition of HDAC enzymes, (b) (4) occurs resulting in cell cycle arrest and apoptosis. In vitro results using cultured cells, demonstrated that belinostat treatment results in cytotoxic activity towards both transformed and normal cells, but transformed cells undergo greater cell cycle arrest and/or apoptosis on a percentage basis. Aberrant expression of HDACs in a wide variety of human tumor types and selective inhibition HDAC isoforms using gene silencing techniques demonstrated cell cycle arrest or cytotoxicity in both in vitro and in vivo models.[2]

Pharmacology studies of belinostat were conducted using in vitro, cell based and in vivo assays by Spectrum Pharmaceuticals, Topotarget, commercial vendors or academic laboratories. Results that explored the mechanism of belinostat action include:

- Belinostat inhibits HDAC1, HDAC2, HDAC3, and HDAC8 (Class I), and HDAC4, HDAC6, HDAC7 and HDAC9 (Class II) enzymes at concentrations < 250 nM using purified recombinant human proteins.[3]
- Belinostat treatment of cultured human tumor or normal cells, or peripheral blood cells collected from mice treated with belinostat showed a rapid increase of acetylated histone proteins.
- Belinostat treatment of cultured tumor cells results in reduced cell viability that correlates with cleavage of poly (ADP-ribose) polymerase (PARP1; a marker of apoptosis), and induced expression of cyclin-dependent kinase inhibitor p21 (CDKN1A; a marker of G1-phase cell cycle arrest).
- Cultured human cancer cells were more sensitive to belinostat-induced cytotoxicity than the cultured normal human cells used.

Safety pharmacology studies conducted included a cardiovascular and respiratory evaluation in anesthetized dogs, an in vitro hERG channel assay and an observational study (Irwin test) in rats. Increased heart rates were observed in the dog cardiovascular study; there were no other findings of toxicity to the cardiovascular, respiratory or central nervous system. Cardiovascular toxicities also were observed in repeat-dose toxicology studies: microscopic findings of cardiomyopathy in the rat and increased heart weight in the dog were observed.

Plasma concentrations showed roughly dose proportional C_{max} and AUC exposures in both rats and dogs. Exposures were typically higher in male rats compared to females, without gender differences noted in dogs. The half-life was between 0.35-1.2 hours in rats and 0.45-1.6 hours in dogs. The major pathway of belinostat metabolism is

[2] West A.C. (2014), *Ibid.*

[3] Experimental evidence regarding HDAC 5, -10 and -11 is not provided in the NDA. A tabular listing published in a review article by Novartis (see Section 4.1 for details) shows that belinostat can inhibit these HDACs also. The details of the experiments conducted for this tabular listing were not reported.

glucuronidation, with the formation of belinostat glucurounide as the primary metabolite and other metabolites which do not have an in vitro cytotoxic effect.

Single and repeat-dose general toxicology studies using IV administered belinostat were conducted in rats and dogs. Repeat-dose toxicology studies with oral administration in rats and dogs (twice daily for 4 weeks) were also conducted. Five-day and 28-day IV studies were conducted using a vehicle/formulation that differed from that used in clinical studies and the to-be-marketed formulation (L-arginine). These studies were supplemented with 7-day repeat-dose bridging studies in rats and dogs using the different formulations. Twenty-four week studies using the L-arginine vehicle/formulation were conducted according to a schedule of 5 days of daily belinostat IV administration followed by 16 days off for 8 cycles (for a total of 24 weeks) with controls and the high dose group containing recovery groups of 2 week duration.

In the 24-week intravenous studies, rats administered doses of 10, 25 or 100 mg/kg (60,150 or 600 mg/m^2) experienced more severe toxicities than dogs that received 10, 25 or 50 mg/kg (200, 500 or 1000 mg/m^2). Toxicities observed following belinostat administration were primarily related to the gastrointestinal system, hematopoietic system, lymphoid system, genitourinary system and the site of injection.

- In rats, mortalities occurred at the high dose in 40% of males and 20% of females primarily during the 6th and 7th cycles of treatment; these animals were terminated early because of poor general health and the severity of their ulcerated tail lesions associated with IV belinostat administration. Microscopic findings in these early decedents included minimal cardiomyopathy and thymus atrophy possibly associated with belinostat administration. Adverse and significant reduction in mean body weight gains in all groups occurred and corresponded with lower mean food consumption. Changes in hematology parameters included dose-dependent decreased total white blood cell and lymphocyte counts, decreased platelets in high dose males, and decreased reticulocyte and red blood cell counts that did not demonstrate a dose-dependency. Urine analysis showed the presence of blood and protein in the low and mid dose groups with fewer occurrences in the high dose group. At terminal necropsy, microscopic findings were limited to tail lesions and thymus atrophy in high dose group animals.

- In dogs, vomiting occurred during or immediately after IV belinostat dosing; other clinical signs included soft feces, salivation, impaired mobility and subdued behavior. Changes in hematological parameters were limited to decreased leukocyte counts, shorter prothrombin times, and slightly higher red cell distribution widths that were also present at the end of the recovery period. Clinical chemistry changes included decreases in alkaline phosphatase levels in all dose groups, and increases in glucose, inorganic phosphorus and urea; the significance of these findings is unclear. Lymphoid atrophy was observed at the highest dose tested in mesenteric, mandibular, spleen, ileum and cecum tissues at the high dose tested. Male dogs from all treatment groups had reduced organ

weights of the testes/epididymides that correlated with delayed testicular maturation in all treatment groups. A dose-dependent increase in mean heart:body weight ratio in males was observed at terminal sacrifice with no microscopic correlates.

Belinostat was positive for genotoxicity in the three assays used: the bacterial reverse mutational test (Ames assay), the in vitro mouse lymphoma cell mutagenesis assay, and in vivo clastogenicity assay in mouse bone marrow cells (micronucleus assay).

Animal studies to evaluate the reproductive, developmental and carcinogenic potential of belinostat were not conducted because belinostat is genotoxic and targets rapidly dividing cells and, therefore, is expected to cause teratogenicity and/or embryo-fetal lethality.

1.3 Recommendations

1.3.1 Approvability

RECOMMEND APPROVAL: The submitted pharmacology and toxicology studies using belinostat support the safety of its use in patients with relapsed or refractory peripheral T-cell lymphoma (PTCL).

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies using belinostat are necessary for the proposed indication.

1.3.3 Labeling

Information in the nonclinical sections of the label reflects findings of studies reviewed within this document; therefore, a separate labeling review is not considered necessary at this time.

2 Drug Information

2.1 Drug

CAS Registry Number
414864-00-9, 866323-14-0

Generic Name
Belinostat

Code Name
PXD101

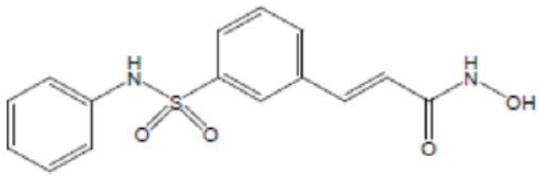
Chemical Name
IUPAC: (E)-*N*-hydroxy-3-[3-(*N*-phenylsulfamoyl)phenyl]prop-2-enamide
CAS name: N-hydroxy-3-[3-[(phenylamino)sulfonyl]phenyl]-2-propenamide

Other: *N*-hydroxy-3-(3-phenylsulphamoylphenyl)acrylamide

Molecular Formula
$C_{15}H_{14}N_2O_4S$

Molecular Weight
318.35 g/mole

Structure or Biochemical Description



Pharmacologic Class
Histone Deacetylase Inhibitor (HDAC)

2.2 Relevant INDs, NDAs, BLAs and DMFs

Clinical investigation of belinostat was conducted under IND 070789. The following DMFs were referenced to support the NDA: [redacted] (b) (4), and 026926.

2.3 Drug Formulation

Belinostat for Injection is supplied as a sterile, yellow lyophilized [redacted] (b) (4). The drug product is a single use vial containing 500 mg of belinostat (drug substance), and 1000 mg/vial of L-Arginine [redacted] (b) (4). The vial is to be reconstituted with 9 mL of Sterile Water for Injection for a 50 mg/mL solution.

2.4 Comments on Novel Excipients

There are no novel excipients used in the manufacture of Belinostat for Injection.

2.5 Comments on Impurities/Degradants of Concern

The CMC Review team identified the following three impurities requiring toxicological evaluation: [redacted] (b) (4)

Table 1 Toxicological Evaluation of Selected Impurity Release Specifications

Impurity	Release Specification	Company Justification derived from RTECS[a]	Recommendation
[redacted] (b) (4)			

(b) (4)

The release specifications for all three were deemed acceptable based on the rationale provided by the Applicant.

2.6 Proposed Clinical Population and Dosing Regimen

Belinostat is indicated for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL).

The recommended dose of belinostat is 1000 mg/m^2 administered over 30 minutes by intravenous (IV) infusion on days 1-5 of a 21-day cycle. Cycles can be repeated until disease progression or unacceptable toxicity.

2.7 Regulatory Background

The original IND for Belinostat was submitted in 2004. FDA provided the Applicant with nonclinical responses to questions in 2010, including an agreement that an embryo-fetal development study was not needed in the NDA for belinostat per the recommendations provided in ICH S9. A preNDA meeting was held in May 2013, and FDA provided agreement that the nonclinical studies to be submitted could support the proposed NDA.

3 Studies Submitted

3.1 Studies Reviewed

Study No.	Title
Primary and Secondary Pharmacology	
PXDIOI-PHM-87	Activity of PXD101 on Tumor and Normal Cells
PXD101-ONC-12	Belinostat pharmacodynamic activity and exposure relationship in preclinical models
PX-CA-06	Effects of Belinostat and Metabolites on Cell Growth
Safety Pharmacology	
455-01	Effects of PXD101 on Cloned hERG Channels Expressed in Mammalian Cells
456-01	Effects of PXD101 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers
2525-006	PXD101: Effects on general Activity and Behavior in the Rat Following Intravenous Administration
1981-012	PXD1010: Cardiovascular and Respiratory Effects in the Anesthetized Dog Following Intravenous Administration
2525-002	Expert Review of ECG: Re-examination of traces from a canine toxicology study (1981/008)
Pharmacokinetics	
1981-035	PXD101: Metabolite Profiling in Dog Plasma Samples Following Oral and Intravenous Administration
Toxicology	
2525-001	PXD101: Cyclic Intravenous Dosing Study in the Rat (8 Cycles over 24 Weeks)
2525-013	PXD101: Cyclic Intravenous Dosing Study in the Dog (8 Cycles over 24 Weeks)

Study No.	Title
1981-024	PXD101: Intravenous Dose range finding Study for 14 Days in the Rat
1981-025	PXD101: Intravenous Dose range finding Study in the Dog
1981-008	PXD101: Cyclic Intravenous Dosing Study for 28 Days in the Dog
1981-007	PXD101: Cyclic Intravenous (infusion) Dosing Study for 28 days in the Rat
1981-018	PXD101: reverse mutation in five histidine-requiring strains of Salmonella typhimurium
1981-022	PXD101: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre^{R} fluctuation technique: Screening study
2525-005	PXD101: Induction of Micronuclei in the Bone Marrow of Treated Rats

3.2 Studies Not Reviewed

Study No.	Title
Pharmacology	
NCI 60 Screen	NCI 60: Human Tumor Cell Line Screen
FMcL In Vitro Summary 11-12-02	PXD101 In Vitro Pharmacology
PXD101-ONC-1	Growth-Inhibitory Activity of PXD 101 on Cell Lines Representing a Variety of Cancer Types.
PXD101-ONC-2	Belinostat anti-cancer effect in vitro in various human cancer cell lines (clonogenic assay, continuous incubation)
PXD101-ONC-4	In Vitro Analysis of PXD 101 in Ovarian Cancer Specimens
PXD101-ONC-5	Time Course for Growth-Inhibitory Activity of PXD101
PXD101-ONC-6	In Vivo Effect of Belinostat in Combination with Various Chemotherapeutic Drugs Using the IP P388 Mouse Ascites Model
PXD101-ONC-7	Preclinical in Vivo Schedule Studies for Belinostat Using the IP P388 Mouse Ascites Model
PXD101-ONC-8	Efficacy Evaluation of PXD101 in combination with Tarceva against the Calu-3 Human Non-Small Cell Lung Carcinoma Xenograft in Nude Mice
PXD101-ONC-9	The In Vitro Effect of Belinostat in Combination with Various Chemotherapeutic Agents (Clonogenic Assay, Continuous Co-Incubation)
PXD101-ONC-10	Dose and Schedule Effect of Belinostat on the Body Weight and White Blood Cell Count in Non-tumor Bearing Male Nude Mice
PXD101-ONC-11	Effect of PXD101 on PC-3 orthotopic tumor growth and metastasis in male nude mice
PXD101-ONC-17	Efficacy Evaluation of Belinostat in Combination with Tarceva against the HCC-827 Human NSCLC Xenograft in Nude Mice
PXD101-ONC-18	Efficacy of PXD101 on A2780 Xenograft in Nude Mice: Dose and Schedule Dependence
PXD101-ONC-21	In Vivo Effects of PXD101 in Combination with Bortezomib Administered on Different Schedules
PXD101-PHM-5	Evaluation of PXD101, and Combinations with Paclitaxel or Carboplatin, in Nude Mice Bearing Established Subcutaneous Human A2780 Ovarian Adenocarcinomas
PX-xen-54	Belinostat+ Yondelis, MesSa (Sarcoma) Xenograft
PX-xen-55	Belinostat + Sorafenib, HEPG2 (Liver Cancer) Xenograft
PX-xen-58	Beliniostat + Paclitaxel + Carboplatin, A2780 (ovarian) Xenograft
PX-xen-61	Beliniostat + Paclitaxel + Carboplatin, A2780 (ovarian) Xenograft
TT-PX-MYCOM-2003-06-V1.0	HDAC Project Myeloma Combinations
TT-PX-MYCOM-2004-04-V1.0	HDAC Project Myeloma Combinations

Study No.	Title
TT-PX-COM-2006-11-v1.0.	HDAC PROJECT Combinations SaOS2 – PXD101 +/- Doxorubicin
TT-PX-COM-2006-12-v1.0.	HDAC PROJECT Combinations U2OS – PXD101 +/- Doxorubicin
TT-PX-COM-2006-13-v1.0.	HDAC PROJECT Combinations MESSA – PXD101 +/- Doxorubicin
TT-PX-COM-2004-04 -v1.0. 2004	HDAC PROJECT Combinations HT29 (colon), MCF-7 (breast), A2780, OVCAR-3 (ovarian), PC3 (prostate) cell lines PXD101 +/- 5-FU
PX-CA-02	Clonogenic Assay Studies on Belinostat as a Substrate for ABC Drug Transporters Mediating Multi-Drug Resistance
PX-WB-01	The effect of Belinostat in combination with Yondelis on DNA double-stand breakage measured by phosphorylation of the histone H2AX
5219	Study of the Effects of PXD101 in Various Cell Biology Assays
4212	In Vitro Study of PXD101 on various receptor binding and enzyme assays
4747	Study of the Effects of Several Compounds on Various Matrix Metalloproteinase Act ivies
5424	In Vitro Pharmacology: TACE Assay
2525-017	Effects of PXD101 on Selected Cytochrome P450 Activities in Human Liver microsomes: Prediction of Drug Interactions
PIQ0026	The Potential of a Test Article to Inhibit Human Cytochrome P450 Enzymes
(b) (4)-TopoTarget-01	In vitro Interaction Studies of one Test Article with P-gp (MDR1/ABCB1) in the vesicular transport assay and in Bidirectional Transport (Papp) Studies on Caco-2 Monolayers
1981-15	Hemolytic Potential and Plasma Compatibility in Rat and Dog
Pharmacokinetics	
CYP0063_R4	Caco-2 Permeability (In Vitro)
2525-007	[^{14}C]-PXD101 Single-dose ADME Study in Dogs
1981-023	PXD101 Single-dose PK Study
53973	PXD101 Single Dose in Dog (Oral and IV)
1981-034	PXD101 Single-dose PK Study
HCH0009	Toxicokinetic/Bioequivalence Study in Dog (Oral v. IV)
2525-009	[^{14}C]-PXD101 Metabolism in microsomes and hepatocytes from rat, mouse, dog, monkey and man
1981-027	PXD101 Repeat Dose in Rat (IV)
(b) (4)-Topo-Target01	PGP-substrate and Pgp-inhibition Study (Vesicular transport and Caco-2)
2525-008	[^{14}C]-PXD101 Single-dose ADME Study in Rats
Single Dose Toxicology	
1535-04542	PXD101: Maximum Tolerated Dose Intravenous Infusion Study in Beagle Dogs
1535-05001	PXD101 and/or L-Arginine: Maximum Tolerated Dose Intravenous Infusion Study in Beagle Dogs
1981-006	PXD101: Single Dose Intravenous Toxicity Study in the Mouse
1981-005	PXD101: Single Dose Intravenous Toxicity Study in the Rat
Repeat Dose Toxicology	
1981-009	PXD1 01: Dose range-finding Intravenous Administration Toxicity Study in the Dog
1981-010	PXD1 01: Dose range-finding Intravenous Administration Toxicity Study in the Rat
FPD-05-0031	Oral Dose-Range Finding Toxicity Study of PXD101 in the Adult Male and Female Wistar Rat after 14-Days Twice-daily Repeat Administration
HCH0001	Oral Maximum Tolerated Dose (MTD) and 16 Day Dose Range Finding Study in the Dog
HCH0002	4 Week Oral (Capsule) Toxicity Study in the Dog with a 7 Week

Study No.	Title
	Treatment-Free Period
HCH0003	PXD101: 4 Week Oral (gavage) Toxicity Study in the Rat with a 14 Day Treatment-Free Period

3.3 Previous Reviews Referenced

The Nonclinical review of the short-term repeat dose toxicology studies in rats and dogs (Study Nos. 1981-024, -025, -008 and -007) as written by Dr. Lilliam Rosario were incorporated into this review.

4 Pharmacology

4.1 Primary Pharmacology

The proposed mechanism of action of belinostat is based on its ability to inhibit histone deacetylase enzymes (HDACs). The hydroxamate region of PXD101 chelates a zinc ion, which is necessary for HDAC activity. Acetylation and deacetylation of histones are controlled by the enzymatic activity of histone acetyltransferases (HAT) and HDACs. HDACs can alter acetylation levels of histone and non-histone proteins. Belinostat and other HDAC inhibitors in general cause the accumulation of acetylated histones and other proteins, resulting in open chromatin structure, cell cycle arrest, apoptosis and a decrease in tumor cell proliferation.

Selective HDAC inhibitors target the classical family members from classes I, II and IV. HDAC inhibitors, like belinostat, act through binding into the active side pocket and chelation of the catalytic zinc-ion located at its base.[4] The enzymatic pocket of HDACs is highly conserved and most HDAC inhibitors either inhibit all HDACs or many of them simultaneous (see table).

Table 2 Inhibition Profile of Pan- and Class-Selective HDAC Inhibitors[4]

	Inhibitor	class I				class II A				class II B		class IV
		HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	HDAC11
pan-inhibitors	TSA						nd				nd	nd
	Vorinostat (SAHA)						nd				nd	nd
	NVP-LAQ824						nd				nd	nd
	Panbinostat						nd				nd	nd
	Belinostat						nd				nd	nd
	PCI-24781					nd	nd	nd	nd			nd
class I inhibitors	MS-275						nd				nd	nd
	MGCD0103								nd		nd	nd
	Depsipeptide			nd	nd		nd	nd	nd		nd	nd
	Apicidin						nd				nd	nd
	Valproic acid					nd	nd				nd	nd
	Trapoxin		nd	nd	nd		nd	nd	nd		nd	nd
	SB-429201		nd			nd	nd	nd	nd	nd	nd	nd
	Bispyridinum diene				nd		nd	nd	nd	nd	nd	nd
	SHI-1:2								nd		nd	nd
	R306465		nd	nd		nd	nd	nd	nd		nd	nd
	SB-379278A		nd			nd	nd	nd	nd	nd	nd	nd
	PCI-34051					nd	nd	nd	nd			nd
	Cpd2		nd	nd		nd	nd	nd	nd		nd	nd
class II inhibitors	APHA derivatives		nd	nd	nd		nd	nd	nd	nd	nd	nd
	Tubacin		nd	nd	nd	nd	nd	nd	nd		nd	nd
	Mercaptoacetamide			nd		nd	nd	nd	nd			nd
	NCT-10a/14a		nd	nd	nd		nd	nd	nd		nd	nd

Depicted are relative inhibitory potency of several pan-, class I selective, and class II selective compounds against HDACs1-11

strong inhibition (EC50 < 5fold x EC50 relative to most sensitive HDAC isoform)

weak inhibition (EC50 > 5fold x EC50 relative to most sensitive HDAC isoform)

no inhibition (EC50 > 100fold x EC50 relative to most sensitive HDAC isoform)

nd no data published

[4] Witt, O. et al. HDAC family: What are the cancer relevant targets? *Cancer Letters* (2009), 277:8-21.

Relevant data provided to support the proposed mechanism of action for belinostat was reviewed. These data were provided in the listed individual Study Reports and publications in peer-reviewed journals.

DATA REVIEWED IN SUBMITTED PUBLISHED LITERATURE:

Belinostat inhibits histone deacetylases

Although by naming convention all deacetylases are HDACs, they do not all act on histones. There are three classes of HDACs (I, II and IV) that are grouped according to their homology to yeast proteins, subcellular location and Zn-dependent enzymatic activities.[5] Class I HDACs (HDACs 1, 2, 3) are usually found in the nucleus and act on histone substrates. Class II HDACs act on non-histone proteins located in the cytoplasm, including α-tubulin, hypoxia-inducible factor (HIF)-1 α, and heat-shock protein (HSP) 90. There are 2 subtypes of class II HDACs: Class IIa HDACs (HDACs 4, 5, 7, and 9) can pass between the nucleus and the cytoplasm and Class IIb HDACs (HDACs 6 and 10) are found in the cytoplasm. The class III HDACs, or sirtuins, are NAD^+-dependent enzymes that have some overlapping functions as the classical HDACs but are not inhibited by conventional HDAC inhibitors (HDACi). The Class IV HDAC enzyme, HDAC11, has characteristics of both the Class I and Class II HDACs.

- Results published that detail the experimental conditions used show that Belinostat inhibits HDACS 1, 2, 3, 4, 6, 7, 8 and 9 using recombinant human proteins at concentrations < 250 nM (Table 3).[6]
- Similar results (Table 4) including the additional HDACs 5, 10 and 11 were published in a review article by Novartis and not in a peer-reviewed scientific journal. The details of the experiments conducted were not reported.[7]

[5] West, A.C. and Johnstone, R.W. *Ibid.*

[6] Khan, N. et al. Determination of the class and isoform selectivity of small molecule histone deacetylase inhibitors. *Biochem. J.* (2008) 409: 581-589.

[7] Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): Successes and challenges. *Cancer Letters* (2009) 280:233-241. **N.B.: This is a review article, and the tabulated data presented in the review is cited from the following meeting abstract and not a peer-reviewed journal article. The methodology used to collect these data is not provided.:** W. Shao, J.D. Growney, Y. Feng, P. Wang, Y. Yan-Neale, G. O'Connor, Potent anticancer activity of the pan-deacetylase inhibitor panobinostat (LBH589) as a single agent in in vitro and in vivo tumor models, in: 99th American Association of Cancer Research Annual Meeting, April 12–16, 2008, San Diego, CA, 2008, Abstract 6244.

Table 3 In vitro effects on HDAC activity using belinostat and other HDAC inhibitors[8]

Inhibitor	rhHDAC...	EC_{50} (nM) 1	2	3	4
TSA		2 ± 0	3 ± 0	4 ± 1	6 ± 2
NVP-LAQ824		5 ± 1	5 ± 1	12 ± 1	23 ± 18
Panobinostat		3 ± 0	3 ± 0	4 ± 1	12 ± 5
ITF2357		28 ± 8	56 ± 13	21 ± 3	52 ± 5
Belinostat		41 ± 6	125 ± 21	30 ± 0	115 ± 16
Vorinostat		68 ± 14	164 ± 45	48 ± 17	101 ± 31
MS-275		181 ± 62	1155 ± 134	2311 ± 803	>10000
MGCD0103		34 ± 17	34 ± 8	998 ± 431	>10000
Apicidin		>10000	120 ± 28	43 ± 7	>10000
VPA		1584000 ± 302 624	3068000 ± 0	3071000 ± 0	ND

Inhibitor	6	7	8	9
TSA	3 ± 1	5 ± 2	456 ± 59	6 ± 5
NVP-LAQ824	7 ± 3	18 ± 4	162 ± 44	6 ± 5
Panobinostat	61 ± 1	14 ± 7	248 ± 11	3 ± 2
ITF2357	27 ± 16	163 ± 8	ND	ND
Belinostat	82 ± 19	67 ± 22	216 ± 43	128 ± 46
Vorinostat	90 ± 26	104 ± 35	1524 ± 463	107 ± 21
MS-275	>10000	>10000	>10000	505 ± 37
MGCD0103	>10000	>10000	>10000	ND
Apicidin	>10000	>10000	575 ± 111	>10000
VPA	>10000	>10000	7442000 ± 2 740000	>10000

Each compound was assayed in triplicate per plate. EC_{50} values were determined from the average of a minimum of two plates. Results are means+/−S.E.M.

[8] Khan, N et al. (2008) *Ibid.*

Table 4 Inhibition of HDAC activity using belinostat (and other HDAC inhibitors; information provided in NDA)[9]

Inhibition of enzyme activity IC_{50} (nM)	Panobinostat (LBH589)	Vorinostat (SAHA)	Belinostat (PXD-101)	MGCD0103
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	12522	598	>30,000
HDAC8	277	1069	157	28,167
HDAC9	5.7	78.1	44.2	1177
HDAC10	2.3	88.4	31.3	54.9
HDAC11	2.7	109	44.2	104

Belinostat reversibly increases acetylation of Histones H3 and H4 and acetylates alpha-tubulin

- In vitro acetylation of histone H3 and H4 in A2780 ovarian tumor cells was maintained for 36 h with continuous exposure to 1 μM belinostat, Figure 2a.[10] Acetylation decreased in cells previously treated after incubation in drug-free medium, Figure 2b.
- In vivo acetylation of histone H4 from peripheral blood mononuclear cells in mice with A2780 human tumor xenografts was observed after a single Intraperitoneal (IP) injection of PDX101 (40 mg/kg) and tumor growth and delay increased with increasing dose of PDX101, Figure 3a and b.
- In vitro acetylation of alpha-tubulin was shown in human ovarian carcinoma OVCAR-3 cells incubated with PXD101.[11]

[9] Atadja, P. et al. (2009) *Ibid.*

[10] Plumb A. et al. Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. *Mol. Cancer Ther.* (2003) 2:721-728

[11] Qian X. et al. Activity of PXD101, a histone deacetylase inhibitor in preclinical ovarian cancer studies. *Mol. Cancer Ther.* (2006) 5:2086-2095

Figure 2 In vitro Acetylation of Histone H3 and H4 from ovarian tumor cells treated with belinostat [12]

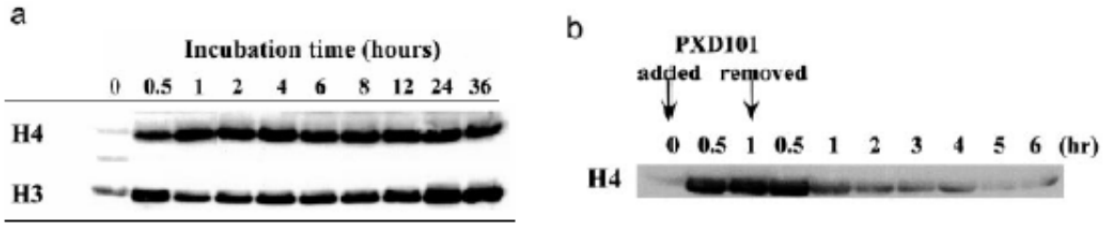


Figure 2: Histone H3 and H4 acetylation determined by Western blot of histones extracted from A2780 cells treated with PXD101 (1 μM) for various times (*a*) and from A2780 cells treated for 1 h with PXD101 and then incubated in drug-free medium for various times (*b*).

Figure 3 In vivo Acetylation of Histone H4 from Peripheral Blood Mononuclear cells isolated from tumor-bearing mice treated with belinostat[13]

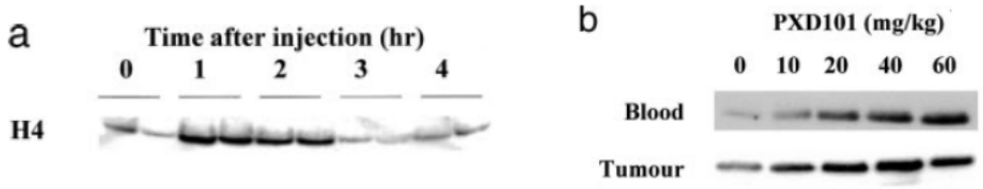


Figure 3: (a) Western blots of histone H4 extracted from peripheral blood mononuclear cells taken from A2780 tumor-bearing mice treated with a single i.p. 40 mg/kg injection of PDX101 returned to baseline levels by 3 h; (b) Western blots of histone H4 2 h after a single i.p. at various doses of PXD101 showed a dose-dependent effect of PDX101 effect.

Figure 4 PXD101 effects on the Acetylation of α-tubulin in cultured cells

(Excerpted from Pharmacology Written Summary Section 2.6.2)[14]

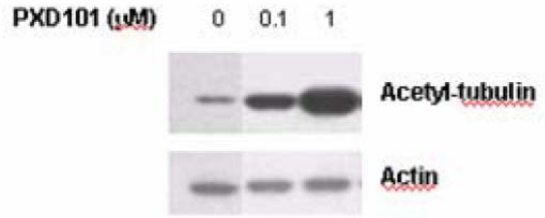


Figure 4: Human ovarian carcinoma OVCAR-3 cells were incubated with different concentrations of PXD101. After 24 h incubation, cell lysates were prepared and analyzed by immunoblotting

PDX101 Induction of Apoptosis and Cyclin-Dependent Kinase Inhibitor p21

Poly (ADP-ribose) polymerase (PARP) is a family of proteins involved in DNA repair and programmed cell death. The cleavage of PARP is an indicator of apoptotic cell death.

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[12] Plumb A. et al. (2003), *Ibid.*
[13] Plumb A. et al. (2003), *Ibid.*
[14] Qian X. et al. (2006), *Ibid.*

The cyclin-dependent kinase inhibitor p21 is induced by both p53-dependent and p53-independent mechanisms and causes cell cycle arrest.

Methods

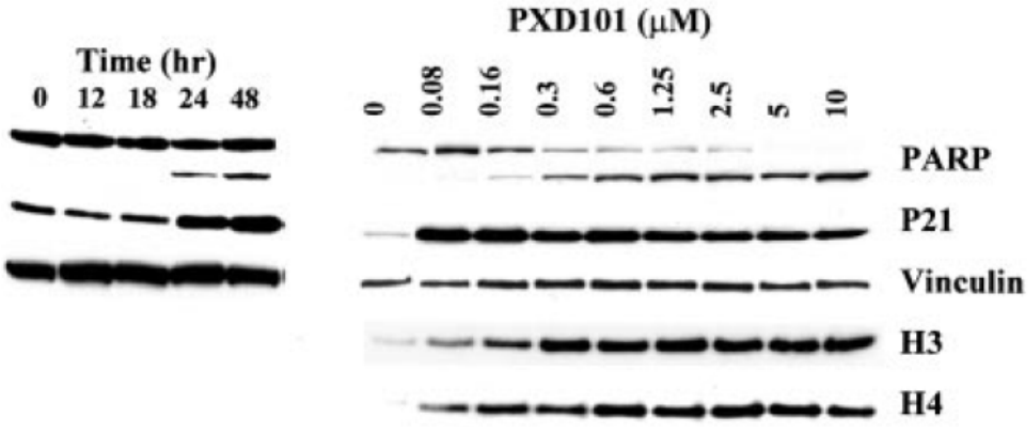
Human colon carcinoma cells (HCT116) were plated in 25-cm^2 flasks at sub-confluent density and allowed to establish for 48 h. PXD101 at different concentrations was added for 24 h after which time cell lysates were prepared. The cleavage of PARP, expression of p21 and acetylation of histones H3 and H4 was examined by immunoblot analysis.[15]

Results

- A temporal and dose-dependent increase in PARP cleavage starting at 0.16 μM supports the hypothesis that belinostat can cause apoptosis in cultured human cells.
- PXD101 induced expression of p21 starting at 0.08 μM.
- Histones H3 and H4 acetylation increased in a dose-dependent manner.

Figure 5 Effects of PXD101 on p21 expression and PARP cleavage in HCT116 cultured cells

(Excerpted from Plumb JA et al)[10]



In the left panel, HCT116 cells were incubated with PXD101 (1 μM) for various times, and PARP and p21 were detected by Western blotting. In the right panel, cells were incubated for 24 h with a range of concentrations of PXD101 (μM); histone H3 and H4 acetylation was also detected. Vinculin is a loading control.

DATA REVIEWED IN SUBMITTED PHARMACOLOGY STUDY REPORTS:

Belinostat leads to cell cycle arrest and apoptosis

A. Report PXD101-PHM-87: Activity of PXD101 on Tumor and Normal Cells

[15] Plumb JA et al., (2003), *Ibid.*

This study assessed the effects of PXD101 on tumor and normal cells of human origin and evaluated growth inhibition, cell cycle arrest and apoptosis.

PDX101 Inhibition of cell growth (prostate)

Methods

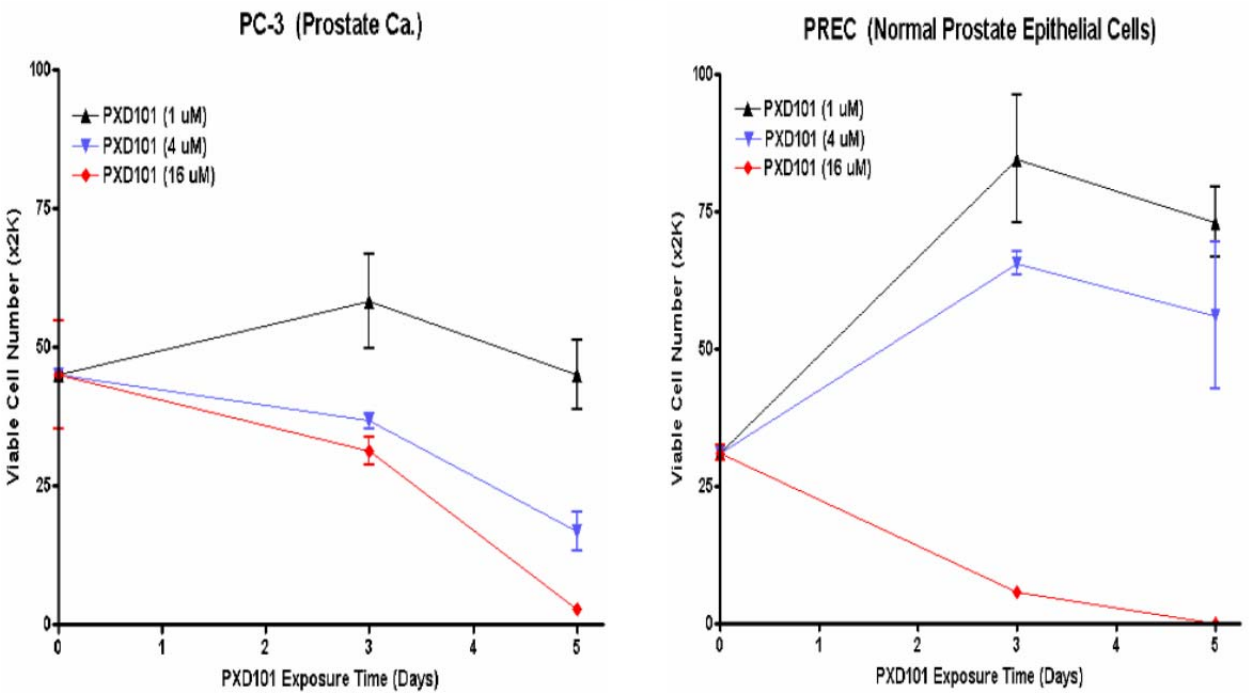
Prostate carcinoma cells (PC-3) and normal prostate epithelial cells (PREC) of human origin were cultured in 6-well plates at sub-confluent density and allowed to attach for 1 day. Cells were treated at different concentrations of PXD101. Cell viability was assessed using trypan-blue dye exclusion and viable cells were counted on a hemocytometer after 3 and 5 days exposure.

Results

- PXD101 at 1 or 4μM did not inhibit the growth of PREC cells by Day 3 although the number of viable cells decreased by Day 5. PDX101 at 16 μM inhibited the growth of human normal prostate cell by Day 2 and 5 (see Figure below).
- PXD101 at 1 μM inhibited the growth of PC-3 cells with no apparent cytotoxicity and reduced the number of viable cells at 4 or 16 μM by Day 3 and 5.
- Tumor cells were more sensitive than normal cells to the effects of PXD101.

Figure 6 Effects of PXD101 in Tumor and Normal Human Prostate Cells

(Excerpted from Study Report PXD101-PHM-87)



PXD101 Effect on normal and cancer cell viability

Methods

Human tumor cells and normal cells were obtained from the (b) (4) or the NCI (Bethesda, MD): HCT-116 (colon carcinoma), SW-620 (colon carcinoma), HT-29 (colon carcinoma), HeLa (cervical carcinoma), SK-MEL-2 (melanoma) and BT-549 (breast carcinoma) NHLF (lung fibroblasts) and HRE (renal epithelial), ARPE-19 (retinal epithelial) and CCD1070sk (skin fibroblasts). Following incubation with vehicle or PXD101, cell viability was measured using trypan blue exclusion, ATP concentrations (b) (4)) or lactate dehydrogenase release (b) (4)).

Results

Cells in the table below were treated with two different concentrations of PXD101 for 3 days, after which time the number of viable and dead cells in the total populations was determined by trypan-blue dye exclusion. At the higher PXD101 concentration used (2.5 mM), 3 out of 4 normal cell lines showed greater viability than the 5/6 tumor cell lines used.

Table 5 PXD101 effect on cell viability of normal and cancer cell lines

(Table excerpted from Study Report PXD101-PHM-87)

		Number of Viable Cells Relative to No-Drug Control (%)		
Cells	**Cell Type**	**No PXD101**	**PXD101 (0.625 µM)**	**PXD101 (2.5 µM)**
	Tumor			
SW-620	Colon carcinoma	100	48	5
SK-MEL-2	Melanoma	100	12	6
HeLa	Cervical carcinoma	100	44	40
BT-549	Breast carcinoma	100	49	8
HCT-116	Colon carcinoma	100	18	8
HT29	Colon carcinoma	100	44	2
	Normal			
NHLF	Primary lung fibroblasts	100	28	6
HRE	Primary renal epithelial	100	56	36
ARPE-19	Retinal epithelial	100	49	31
CCD1070sk	Skin fibroblasts	100	77	30

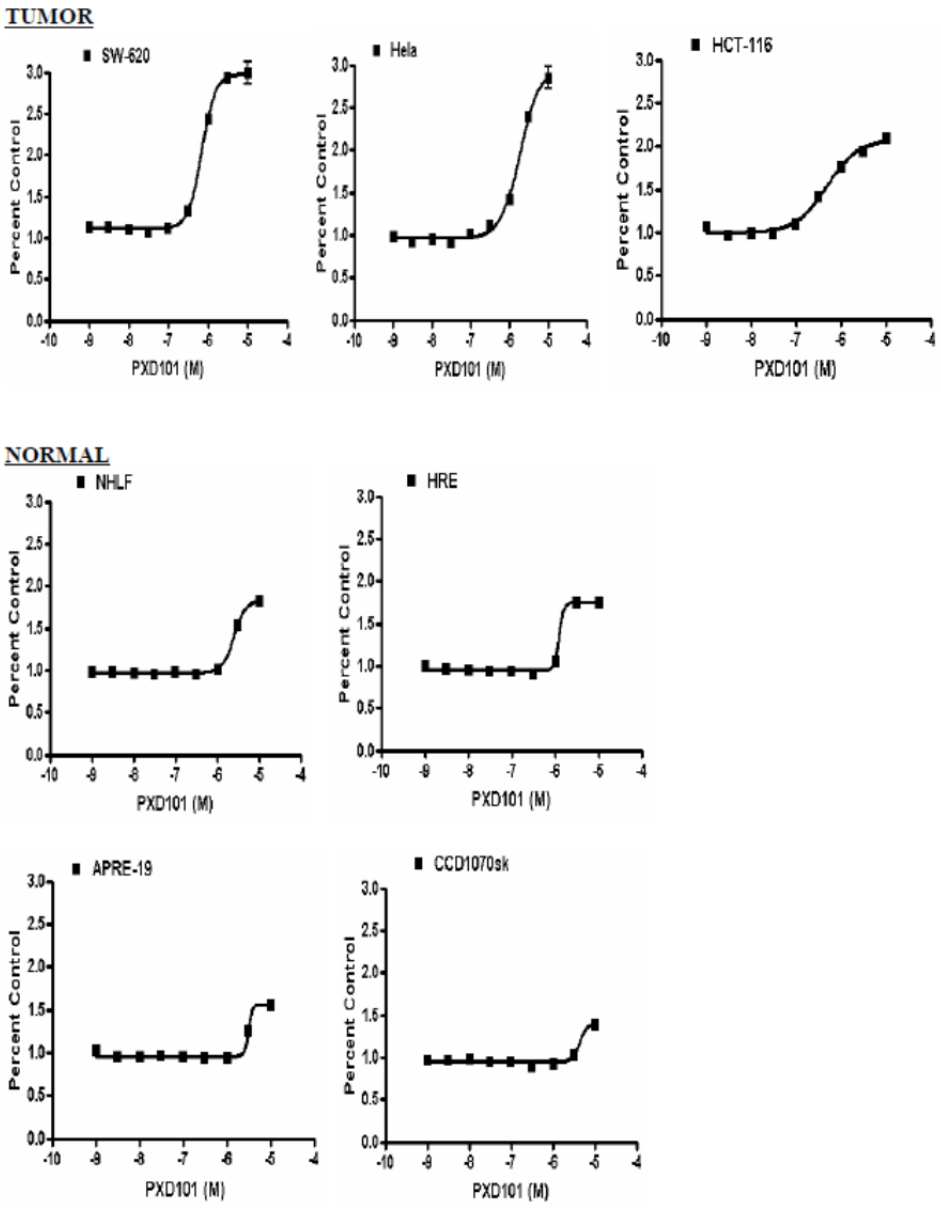
An assay that evaluated PXD101 effect on ATP metabolism as another measure of cell growth/viability of these 4 normal and 3 of the tumor cell lines showed that PXD101 inhibited all of the cell types with approximately similar IC_{50} values (0.3-1.2 µM).

The cytotoxicity of these cell types was then evaluated using a lactate dehydrogenase (LDH) release assay, which measures the LDH present in cell culture media that was released from dead or dying cells. This experiment showed that PXD101 induced a greater level of LDH release in the cancer cell lines than in the normal cells, and the EC50 PXD101 concentrations were lower also. In the figure below, cells were exposed to the indicated PXD101 concentration for 3 days and then the culture media was

examined for LDH. Results are reported as fold-increase relative to untreated control cells, with a value of 2.0 indicating a 2-fold (100%) increase relative to controls.

Figure 7 Cell viability following PXD101 incubation (LDH-Release)

(Figure excerpted from Study Report PXD101-PHM-87)



PXD101 Effects on cell cycle

Methods

PREC and 9 different tumor cells were seeded in 90 mm tissue culture dishes at sub-confluent density and allowed to attach overnight then exposed to 0.5, 2 or 8 µM PXD101. Cells were processed after 24 or 48 h of exposure using propidium iodide for staining and flow cytometry with Cell Quest DNA and ModFit LT cell cycle analysis

software. Based upon DNA content, cells were categorized with respect to cell cycle as: Sub G1 (<2N DNA content), G0/G1 (2N DNA content), and S phase (2N and 4N DNA content), G2/M (4N DNA content).

Results

- Exposure of tumor cells to different concentrations of PXD101 resulted in an increase in SubG1 and G2/M populations and a reduction in G0/G1 and S-phase populations.
- Exposure of normal prostate cells to PXD101 resulted in less SubG1 staining indicating that PXD101 may be less cytotoxic to normal than tumor cells. A reduction in S-phase and increase in G2/M phase is noted.

Table 6 Percent of Cells in the different cell cycle stages after treatment with PXD101

Cells	Cell Type	PXD101 concentration (μM)	Exposure (Hour)							
			24				48			
			SubG1	G0/G1	S	G2/M	SubG1	G0/G1	S	G2/M
MiaPaca-2	Pancreatic carcinoma	0	8	51	28	13	10	45	30	15
		8	**73**	4	0	**23**	**38**	10	0	**52**
BxPC-3	Pancreatic carcinoma	0	19	49	16	16	13	36	25	26
		8	**57**	11	0	**32**	**31**	20	0	**49**
Panc-1	Pancreatic carcinoma	0	19	36	28	17	10	38	29	23
		8	**52**	21	2	**25**	**59**	16	3	22
A549	Lung carcinoma	0	2	36	46	16	14	37	31	18
		8	ND	ND	ND	ND	**54**	12	3	**31**
NCI-H460	Lung carcinoma	0	3	39	42	16	11	43	31	15
		8	**17**	26	0	**57**	**50**	15	2	**33**
HCC-827	Lung carcinoma	0	24	22	32	22	11	40	31	18
		8	**93**	2	0	5	**84**	2	0	**14**
PC-3	Prostate carcinoma	0	16	41	24	19	3	54	24	19
		8	**13**	31	1	**55**	**22**	30	1	**47**
DU145	Prostate carcinoma	0	3	42	24	19	3	54	24	19
		8	**60**	31	1	**55**	**22**	30	1	**47**
LNCaP	Prostate carcinoma	0	31	54	12	3	22	51	19	8
		8	ND	ND	ND	ND	**54**	23	4	**19**
PREC	Normal prostate	0	5	70	13	12	7	65	16	12
		8	9	66	1	**24**	**20**	60	0	**20**

The cell cycle distribution of each cell population analyzed is shown. Cells in SubG1 are considered dead or dying. N.D. = not done.
PXD101 concentrations used were 0.5, 2 and 8 μM; only the results for control and the 8 μM are shown.

B. Report # PXD101-ONC-12: Belinostat pharmacodynamic activity and exposure relationship in preclinical models

Belinostat was tested for its ability to inhibit cell growth in soft agar (anchorage-independent growth), and to inhibit growth of using a mouse tumor xenograft model of ascities. The P388 mouse leukemia and the A2780 human ovarian cancer cell lines

were used. The pharmacodynamic (PD) endpoint of histone H4 acetylation in tumor samples was also measured.

Belinostat treatment of a mouse ascites model

Methods

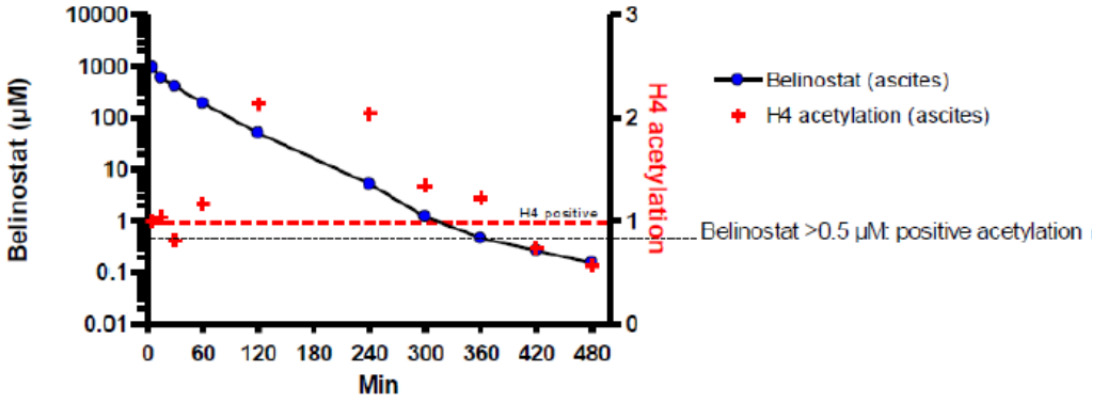
Mice (B6D2F1, (b) (4) were injected intra-peritoneally (IP) with 10^6 P388 mouse leukemia cells in 200 µL. Mice received a single IP dose of 80 mg/kg belinostat on Day 7 and 3 mice/time point were sacrificed at 5, 15, 30 minutes and 1, 2, 4, 5, 6, 7 and 8 hours. Ascites fluid was collected from the abdominal cavity of these mice and centrifuged. Belinostat concentration was measured in the ascites supernatants and H4 histone acetylation was evaluated in the cell pellet using immunoblotting. Weight gain of >20% was defined as moribund state and mice were sacrificed. Individual survival days were recorded and median survival calculated.

Results

H4 acetylation in ascites cells collected from 1 to 6 h after the last dose of belinostat was above the baseline. H4 Acetylation began to decline after 4 hours. Belinostat concentration in ascites fluid declined from approximately 800 µM to 0.5 µM, and the decline correlated with a decrease in H4 acetylation after 4 hours. H4 acetylation levels fell below baseline when belinostat ascites fluid concentrations were approximately <0.5 µM.

Figure 8 H4 Acetylation in tumor ascites cell from Belinostat treated IP P388 Mouse ascites model

(Excerpted from Study PXD101-ONC-12)



Blue bullets: belinostat concentration in ascites fluid in µM (left y-axis).

Red crosses: relative H4 acetylation in tumor cells isolated from ascites fluid (Western blot). 1: acetylation after 5 min (background); 0: no acetylation (less than background); >1: positive acetylation.

In a separate study (PXD101-ONC-7), different schedules of belinostat treatment were evaluated using the IP P388 mouse ascities model. The end-point measured was survival. The schedules tested varied the time intervals between doses (daily, every other day, or multiple doses per day (x2, x3, x4, x8). The results listed in Table 7

that supported a relationship between long treatment duration and anti-tumor effect.

- A single belinostat dose did not improve survival.
- Daily single doses for 5 or 10 days increased survival in a dose-dependent manner.
- Multiple daily doses improved survival in a dose-dependent manner.

Table 7 Percent Increased Survival (P388 ascites model)

(Tables copied from the Pharmacology Written Summary)

Belinostat once daily treatment

Schedule	Belinostat (mg/dose)			Source
	200	320	-	
qd3	0	-44%	-	PXD101-ONC-7, Table 2
qd3, 10, 17	-9%	-	-	
	20	**40**	**80**	
qd 3-7	11%	11%	30%	PXD101-ONC-7, Table 3
qd 3-12	18%	18%	27%	PXD101-ONC-7, Table 4
qd 3,5,7	60%	30%	40%	PXD101-ONC-7, Table 5
qd3-7,10-14 (17-21)	-	45%	41%	PXD101-ONC-7, Table 6

qd: once daily. Intraperitoneal treatment.

Belinostat treatment, multiple doses per day

Schedule	Belinostat (mg/dose) (Accumulated Daily Dose)					Source
	10	20	40	80	160	
qid 3	-	-	30% (160)	31% (320)	44% (640)	PXD101-ONC-7, Table 7
oid 3	22% (80)	56% (160)	50% (320)	56% (640)	-	
oid 3, 7	30% (80)	69% (160)	90% (320)	-	-	

qid: four times daily; oid: eight times daily; Intraperitoneal treatment.

Belinostat treatment, multiple doses, multiple days

Schedule	Belinostat, mg/dose (Accumulated Daily Dose)			Source
	20	40	80	
qd 3-7	11% (20)	11% (40)	30% (80)	PXD101-ONC-7, Table 8
bid 3-7	33% (40)	44% (80)	-	
tid 3-7	67% (80)	102 (120)	-	

qd: once daily, bid: twice daily, tid: three times daily. Intraperitoneal treatment.

Belinostat treatment of a human ovarian carcinoma xenograft

Methods

Female NMRI mice were injected subcutaneously with 10^7 A2780 human ovarian cancer cells. When tumors reached 1000 mm3, mice were randomized and treated with belinostat formulated in L-arginine and injected IV into the tail vein at 10 ml/kg. Mice were treated once daily for 7 days (0, 30, 60 mg/kg) or 3x daily (10, 20 mg/kg). Tumor

biopsies were collected and kept in formalin; plasma and tumor tissue were collected after sacrifice.

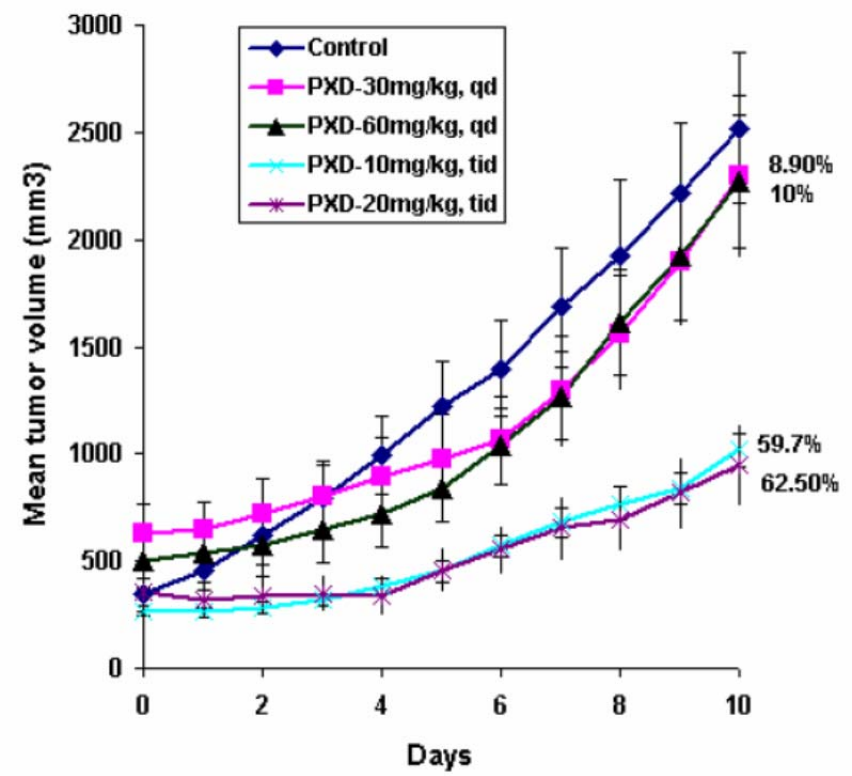
Results

The half-life of belinostat in mice is <50 min, therefore more frequent treatment was tested.

- Final tumor volumes were reduced after 1 day using more frequent dosing.

Figure 9 Mouse tumor xenograft model A2780 ovarian cancer

(Excerpted from Study PXD101-ONC-12)

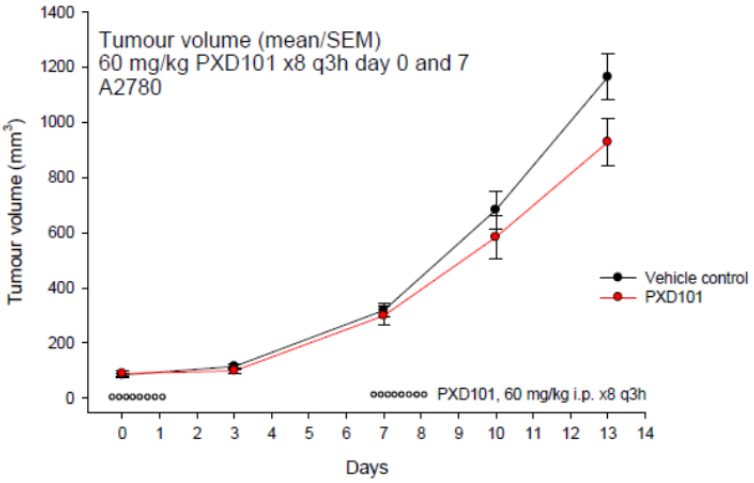


qd: dosing once daily; tid: dosing x3 daily; mice were dosed for 7 days

- Curiously, belinostat treatment once daily for 7 days at 60 mg/kg not exhibit an anti-tumor effect

Figure 10 Mouse tumor xenograft model (A2780 ovarian cancer cells)

(Excerpted from Study PXD101-ONC-12)



4.2 Secondary Pharmacology

D. Report # PX-CA-06: Effects of Belinostat and Metabolites on Cell Growth

This study investigated the pharmacological activity of the five major human belinostat metabolites. The major pathway of belinostat metabolism in humans appears to be through glucoronidation.[16]

Methods

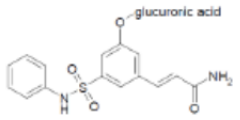
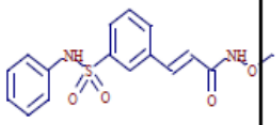
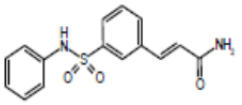
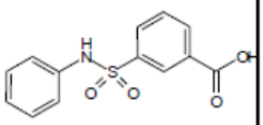
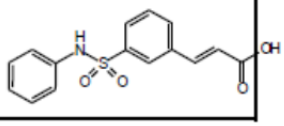
Belinostat metabolites
Identified human metabolites and their relative exposure were calculated from re-analyzed results of human plasma samples obtained in the clinical study PXD101-CLN8 from subjects that received 1000 mg/m2 IV.

[16] Wang LZ, et al. Glucuronidation by UGT1A1 Is the Dominant Pathway of the Metabolic Disposition of Belinostat in Liver Cancer Patients, (2013), 8:e54522.

Table 8 Relative Exposure of Human Belinostat Metabolites in Cancer Patients

(Excerpted from Study Report PX-CA-06)

Id	Mol weight (g/mol)	AUC inf (ng/ml*hr) Mean	AUC inf (uM*hr) Mean	Exposure relative to belinostat Fold
Belinostat	318	12500	39	1.0
Belinostat glucuronide	494	315000	638	16.2
Methylated belinostat	332	15000	45	1.1
Belinostat amide	302	11800	39	1.0
3-ASBA	277	29000	105	2.7
Belinostat acid	303	6500	21	0.5

Id	Alias	Classification in humans	Structure
Belinostat glucuronide	M18 TP201806	Major	
Methylated belinostat	PX106507	Major	
Belinostat amide	M21 PX118624	Major	
3-ASBA	M24 3-(AnilinoSulfonyl)BenzeneCarboxylic Acid TP201859	Major	
Belinostat acid	M26 PXD101-6 PX118623	Major	

WST-1 proliferation assay

Three cell lines, HeLa (cervix cancer), HCT-116 (colon cancer) and MCF-7 (breast cancer) were tested. Cells were incubated with different concentration of belinostat or

metabolites for 48 hours. The number of viable cells was then assessed using the Cell Proliferation Reagent WST-1 (tetrazolium salt).

Clonogenic assay
The same three cell lines that were tested in the WST-1 assay were also tested in a clonogenic assay (anchorage-independent growth).

Results
The inhibition of cell proliferation in vitro by belinostat metabolites was much lower compared to parent in HeLa (IC_{50} = 0.7 µM) and HCT-116 cells (IC_{50}= 0.3 µM). WST-1 assay results for belinostat metabolites were similar in MCF-7 cells.

Table 9 Inhibition of Cell Proliferation and Anchorage Independent Growth of Belinostat and the Major Human Metabolites

(Excerpted from the Pharmacology Written Summary)

Metabolite (designation) [Id]	Classification in Humans Exposure [a]	WST-1 IC_{50} in µM	Clonogenic Assay IC50 in µM		
		HeLa	HeLa	MCF-7	HCT-116
Belinostat (PXD101)	Parent	0.7	0.40	1.30	0.51
Belinostat glucuronide (TP201806) [M18]	Major 10-fold higher than belinostat	>100	26	>300	41
3-ASBA (TP201859) [M24	Major 1.5-fold higher than belinostat	>100	>300	>300	>300
Belinostat amide (PX118624) [M21] (also impurity)	Major Exposure like belinostat	>100	97	148	224
Belinostat acid (PXD101-6) [M26] (also impurity)	Major 3-fold lower than belinostat	>90	>300	>300	>300
Methyl belinostat (PX106507)	Major 3-fold lower than belinostat	ND	105	54	199

Three belinostat human metabolites displayed some activity in the clonogenic assay:
- belinostat glucoronide (IC_{50}: 26 µM)
- methylated belinostat (IC_{50}: 54 µM)
- belinostat amide (IC_{50}: 97 µM)

Belinostat glucoronide, the major human metabolite representing approximately 16-fold exposure compared to belinostat, was the most active belinostat metabolite in the clonogenic assay. However, the glucoronide metabolite activity is between 65- and 230-fold less than the activity of belinostat in the 3 cell lines tested.

4.3 Safety Pharmacology

Study title: PXD101: Cardiovascular and Respiratory Effects in the Anaesthetized Dog Following Intravenous Administration

Study no.:	1981-012
Study report location:	Section 4.2.1.3
Conducting laboratory and location:	(b) (4)
Report Issued:	October 2004
GLP compliance:	OECD GLP (issued 1998)
QA statement:	Yes
Drug, lot #, and % purity:	PXD101, PR1(1)-67, 96.9%

Key Study Findings

- Anesthetized dogs treated with 35 mg/kg experienced increased heart rate.
- Transient small increases in respiration rate occurred following 15 and 35 mg/kg administration, but were not significantly different from vehicle controls.

Methods

Doses:	0, 7, 15, 35 mg/kg
Frequency of dosing:	Single ascending doses administered at 30 min intervals
Route of administration:	Intravenous; 15 min
Dose volume:	1 ml/kg
Formulation/Vehicle:	L-Arginine in Water for Injection
Species/Strain:	Beagle dog
Number/Sex/Group:	2/sex/group (Group 1: vehicle; Group 2: treatment group)

Vehicle or belinostat were administered IV over 15 minutes in ascending doses at intervals of at least 30 minutes. The 4 dogs in the vehicle group received 3 doses. The 4 dogs in the belinostat group received 7, 15 and 35 mg/kg

- Systolic, diastolic and mean arterial blood pressure
- Heart rate
- Left ventricular pressure and its derivative, dP/dt_{max}
- Mean femoral artery blood flow.
- RR, QRS, PR, QT and QTc-intervals, and the peaks of the R, P and T-waves of the ECG complex
- Peak inspiratory and expiratory flow, tidal volume, minute volume and rate of respiration

Bioanalytical sampling for PK calculations were also collected, at 0, 5, 15 and 30 min post-infusion.

Results
Changes in cardiovascular parameters following PXD101 administration were limited to heart rate.

Table 10 Heart Rate Changes in Cardiovascular Safety Study (Dogs)

	Heart rate (bpm)	
Time (min)	Vehicle	PXD101, 35 mg/kg
baseline	92±7	85±6
2	103±11	129±13
10	103±9	133±21
20	109±10	129±10
30	103±9	116±13

There were no changes in the other parameters measured that indicated a difference between vehicle and PXD101 treatment.

Study title: Effects of PXD101 on Cloned hERG Channels Expressed in Mammalian Cells

Study no.:	455-01
Study report location:	Section 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 5, 2004
GLP compliance:	A signed statement is provided, but actual GLP status is unclear
QA statement:	Yes
Drug, lot #, and % purity:	PXD101, batch #PRQ1(3)-10 (Lot #8), 100%

Key Study Findings

- PXD101 inhibited hERG current at the two highest concentrations used: by 20.1% ±0.5% at 270 microM (n=3) and at 7.5±0.1% at 105.5 μM (n=3). An IC_{50} was not reached.

Methods

Doses:	8.4, 105.5 and 270 μM Test concentration analysis indicated they differed than expected: 10, 100, 300 μM (terfenadine 60 nM was used as a positive control)
Formulation/Vehicle:	0.3% DMSO in HEPES-buffered physiological saline
Species/Strain:	HEK-293 cells transfected with hERG cDNA were obtained from (b) (4)

Results

Patch clamp recordings were measured on three cells for each PXD101 concentration. Inhibition of hERG current was observed using concentrations of 105.5 and 270 μM (7.5±0.1% and 20.1% ±0.5%, respectively). The low concentration used inhibited hERG current at levels that were comparable to the vehicle control. The positive control (terfenadine, 60 nM) inhibited hERG by 74.1±1.8%.

Study title: Effects of PXD101 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers

Study no.:	456-01
Study report location:	Section 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 5, 2004
GLP compliance:	A signed statement is provided, but actual GLP status is unclear
QA statement:	Yes
Drug, lot #, and % purity:	PXD101, Batch #PRQ1 (3)-10, Lot #8, 100%

Key Study Findings

- PXD101 induced shortening of cardiac action potentials, suggesting mixed ion channel effects that would involve inhibition of depolarizing currents (i.e., Na and Ca).

Methods

Doses:	0.01, 0.05, 0.1 mg/ml
Formulation/Vehicle:	0.3% DMSO in Purkinje fiber Tyrodes solution

The effects of PXD101 on action potential parameters were compared to time-matched vehicle control sequences for statistical significance. Rate-dependent effects were measured at the following cycle lengths:

2 sec (simulates bradycardia)
1 sec (simulates normocardia)
0.5 sec (simulates tachycardia)

Results

PXD101 induced significant changes in duration of the action potential measured from the initial upstroke to the point of return to 60% of the initial resting potential at all three cycle lengths. PXD101-induced action potential shortening was physiologically significant and suggests mixed ion channel effects involving additional inhibition of depolarizing currents (sodium and calcium channels). PXD101 did not significantly change the resting membrane potential, action potential amplitude or the maximum rate of depolarization when compared to time-matched vehicle control values.

Study title: Effects on General Activity and Behavior in the Rat Following Intravenous Administration

Study no.:	2525-006
Study report location:	Section 4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 25, 2004
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	PXD101, PRQ1(3)-10, 100.5%

Key Study Findings

- PXD101 did not result in significant behavioral or physiological changes in rats when compared to vehicle-treated animals.

Methods

Doses:	10, 25 or 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	IV
Dose volume:	10 ml/kg
Formulation/Vehicle:	L-arginine at same concentrations as PXD101
Species/Strain:	Rat/Crl:WI[Glx/BRL/Han]
Number/Sex/Group:	6 males/group
Age:	6 weeks
Weight:	174-211 g

Observations and Results

Observations were made at 1, 5, 15, 30, 60 and 120 minutes post-infusion and daily 7 days thereafter.

- There were no remarkable behavioral or physiological changes following belinostat administration.
- The positive control chlorpromazine (3 mg/kg) produced marked changes in all animals that were apparent beginning within 5 minutes postdose.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: PXD101: Metabolite Profiling in Dog Plasma Samples Following Oral and Intravenous Administration

Study no.:	1981-035
Study report location:	Section 4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 12, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PXD101; Lot # PR1(1)-67; Purity 96.9%

Methods:

Dog plasma samples were generated from Study #53973 and were previously analyzed under (b) (4) reverence number 1981-034 using LC-MS/MS. Two samples per animal per dose route were used:

- Oral dose Day 1 (11/19/03) animals 1 and 2: 30 minutes and 90 minutes
- IV dose Day 15 (12/03/03) animals 1 and 2: 15 minutes and 60 minutes

Results:

The following metabolites were detected as identified based entirely on the molecular masses determined suing full scan single MS. Product ion MS/MS was not performed on the samples. The relative amounts of the metabolites were not determined.

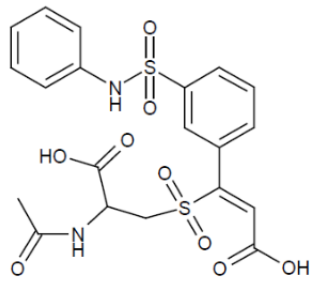
Table 11 PXD101 Metabolites Detected in Dog Plasma

(Table Excerpted from the Study 1981-035)

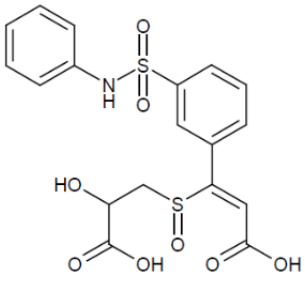
Rt	Mwt	Oral (Day 1)				IV (Day 15)				Metabolite Codes
		dog 1 30 min	dog 2 30 min	dog 1 90 min	dog 2 90 min	dog 1 15 min	dog 2 15 min	dog 1 60 min	dog 2 60 min	
19.3	496					✓		✓	✓	M2
21.2	439					✓		✓	✓	M11
21.6	423		✓		✓	✓		✓	✓	M12
27.7	318	✓	✓	✓	✓	✓	✓	✓	✓	M6 (PXD101)
28.9	302	✓		✓		✓	✓	✓	✓	M7

Figure 11 PXD101 Metabolite Structure

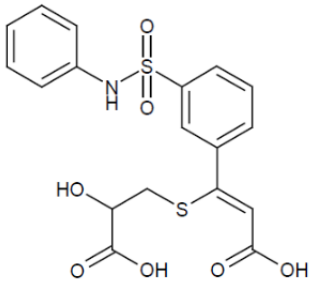
M2



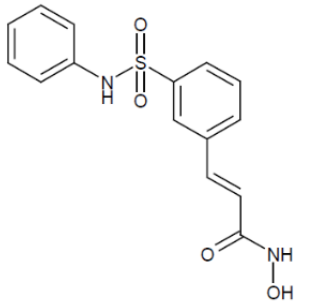
M11



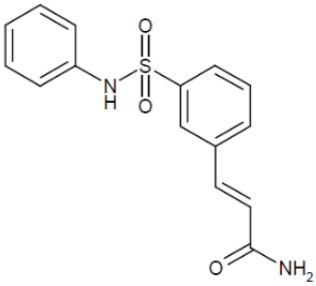
M12



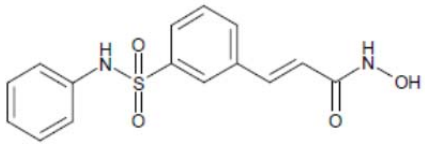
M6



M7



PXD101 Structure



5.2 Toxicokinetics

Refer to individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose studies were not reviewed.

6.2 Repeat-Dose Toxicity

Study title: PXD101: Cyclic Intravenous Dosing Study in the Rat (8 Cycles over 24 Weeks)

Study no.:	2525-001
Study report location:	Section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 17, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PXD101; Lot # PRX1(6)-54; Purity 99.3%

Key Study Findings

- Mortality at 100 mg/kg/day was 40% in male and 20% in female rats. Microscopic findings in early decedents included minimal cardiomyopathy and thymus atrophy possibly associated with PDX101 administration. Tail lesions observed in all early decedents was associated with intravenous dosing.
- PDX101-related, adverse and significant reduction in mean body weight gain in all groups and corresponded with lower mean food consumption.
- Decreased RBCs, reticulocytes, and WBCs indicate that PXD101 may cause bone marrow suppression at 100 mg/kg/day.
- A dose-proportional and significant reduction in alanine aminotransferase values occurred in males at all doses and in females at 25 and 100 mg/kg/day. There were no microscopic correlates in the liver.
- High number of rats in the 10 and 25 mg/kg/day groups presented urine with protein and blood. The incidence was lower to absent in the 100 mg/kg/day.
- Gross lesions included small thymus in 29% of males at 100 mg/kg/day that corresponded with microscopic findings of minimal atrophy.
- Microscopic findings at terminal sacrifice possibly associated with PDX101 administration included minimal thymus atrophy in 48% of male rats

Methods

Doses:	0, 10, 25 or 100 mg/kg/day. Dose selection based on 1-cycle toxicology study in rats
Frequency of dosing:	8 cycles of 5 days treatment followed by 16 days of drug-free period. Dosing for females was staggered by 1 week.
Route of administration:	Intravenous infusion over ~15 minutes
Dose volume:	10 mL/kg at a rate of 0.5 mL/minute
Formulation/Vehicle:	L-Arginine in water for injection on a ratio of 2:1 PXD101 by weight
Species/Strain:	Rats CRL:WI(GLX/BRL/Han)
Number/Sex/Group:	Control and high-dose groups 15 rats/sex/group; other groups 10 rats/sex/group
Age:	Males: 28 to 35 days; Females: 21 to 28 days
Weight:	Males: 173.4 to 231.8 g; Females: 129.3 to 183.1 g
Satellite groups:	18 rats/sex/group in drug-treated groups for TK analysis
Blood samples:	Blood was withdrawn from satellite groups on dosing Days 1, 5 and 151 after 5, 15 and 30 min and 1, 2, 6, and 24 hours after dosing
Unique study design:	10 rats/sex/group were terminated after administration of the last dose in cycle 8th and 5 rats/sex/group were kept for 16 days recovery
Deviation from study protocol:	Blood samples collected for hematology analysis on cycle 6 included samples from 5 rats/sex in the control and 100 mg/kg/day groups assigned for recovery at the end of the study

Observations

Table 12 Experimental Design in Rats

Group #	PXD101 Dose level (mg/kg/day)	Animals / group					
		Terminal		Recovery		TK Groups	
		M	F	M	F	M	F
1	0	10	10	5	5	-	-
2	10	10	10	-	-	8	8
3	25	10	10	-	-	8	8
4	100	10	10	5	5	8	8

Table 13 Treatment Cycles in Rats

Cycle of Study		Day of Study	Week of Study
Cycle 1	Treatment	1-5	1
	Recovery	6-21	1-3
Cycle 2	Treatment	22-26	4
	Recovery	27-42	4-6
Cycle 3	Treatment	43-47	7
	Recovery	49-63	7-9
Cycle 4	Treatment	64-68	10
	Recovery	69-84	10-12
Cycle 5	Treatment	85-89	13
	Recovery	90-105	13-15
Cycle 6	Treatment	106-110	16
	Recovery	111-126	16-18
Cycle 7	Treatment	127-131	19
	Recovery	132-147	19-21
Cycle 8	Treatment	148-152	22
	Recovery	153-168	22-24

Results

Mortality

Three rats from the control and the 100 mg/kg/day groups were replaced during the first dosing cycle because of treatment-related severe tail vein lesions. Thereafter, a total of 8 rats from the main groups and 5 rats from the satellite groups died or were euthanized because of tail vein lesions, Table 14.

Table 14 Early Decedents

Group/sex	Week of Death	Dosing Cycle	Comments/Clinical signs
Main Study			
1M	4	2	Found dead after dosing; no clinical signs
4M	7	3	Euthanized; ulcerated tail, moribund
4M	20	7	Euthanized; ulcerated tail, moribund
4M	20	7	Euthanized; ulcerated tail, moribund
4M	20	7	Euthanized; ulcerated tail, moribund
1F	4	2	Found dead after dosing; no clinical signs
4F	14	5	Euthanized; ulcerated tail, moribund
4F	17	6	Euthanized; shedding/peeling tail skin; hard, swollen, ulcerated tail; moribund
Satellite Study			
4M	2	1	Euthanized
4M	21	7	Euthanized
4F	6	2	Euthanized
4F	17	6	Euthanized
4F	17	6	Euthanized

Clinical Signs

Tail lesions were the most frequent clinical signs; the 100 mg/kg/day group had the highest number of animals with tail lesions, **Error! Reference source not found.**.

Clinical signs of protruding eyes, and staining/thinning fur were present more frequently in females.

Table 15 Number of Rats with Clinical Signs

Clinical Signs		PXD101 Dose (mg/kg/day)							
		0		10		25		100	
	Gender # rats/group	M 15	F 15	M 10	F 10	M 10	F 10	M 15	F 15
Tail sore/lesions Ulcerated, other abnormalities		-	1	1	-	1	1	11	9
Teeth abnormalities Pale/white patches lower/upper incisors		-	-	1	-	-	-	1	-
Thinning fur, dorsal		3	4	-	2	-	1	-	6
Staining, dorsal		-	4	-	4	-	1	-	1
Protruding eyes		-	-	-	1	-	2	-	4

Body Weight

Mean body weight gain during the study, Days 1 through 155, was lower for rats in the 10, 25 and 100 mg/kg/day groups compared to control. Mean body weight gain was lower during the 5 day-treatment compared to control while it was comparable to control rats during the 16 days-treatment free. The lower body weight gain corresponded with lower mean food consumption in the 10, 25 and 100 mg/kg/day groups.

Table 16 Percent Change in Mean Body Weight Gain Compared to Control for Days 1 through 155 (Rats)

Gender	PXD101 Dose (mg/kg/day)		
	10	25	100
Males	-9.7	-14.4 **	-14.0 *
Females	-13.1 *	-9.7	-4.5

*p<0.05; **p<0.01

Food Consumption

Mean food consumption during the study, Days 1 through 154, was lower for rats in the 10, 25 and 100 mg/kg/day groups compared to control. Mean food consumption was lower during the 5 day-treatment compared to control while it was comparable to control rats during the 16 days-treatment free.

Table 17 Percent Change in Mean Food Consumption Compared to Control for Days 1 through 154 (Rats)

Gender	PXD101 Dose (mg/kg/day)		
	10	25	100
Males	-4.1	-9.8	-4.9
Females	-4.7	-9.7	-4.5

Ophthalmoscopy

Unremarkable

ECG

Not conducted

Hematology

Blood samples for clinical pathology analysis were taken at the end of the 5-day treatment for cycles 2, 4, 6 and 8 and at the end of the recovery period. Blood samples for cycle 6 included those from animals assigned for recovery at the end of the study.

Hemoglobin concentration, red blood cell (RBC) counts and packed cell volume were in general 3% to 10% lower compared to control for rats in the 10 and 25 mg/kg/day during most of the treatment cycles. Effects in the 100 mg/kg/day group were not significantly different from controls except for males during cycle 2. There was variability in reticulocytes counts. Reticulocytes were significantly increased in the 10 mg/kg/day males during cycles 2-8 and in females during cycles 2-4. Reticulocyte counts were 15.8 % lower in 100 mg/kg/day males and significantly lower in females during cycles 6-8. Reticulocyte counts were higher in females and significantly higher in males in the 100 mg/kg/day group during the recovery phase.

White blood cells (WBC) counts, including neutrophils and lymphocytes, were lower in all groups compared to controls with statistical significance in the 25 and 100 mg/kg/day groups. WBCs returned to normal values or were significantly increased during the recovery phase.

Platelet counts showed a trend to be lower in all groups compared to control and all cycles reaching statistical significance for males during cycle 6. Mean platelet volume showed a trend for higher values compared to control with statistical significance in males at 10 and 25 mg/kg/day.

In general, hematological results of decreased RBC, reticulocytes, and WBC indicate that PXD101 may cause bone marrow suppression at 100 mg/kg/day.

Table 18 Hematological Parameters Percent Change as Compared to Controls

Parameter		PXD101 Dose (mg/kg/day)					
		10		25		100	
		M	F	M	F	M	F
Hemoglobin							
	Cycle 2	↓**3.9***	↓7.4	↓1.3	↓6.0	↓1.3	↓0.7
	Cycle 4	↓5.9	↓**7.2****	↑1.3	↓**4.6***	↑4.6	↓0.7
	Cycle 6	↓3.3	↓**4.6****	↑0.7	↓1.3	↑2.6	↓0.7
	Cycle 8	↓2.0	↓5.3	↑1.3	↓2.7	↑2.0	↓0.7
	Recovery	ND	ND	ND	ND	↑2.0	↓2.4
Red Blood Cell							
	Cycle 2	↓**6.9****	↓**7.0****	↓2.9	↓**6.0***	↓**6.5***	↓1.7
	Cycle 4	↓**10.2***	↓**6.8***	↓1.9	↓**6.3***	↓4.0	↓1.7
	Cycle 6	↓**5.9****	↓3.3	↓1.6	↓2.6	↓1.6	↓3.3
	Cycle 8	↓5.6	↓6.2	↓1.1	↓3.3	↓3.4	↓0.7
	Recovery	ND	ND	ND	ND	↑1.9	↑2.2

Parameter	PXD101 Dose (mg/kg/day)					
	10		25		100	
	M	F	M	F	M	F
Absolute Reticulocytes						
Cycle 2	↑**30****	↑**45****	↑15	↑25	0	↑25
Cycle 4	↑89.5	↑**41.2***	↑10.6	0	↓15.8	↑5.9
Cycle 6	↑**21.1***	0	↑5.3	↓11.1	↓15.8	↓**33.3****
Cycle 8	↑21.1	↑5.9	↑15.8	↓11.8	↓15.8	↓23.5
Recovery	ND	ND	ND	ND	↑**53.3***	↑10.0
White Blood Cell						
Cycle 2	↓9.9	↓2.3	↓**31.0****	↓14.0	↓**38.0****	↓30.2*
Cycle 4	↓11.4	↓11.1	↓**40.0*****	↓16.7	↓**38.6*****	↓**30.6****
Cycle 6	↓6.2	↓14.6	↓24.6	↓**22.0***	↓**32.3****	↓**29.3****
Cycle 8	↓10.5	↓3.2	↓**42.5****	↓12.9	↓17.5	↓**22.6***
Recovery	ND	ND	ND	ND	↑7.3	↑34.4
Neutrophils						
Cycle 2	↓9.1	↓16.7	↓18.2	↓33.3	↓36.4	↓50.0
Cycle 4	↓35.7	0	↓**50.0***	↓16.7	↓**50.0***	↓33.3
Cycle 6	↑23.1	↓37.5	↑7.7	↓50.0	↑38.5	0
Cycle 8	↓**36.4***	0	↓**36.4***	↓20.0	↑27.3	↓20.0
Recovery	ND	ND	ND	ND	↑27.3	↑28.6
Lymphocytes						
Cycle 2	↓12.1	↓8.3	↓**34.5****	↓13.9	↓**39.7****	↓**30.6***
Cycle 4	↓3.9	↓10.7	↓**36.5****	↓14.3	↓**34.6****	↓**28.6***
Cycle 6	↓18.0	↓9.7	↓**32.0***	↓16.1	↓**34.0****	↓**38.7*****
Cycle 8	↓6.8	↓4.0	↓**45.5****	↓12.0	↓18.2	↓**28.0***
Recovery	ND	ND	ND	ND	↑2.4	↑**43.5***
Mean Myelogram						
Terminal necropsy	ND	ND	ND	ND	↓15.6	↓6.5
Recovery necropsy	ND	ND	ND	ND	↓3.7	↑2.4
Platelets						
Cycle 2	↓9.7	↑0.6	↓9.4	↑6.3	↓13.2	↑7.7
Cycle 4	↓6.8	↓0.6	↓14.0	↑2.1	↓19.3	↑2.4
Cycle 6	↓**13.9****	↓6.0	↓**15.9*****	↓4.0	↓**15.7*****	↓3.0
Cycle 8	↓12.9	↓0.1	↓24.0	↑1.5	↓13.3	↑6.5
Recovery	ND	ND	ND	ND	↑2.3	↑8.9
Mean Platelet Volume						
Cycle 2	↑4.1	↑4.1	↑5.5	↑2.7	↑5.5	↑2.7
Cycle 4	↑**7.3****	↑6.7	↑**7.3****	↑8.0	↑7.3	↑9.3
Cycle 6	↑3.8	↓1.4	↑**6.3***	↑1.4	↑3.8	↑1.4
Cycle 8	↑2.7	↑4.0	↑6.9	↑2.7	↑6.9	↓1.3
Recovery	ND	ND	ND	ND	↑3.2	↑1.4
Prothrombin time						
Cycle 2	0	↑2.1	↑0.5	↑**5.1***	↑6.2	↑3.6
Cycle 4	↓3.8	↓3.3	0	↑1.0	↑1.0	↑6.2
Cycle 6	↓0.5	↓1.8	0	↑1.8	↑7.1	↑4.6
Cycle 8	↑1.4	↓2.7	↑2.7	↑0.9	↑4.5	↑8.1
Recovery	ND	ND	ND	ND	↑4.4	↑2.6
Activated Partial Thromboplastin time						
Cycle 2	↑0.5	↓9.7	↑5.1	↓8.2	↑0.5	↓3.1
Cycle 4	0	↓4.3	↑0.5	↓3.2	↑19.1	↑2.7
Cycle 6	↓3.1	↓10.7	↑5.3	↑2.8	↑3.1	↓2.3
Cycle 8	↓1.3	↓8.5	↑1.8	↓3.8	↓1.3	↓1.4
Recovery	ND	ND	ND	ND	↓1.8	↓8.6

ND= not determined; * P<0.5, ** P<0.01, *** P<0.001. Values in **bold** denotes statistical significance

Clinical Chemistry

Alanine aminotransferase (ALT) decreased in animals at all dose levels except females at 10 mg/kg/day. ALT values were significantly lower in the 25 and 100 mg/kg/day groups. The biological significance is unclear given the absence of microscopic findings in the liver. During cycle 4, values for albumin, albumin:globulin ratio, and glucose were higher compared to control values for males in the 25 and 100 mg/kg/day groups, while values for globulin and total cholesterol were lower compared to control values for the same group of animals during cycle 4. These parameters were comparable to control values at all other intervals.

Table 19 Clinical Chemistry Parameters Percent Change Compared to Control

Parameter	PXD101 Dose (mg/kg/day)					
	10		25		100	
	M	F	M	F	M	F
Alanine Aminotransferase						
Cycle 2	↓3.6	↑2.5	**↓16.4***	↓10.0	**↓25.5*****	↓17.5
Cycle 4	↓6.5	0	↓15.2	↓10.3	**↓21.7****	↓20.5
Cycle 8	↓12.7	0	**↓25.5*****	**↓10.2***	**↓43.6*****	**↓30.8****
Recovery	ND	ND	ND	ND	↑24.5	↓7.3

ND= not determined; * P<0.5, ** P<0.01, *** P<0.001. Values in **bold** denote statistical significance

Urinalysis

Qualitative analysis of urine collected during cycles 4 and 8 showed a high percent of rats in the 10 and 25 mg/kg/day groups with presence of blood. The presence of protein and urine colored red or brown was associated in most cases. The incidence was lower to absent in the 100 mg/kg/day for both male and female rats.

Table 20 Percent Animals with Changes in Urine Analysis Parameters

Parameter	PXD101 Dose (mg/kg/day)							
	0		10		25		100	
	M	F	M	F	M	F	M	F
Protein (approximately ≥1.0 g/L)								
Cycle 4	0	0	**100**	**80**	**70**	**50**	0	0
Cycle 8	10	0	**70**	**60**	**100**	**70**	**22**	0
Recovery	0	0	0	0	0	0	0	0
Blood (approximately ≥50x10^{b} RBC/L)								
Cycle 4	11	11	**100**	**100**	**100**	**100**	**33**	**22**
Cycle 8	30	30	**90**	**100**	**100**	**100**	**57**	**40**
Recovery	0	0	0	0	0	0	0	0
White Blood Cell (approximately ≥11-30 WBC/field)								
Cycle 4	0	0	**50**	10	**80**	0	**22**	0
Cycle 8	0	0	0	10	0	**20**	0	0
Recovery	0	0	0	0	0	0	0	0

Parameter	PXD101 Dose (mg/kg/day)							
	0		10		25		100	
	M	F	M	F	M	F	M	F
Bacteria amorphous debris (some to heavy presence/field)								
Cycle 4	20	20	**50**	20	**60**	0	**80**	0
Cycle 8	67	70	**80**	80	70	70	43	80
Recovery	0	0	0	0	0	0	0	0

Values in **bold** denotes higher incidence compared to control group

Gross Pathology

Early decedents:

- Tail lesions at the injection site (sore, firm, large, flaky, black and soft) were present in 4/4 males and 2/2 females from the 100 mg/kg/day and corresponded with observed clinical signs. One male presented a large and raised area in the liver and one female was observed with mottled liver.

Terminal sacrifice:

- Tail lesions, sore, flaky, thick or abnormal appearance, observed in most rats from the 100 mg/kg/day group.
- Protruding eyes observed in 10%, 30% and 50% in females from the 10, 25 and 100 mg/kg/day, respectively, and corresponded with clinical signs for these groups. No microscopic correlates.
- Small thymus recorded in 29% of males in the 100 mg/kg/day group and thymus with red spots in 16% of rats in the 10 mg/kg/day.
- Mottled liver present in 10% of males from the 25 mg/kg/day group and a pale liver in 10% of males from each the 10 and 25 mg/kg/day groups.
- Pale kidney observed in10% and 29% of males in the 10 and 100 mg/kg/day groups and pelvic dilation in 10% of rats from each group except control.

Recovery sacrifice, (100 mg/kg/day group):

- Distension/red in the urinary bladder in 75% of males.
- Distension of the uterus in 20% of females.
- Red spots in mandibular lymph nodes in 22% of rats.
- Tail sores tail in 44% of rats.

Organ Weights

Unremarkable

Histopathology

Adequate Battery: YES

Peer Review: NO

Histological Findings in Early Decedents

No relevant microscopic findings were observed in one male and one female from the control group. Minimal cardiomyopathy was observed in 1/4 males and minimal thymus atrophy in 1/4 males and1/2 females from the 100 mg/kg/day groups. Spleen hematopoiesis was observed only in animals from the 100 mg/kg/day groups.

Histological Findings Terminal Sacrifice Animals

Minimal testes tubular atrophy was observed in 1/7 males and minimal thymus atrophy in 3/7 males from the 100 mg/kg/day group. Spleen hematopoiesis was observed only in the controls or the high dose group. Tail lesions of different severity were present in animals from all groups.

Histological Findings Recovery Sacrifice Animals

Minimal heart cardiomyopathy was observed in one male at the 100 mg/kg/day and one female from the control group; also, an inflammatory cell foci in the heart was observed in one male at the 100 mg/kg/day.

Table 21 Microscopic Findings in Rats

PXD101 Dose (mg/kg/day)	Males				Females			
	0	10	25	100	0	10	25	100
Early Decedents								
Number of Animals in Group	**1**	**0**	**0**	**4**	**1**	**0**	**0**	**2**
Heart -cardiomyopathy				1a				
Spleen -Hematopoiesis				1a, 1b	1p			1b
Ovary - congestion/hemorrhage					1p			
Thymus -atrophy -congestion/hemorrhage				1a				1a
Tail lesions -phlebitis, dermatitis, edema, hemorrhage				2c, 1d				1c, 1d
Terminal Sacrifice								
Number of Animals in Group	**8**	**10**	**10**	**7**	**9**	**10**	**10**	**8**
Heart -inflammatory cell foci	1a				1a			
Spleen -Hematopoiesis	7a, 1b			5a, 1b	5a, 3b			4a, 3b, 1c
Testes -tubular atrophy				1a				
Thymus -atrophy -congestion/hemorrhage			2p	3a				1p
Tail lesions - phlebitis, dermatitis, edema, hemorrhage	4a	7a, 1b	6a, 1b	3a, 3b, 1c	4a, 1b	4a	2a, 1b	5a, 3b
Recovery								
Number of Animals in Group	**5**	**0**	**0**	**4**	**5**	**0**	**0**	**5**
Heart								

-cardiomyopathy -inflammatory cell foci				1a 1a	1a			
Spleen -Hematopoiesis	2a			2a, 1b	2a, 3b			4a
Testes - tubular atrophy	1e							
Tail lesions -phlebitis, dermatitis, edema, hemorrhage	1a			2a, 1b	2a			1b

a, minimal; b, slight; c, moderate; d, marked; e, severe; p, present

Special Evaluation

None

Toxicokinetics

Systemic exposure to PDX101, as measured by Cmax and AUC (0-24h), increased with dose in an approximately proportional or greater than proportional manner. Systemic exposure was similar in both male and female rats except at the high dose of 100 mg/kg/day. After repeated exposure, Cmax and AUC (0-24h) values increased more than 1.5x, indicating accumulation. Tmax values were similar in both males and females and occurred at 0.08 hours.

Table 22 PDX101 Toxicokinetic Parameters in Rats

(Table Excerpted from Study Report 2525-001)

Dose Group	Dose (mg/kg/day)	Sex (n=3)	C_{max} (µg/mL)			$AUC_{(0-24h)}$ (µg.h/mL)		
			Day 1	Day 5	Day 151	Day 1	Day 5	Day 151
2	10	M	1.14	1.25	2.63	0.27	0.28	0.71
		F	1.85	1.64	2.48	0.40	0.39	0.53
3	25	M	3.13	4.66	7.64	0.81	0.97	2.22
		F	4.56	4.36	6.15	1.01	1.13	1.76
4	100	M	14.06	17.71	30.34	5.87	11.07	18.45
		F	23.87	23.65	37.10	6.81	7.17	12.09

Dosing Solution Analysis

Dose formulations of PXD101 were analyzed for homogeneity and stability at the lowest and highest dose concentrations on Cycle 1 Day1 when stored at room temperature for 7 days. Concentration verification analyses were conducted for dose formulations from Weeks 1 and 22. Results showed that dose formulations were homogenous and stable under the conditions tested and that concentration of the dose formulations were within 90% to 110% of the nominal values.

Appears this way on original

Study title: PXD101: Cyclic Intravenous Dosing Study in the Dog (8 Cycles over 24 Weeks)

Study no.:	2525-013
Study report location:	Section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 26, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PXD101 (belinostat); Lot # PRX1(6)-54; Purity 99.3%

Key Study Findings

- Clinical signs during and/or immediately after dosing included vomiting, soft/liquid/mucoid feces, salivation, impaired mobility and subdued behavior. Changes in hematological parameters included:
  - Increases in absolute reticulocytes (all dose levels), red cell distribution width (50 mg/kg/day), platelets (males at all dose levels) and activated partial thromboplastin time values (males at 25 and 50 mg/kg day).
  - Decreases in white blood cells, lymphocytes (all dose levels), neutrophils and prothrombin time (females at 50 mg/kg/day).
- Changes in clinical chemistry parameters included:
  - Decreases in alkaline phosphatase at all dose levels in both sexes.
  - Increases in glucose and inorganic phosphorus at 50 mg/kg/day.
  - Increases in urea at all dose levels in females and in 50 mg/kg/day males.
- Changes in organ-to-body weight ratios included:
  - Decreased spleen, liver (females), prostate and testes/epididymides at all dose levels
  - Dose-dependent increase in heart (males).
- Microscopic findings included:
  - Lymphoid atrophy in the mesenteric, mandibular, spleen, ileum, and cecum tissues in dogs given 50 mg/kg/day.
  - Focal nephropathy and inflammatory cell foci in the kidney was observed in 33% of dogs.
  - Exfoliated cells (spermatids) in the epididymides present in males at all dose levels including those graded as pubescent suggesting a belinostat-related delay of testicular maturation in male dogs.
  - Microscopic findings were still present at recovery sacrifice.

Methods

Doses:	0, 10, 25 or 50 mg/kg/day. Dose selection based on 2-cycle toxicology study in dogs
Frequency of dosing:	8 cycles of 5 days treatment followed by 16 days of drug-free period
Route of administration:	Intravenous infusion over ~15 minutes between alternate cephalic veins
Dose volume:	5 mL/kg
Formulation/Vehicle:	L-Arginine in water for injection on a ratio of 2:1 PXD101
Species/Strain:	Beagle dogs
Number/Sex/Group:	Control and high-dose groups 5 dogs/sex/group; other groups 3 dogs/sex/group
Age:	4 months old (16 to 17 weeks)
Weight:	Males: 6.9 to 9.6 kg; Females: 6.1 to 8.0 kg
Satellite groups:	None
Unique study design:	3 dogs/sex/groups were terminated after administration of the last dose in cycle 8th and 2 dogs/sex/groups were kept for 16 days recovery
Deviation from study protocol:	One male and one female in the 50 mg/kg/day received only 94% and 83% of the intended dose on one occasion

Group #	PXD101 Dose level (mg/kg/day)	Animals / group			
		Terminal		Recovery	
		M	F	M	F
1	0	3	3	2	2
2	10	3	3	-	-
3	25	3	3	-	-
4	50	3	3	2	2

Results

Mortality

No deaths occurred during the study.

Clinical Signs

Post-dose clinical signs most frequently observed in the 25 and 50 mg/kg/day groups included:

- Vomiting during dosing, soft/liquid/mucoid feces.
- Few cases of salivation, impaired mobility, and subdued behavior.

Clinical signs most frequently observed during the first 3-cycles in the 25 and 50 mg/kg/day groups included:

- Swollen head and/or legs

- Soft/liquid/mucoid feces, which also occurred during the course of the study in few animals

Body Weights

Changes in mean body weight and mean body weight gain were unremarkable.

Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Recordings were taken using the fixed limb leads I, II and III and the augmented leads aVR, aVL and aVF. Heart rates were derived from lead II. No other numerical data was derived because there were no PXD101-related changes in heart rates.

Hematology

Blood samples for hematology analysis were taken pre-treatment, at the end of the 5-days of administration of PXD101 in Cycles 2, 4, 6 and 8 and at the end of the recovery period.

- Higher reticulocyte counts compared to control occurred in 10 and 25 mg/kg/day male and female dogs and in 50 mg/kg/day females in all cycles. At recovery sacrifice, 50 mg/kg/day male and female dogs presented higher reticulocyte counts indicating stimulation of RBC.
- Red cell distribution width (RCDW) showed a trend for increases in 10 and 25 mg/kg/day dogs at all cycles and increased values were significantly higher in the 50 mg/kg/day dogs from cycle 4 through 8. At recovery sacrifice, 50 mg/kg/day male and female dogs presented higher RCDW values compared to control.
- Neutrophil values were variable at all dose levels reflecting the normal variation in dogs because of handling. White blood cells and lymphocytes values were decreased at all dose levels in both male and female dogs with significant lower values in 50 mg/kg/day females in cycle 6 and 8. At recovery sacrifice, 50 mg/kg/day male and female dogs presented similar values or higher values, respectively, compared with control dogs.
- Platelet values increased in male dogs at all dose levels and cycles compared with control. On the contrary, platelet values in female dogs were in general variable but lower at all dose levels in cycle 8 compared with control. At recovery sacrifice, 50 mg/kg/day dogs presented higher values compared with control dogs.
- Prothrombin time values were in general lower compared with control with significant decreased values in 50 mg/kg/day females in cycle 2 and 4 and values.
- Activated partial thromboplastin time (APTT) was in general increased in male dogs at all dose levels and cycles. A 10% percent higher APTT represents an increase in approximately 1.5 seconds and the significant increase of 27.9%

APTT in 25 mg/kg/day males during cycle 6 represents an increase in approximately 4 seconds.

Table 23 Percent Change in Hematological Parameter Values Compared with Control

Parameter		PXD101 Dose (mg/kg/day)					
		10		25		50	
		M	F	M	F	M	F
Absolute Reticulocytes							
	Cycle 2	↑**25.0**	0.0	↑**12.5**	↑**25**	0.0	↑**37.5**
	Cycle 4	↑**42.9**	↑**100.0**	↑**14.3**	↑**60.0**	0.0	↑**60.0**
	Cycle 6	↑**50.0**	↑**66.7**	↑**33.3**	↑**33.3**	↑**16.7**	↑**50.0**
	Cycle 8	↑**33.3**	↑**40.0**	↑**16.7**	↑**20.0**	↓**16.7**	↑**20.0**
	Recovery	NA	NA	NA	NA	↑**130.0**	↑**75.0**
Red Cell Distribution width							
	Cycle 2	↑1.5	↑1.5	↓2.3	0.0	↑5.3	↑5.3
	Cycle 4	↑1.5	↑2.3	↓1.5	↑2.3	↑**5.3***	↑**6.9***
	Cycle 6	↑3.9	↑2.4	↑0.8	↑1.6	↑**7.9****	↑**6.3****
	Cycle 8	↑4.9	↑2.4	0.0	↑1.6	↑**6.5****	↑**5.6***
	Recovery	NA	NA	NA	NA	↑**9.7**	↑**9.8**
White Blood Cells							
	Cycle 2	↓**17.8**	↑7.0	↓7.4	↑6.1	↓**18.5**	↓**10.4**
	Cycle 4	↑2.9	↑7.8	↓1.0	↓1.9	↑**18.4**	↓**17.5**
	Cycle 6	↓9.6	↑**20.0**	↓**19.2**	↓**15.0**	↓**15.4**	↓**22.0**
	Cycle 8	↓6.6	↓0.9	↓7.7	↓**27.0**	↓5.5	↓**28.7***
	Recovery	NA	NA	NA	NA	↓**25.0**	0.0
Neutrophils							
	Cycle 2	↓**23.1**	↑1.6	↓6.4	↑**19.4**	↓**10.3**	↑4.8
	Cycle 4	↑**18.2**	↑**17.3**	↑7.3	↑**15.4**	↑**61.8***	↓5.8
	Cycle 6	↑1.8	↑**39.2**	↓**14.3**	↓**13.7**	↑1.8	↓7.8
	Cycle 8	0.0	↑2.9	↑4.1	↓**26.5**	↑**14.3**	↓**19.1**
	Recovery	NA	NA	NA	NA	↑5.5	↓**30.0**
Lymphocytes							
	Cycle 2	↓6.8	↑**16.3**	0.0	↓9.3	↓**25.0**	↓**27.9**
	Cycle 4	↓**13.5**	↓2.4	↓5.4	↓**22.0**	↓**29.7**	↓**29.3**
	Cycle 6	↓**21.6**	↓2.5	↓**18.9**	↓**12.5**	↓**29.7**	↓**37.5***
	Cycle 8	↓**12.5**	↓**10.5**	↓**18.8**	↓**29.0**	↓**25.0**	↓**44.7****
	Recovery	NA	NA	NA	NA	↑5.5	↓**30.0**
Platelets							
	Cycle 2	↑**30.4**	↑0.6	↑**40.6**	↓5.4	↑**17.0**	↑3.7
	Cycle 4	↑**40.60**	↑6.0	↑**52.6***	↓1.0	↑**22.9**	↑0.7
	Cycle 6	↑**12.0**	0.0	↑**16.3**	↓3.1	↑**18.2**	↓4.1
	Cycle 8	↑**22.8**	↓**16.7**	↑**22.4**	↓**12.7**	↑**14.9**	↓**10.0**
	Recovery	NA	NA	NA	NA	↑**25.2**	↑**13.3**
Prothrombin time							
	Cycle 2	↓1.6	↓3.1	↓3.2	↓6.3	0.0	↓**9.4***
	Cycle 4	↓6.3	↓7.9	↓6.3	↓4.8	↓4.7	↓7.9
	Cycle 6	↓5.9	↓4.4	↓5.9	↓5.9	0.0	↓**10.3***
	Cycle 8	↓7.8	↑1.6	↓4.7	↓4.8	↓4.7	↓6.5
	Recovery	NA	NA	NA	NA	↑7.5	↓2.4
Activated Partial Thromboplastin time							
	Cycle 2	↑**15.7**	↓5.3	↑**27.7**	↓**18.8**	↑**23.3**	↓2.9
	Cycle 4	↑9.1	↓7.0	↑**24.0**	↓**12.3**	↑**13.6**	↓6.4
	Cycle 6	↑4.8	0.0	↑**27.9***	↓**18.1**	↑**17.7**	↓4.3
	Cycle 8	↑6.2	↓2.2	↑**20.6**	↓**11.1**	↑**16.4**	↓0.5
	Recovery	NA	NA	NA	NA	↑**13.1**	↓6.2

Values in **bold** indicates ≥10% variation and/or statistically different compared to control
*P<0.5; **P<0.01. NA= not applicable

Clinical Chemistry

Blood samples for hematology analysis were taken pre-treatment and at the end of the 5-days administration of PXD101 in Cycles 2, 4, 6 and 8 and at the end of the recovery period.

- All dogs in the 10, 25 and 50 mg/kg/day groups showed consistently lower alkaline phosphatase (AP) values ranging from 7% to 46.6%. AP values were significantly lower in the 50 mg/kg/day group compared to controls during cycles 2 through 6. The biological significance of lower AP values across all PXD101-treated dogs is unknown. Liver-to-body weight ratios were lower in the 10, 25 and 50 mg/kg/day groups and ranged from 4% to 14%. However, there were no microscopic findings. Values for inorganic phosphorus were variable with significantly higher values in the 50 mg/kg/day dogs in different cycles. At recovery sacrifice, inorganic phosphorus values in male dogs were lower compared to control.
- All dogs in the 10, 25 and 50 mg/kg/day groups showed consistently high glucose values with highly significant lower values in the 50 mg/kg/day group compared to controls. At recovery sacrifice, glucose values were similar when compared with controls.
- Female dogs in the 10, 25 and 50 mg/kg/day groups showed consistently high urea values compared to controls with highly significant lower values during cycle 8. At recovery sacrifice, urea values were slightly higher in males but were similar in females compared with control dogs.

Table 24 Percent Change in Clinical Chemistry Parameter Values Compared with Control

Parameter	PXD101 Dose (mg/kg/day)					
	10		25		50	
	M	F	M	F	M	F
Alkaline Phosphatase						
Cycle 2	↓**20.2**	↓**11.3**	↓**22.3**	↓**24.5**	↓**38.7****	↓**40.8****
Cycle 4	↓**32.7**	↓**12.0**	↓**25.1**	↓**25.3**	↓**45.7****	↓**41.1***
Cycle 6	↓**26.2**	↓**12.1**	↓**23.3**	↓**25.3**	↓**46.6***	↓**38.7**
Cycle 8	↓**20.9**	↓7.0	↓**22.1**	↓**21.5**	↓**42.6**	↓**32.8**
Recovery	NA	NA	NA	NA	↓3.0	↑8.1
Phosphorous						
Cycle 2	↑4.4	↓4.0	↓4.3	↓4.0	↑**13.0***	0.0
Cycle 4	↑9.5	↓9.5	↑9.5	0.0	↑4.8	↓**14.3**
Cycle 6	↑5.3	↓**11.1**	↓5.3	↑5.6	↑**15.8*****	↑**11.1****
Cycle 8	↑5.9	↓6.3	0.0	↑**12.5***	↑5.9	↑**18.8*****
Recovery	NA	NA	NA	NA	↓**20.0**	↓6.3
Glucose						
Cycle 2	↑**11.7***	↑1.6	↑**11.7****	↑4.8	↑**16.7*****	↑**11.1***
Cycle 4	↑9.3	↑3.7	↑1.9	↑7.4	↑**13.0**	↑**6.2***
Cycle 6	↑**14.6****	↑3.5	↑**16.4****	↑5.3	↑**12.7****	↑**14.0*****
Cycle 8	↑8.9	↑7.3	↑8.9	↑**12.7***	↑**12.5****	↑**9.1***
Recovery	NA	NA	NA	NA	↑7.3	↑4.4

Parameter		PXD101 Dose (mg/kg/day)					
		10		25		50	
		M	F	M	F	M	F
Urea							
	Cycle 2	↑6.3	**↑41.9***	↑6.3	**↑16.1**	**↑15.6**	**↑25.8**
	Cycle 4	0.0	**↑35.3**	↑5.7	**↑32.4**	0.0	**↑35.3**
	Cycle 6	↑2.6	**↑39.5**	↑7.9	**↑42.1**	**↑18.4**	**↑39.5***
	Cycle 8	**↑14.6**	**↑50.0****	↑4.9	**↑47.6****	**↑14.6**	**↑35.7***
	Recovery	NA	NA	NA	NA	**↑15.4**	↑4.1

Values in **bold** indicates ≥10% variation and/or statistically different compared to control
*P<0.5; **P<0.01; ***P<0.001. NA= not applicable

Urinalysis

Unremarkable

Gross Pathology

Macroscopic findings at terminal sacrifice were in general unremarkable. One male in the 10 mg/kg/day presented moderate dark areas in all lobes of the lung and a raised area that corresponded microscopically with hemorrhage, pneumonia and fibrosing alveolitis. Macroscopic findings at recovery sacrifice were unremarkable.

Organ Weights

Organ-to-body weight ratios for the spleen were decreased in all male groups and in the 25 and 50 mg/kg/day female groups at terminal sacrifice when compared to controls. Lower weight ratios for the spleen corresponded with microscopic findings of spleen atrophy in one male at 50 mg/kg/day and females at 25 and 50 mg/kg/day. Ratios for the spleen were also decreased in the 50 mg/kg/day males and females at the recovery sacrifice.

The lower ratios for the prostate and testes/ epididymides in all male groups at the terminal and recovery sacrifice corresponded with minimal testes tubular atrophy and exfoliated cells in the epididymides in some dogs. All dogs were sexually immature at the beginning of the study.

Mean organ:body weight ratios for the liver were decreased in all male and female groups and showed a dose-related decrease in female dogs at terminal sacrifice; ratios were also decreased at recovery sacrifice. Ratios for the heart were higher in the 25 and 50 mg/kg/day males at terminal sacrifice with no microscopic correlates. Ratios for the kidney were decreased in all female groups with no microscopic correlates.

Table 25 Percent Change in Organ to Body Weight Ratios Compared to Control

PXD101 Dose (mg/kg/day)	Males			Females		
	10	25	50	10	25	50
Terminal Sacrifice						
Number of Animals in Group	**3**	**3**	**3**	**3**	**3**	**3**
Kidney	↓3.2	↑0.3	↓0.8	↓7.5	**↓13.2**	↓8.8
Spleen	**↓47.5**	**↓58.4**	**↓49.4**	**↑16.7**	**↓14.1**	**↓27.4**
Liver	↓9.9	↓8.2	↓3.9	↓6.8	**↓11.8**	**↓13.5***
Heart	1.0	**↑12.3**	**↑16.4**	↑1.3	↓8.7	1.0
Prostate	**↓39.9**	**↓25.8**	**↓35.9**	NA	NA	NA
Testes/epidydimides	**↓28.2***	**↓27.5**	**↓41.9****	NA	NA	NA
Recovery						
Number of Animals in Group	**0**	**0**	**2**	**0**	**0**	**2**
Kidney			**↓10.1**			↓8.3
Spleen			**↓10.0**			**↓21.4**
Liver			↓3.6			**↓11.5**
Heart			↑2.1			↓9.1
Prostate			**↑45.1**			NA
Testes/epidydimides			**↓26.9**			NA

Values in **bold** indicates ≥10% variation and/or statistically different compared to control
*P<0.5; **P<0.01

Histopathology

Adequate Battery: YES
Peer Review: NO
Histological Findings Terminal Sacrifice Animals
Lymphoid atrophy was observed in the mesenteric, mandibular, spleen, ileum, and cecum in dogs given 50 mg/kg/day. Focal nephropathy and inflammatory cell foci in the kidney was observed in one male and one female in the 50 mg/kg/day group. All dogs were sexually immature at the beginning of the study and presented different stages of sexual maturation at terminal and recovery sacrifice. One dog each from the control and the 25 mg/kg/day groups presented minimal tubular atrophy in the testes as well as one dog each from the control and the 50 mg/kg/day group at recovery sacrifice. Exfoliated cells (spermatids) in the epididymides were present in males given 10, 25 or 50 mg/kg/day including those graded as pubescent. These findings may suggest a PXD101-related delay of testicular maturation in male dogs.

Histological Findings Recovery Sacrifice Animals
Lymphoid atrophy was observed in the mesenteric, mandibular, and spleen tissues as well as focal nephropathy and inflammatory cell foci in the kidney in dogs given 50 mg/kg/day. Two male dogs graded as pubescent presented exfoliated spermatids in the epididymides. These findings suggest lack of recovery during the 16-days drug-free period after the 8th cycle administration of PDX101.

Table 26 Microscopic Findings in Dogs Treated for 8 Cycles

PXD101 Dose (mg/kg/day)	Males				Females			
	0	10	25	50	0	10	25	50
Terminal Sacrifice								
Number of Animals in Group	3	3	3	3	3	3	3	3
Mesenteric Lymph node -atrophy -lymphangiectasis				2a				3a 1a
Mandibular Lymph node -atrophy				2a			2a	3a
Spleen -lymphoid atrophy				1a			2a	1a
Lungs -congestion/hemorrhage -pneumonitis -fibrosing alveolitis -granuloma		1p 1a 1a	1p	1a		1p 1a		1p
Kidney - focal nephropathy - inflammatory cell foci				1a				1a
Ileum -lymphoid atrophy				2a			1a	1a
Cecum -lymphoid atrophy								1a
Ovary -mature -pubescent -immature					1p 1p 1p	3p	3p	2p 1a
Testes -mature -pubescent -tubular atrophy	3p 1a	1p 2p	1p 2p 1a	3p				
Epididymis -exfoliated cells		1a, 2b	1a, 2b	3b				
Thymus -atrophy				3a			1a	1a
Recovery								
Number of Animals in Group	2	0	0	2	2	0	0	2
Mesenteric Lymph node -atrophy								1a
Mandibular Lymph node -atrophy								1a
Spleen -lymphoid atrophy								1a
Lungs -congestion/hemorrhage				2p	1p			1b
Kidney - focal nephropathy - inflammatory cell foci				1a				1b
Ovary -mature -pubescent -immature					1p 1p			2p
Testes								

-mature -pubescent -tubular atrophy	2p 1a			2p 1a				
Epididymis -exfoliated cells				2b				

a, minimal; b, slight; c, moderate; d, marked; e, severe; p, present

Special Evaluation

None

Toxicokinetics

Systemic exposure to PDX101, as measured by Cmax and AUC (0-24h), increased in an approximately dose proportional manner. Systemic exposure was similar in both males and females. After repeated exposure, AUC (0-24h) values increased approximately 1.5x to 1.7x indicating some accumulation over the 8-cycle treatment. Tmax values were similar in both males and females and occurred at 0.08 hours.

Table 27 PXD101 Toxicokinetic Parameters in Dogs

PXD101 Dose (mg/kg/day)	Sex (n=3)	Cmax (µg/mL)			AUC(0-24h) (µg*h/mL)		
		Day 1	Day 5	Day 151	Day 1	Day 5	Day 151
10	M	2.9	2.9	4.2	1.3	1.7	2.7
	F	3.9	4.5	3.8	1.6	2.3	2.2
25	M	10.5	8.3	10.3	4.3	4.2	6.1
	F	8.5	8.4	11.2	4.4	4.3	5.8
50	M	17.7	18.5	21.0	9.0	9.8	12.5
	F	20.0	20.3	23.4	8.7	9.8	13.8

Dosing Solution Analysis

Dose formulations of PXD101 were solutions and were previously analyzed for homogeneity and stability under the rat study #2525-001. Concentration verification analyses were conducted for dose formulations from Weeks 1, 12 and 21 to coincide with TK analysis. Results showed that concentration of the dose formulations were within 90% to 110% of the nominal values.

The following repeat dose toxicology studies were reviewed by Dr. Lilliam Rosario:

Study title: **PXD101: Cyclic Intravenous (infusion) Dosing Study for 28 days in the Rat**

Key study findings:

- Due to the extent and severity of findings at the injection sites, treatment was terminated during (males) or before (females and satellite animals) the second dose cycle.
- Increased reticulocyte and platelet counts were seen in animals treated at 100 mg/kg/day. Slightly low lymphocyte counts were seen in males receiving 10 or 25 mg/kg/day and in males and females receiving 100 mg/kg/day. Slightly low lymphocyte counts were also seen after Cycle 2 in treated males.
- Administration of PXD101 was associated with local toxicity at the intravenous infusion sites. At the highest dosage, the changes were sufficiently severe to prevent further dosing.

Study no.: 1981/007
Volume #2 and page #454
Conducting laboratory and location: (b) (4)

Date of study initiation: 03 January 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Batch number	Purity (%)	Date of receipt at (b) (4)
PR1(1)-67	96.9	3 August 2001

The control article and the vehicle for the test article was 10% w/v ethanol, 15% v/v PEG200 in 0.1 M TRIS buffer (pH 8.5)

Methods
Intravenous administration to rats for 2 cycles of treatment; each cycle included 7 days treatment followed by 14 days of recovery before the next treatment cycle.

Doses:

			Main study		Treatment-free		Satellites#	
Group Number	Description	Dose (mg/kg/day)	M	F	M	F	M	F
1	Control	0	10	10	5	5	-	-
2	Low	10	10	10	-	-	24	24
3	Intermediate	25	10	10	-	-	24	24
4	High	100[A]	10	10	5	5	24	24

[A] Males terminated treatment after four doses in Cycle 2 (Day 26); females and satellite animals terminated treatment before Cycle 2 (Day 22).

Species/strain: rats of the Crl: WI(G1x/ BRL/Han) BR strain ((b) (4)

Number/sex/group or time point (main study): See table above
Route, formulation, volume, and infusion rate: IV; 20 mL/kg administered by slow intravenous infusion at a rate of 0.25 mL/minute
Satellite groups used for toxicokinetics or recovery: See table above
Age: 8-9 weeks old at start of dosing
Weight: males: 243.0 and 310.8 g and females: 146.9 and 213.1 g

Observation and Times:
Clinical signs: Daily.
Body weights: Day-l, twice weekly thereafter and before necropsy.
Food consumption: weekly.
Ophthalmoscopy: not conducted
EKG: not conducted
Hematology: Days 8 and 29
Clinical chemistry: Day 29
Urinalysis: Day 29
Gross pathology: Day 29 or Day 43
Organ weights: Day 29 (end of treatment) or Day 43 (recovery)
Histopathology: Day 29 (end of treatment) or Day 43 (recovery)
Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)
Toxicokinetics: On Days 1 and 7 (Cycle 1) and Days 22 and 28 (Cycle 2), blood samples were collected from three satellite animals/group/sex at 15, 30 and 45 minutes and 1, 2 and 6 hours after dosing

Results:

Mortality: One low dose female was sacrificed during the study due to the extent of treatment- related tail/injection site lesions. On Day 23, Animal number 70 (Group 2F, main study) was sent to necropsy due to tail damage. Due to the poor condition of their tails Group 4 satellite animals were euthanized on Day 14.

Clinical signs:
Marked changes were seen at the dose sites in animals receiving 100 mg/kg/day, particularly among females. One female receiving 10 mg/kg/day was similarly affected and required sacrifice on humane grounds.

After completion of the first dose cycle, three females and one male receiving the highest dose showed signs of sores/lesions, blackened tissue and damaged missing tails. By the start of the second dose cycle (i.e., 14 days after the last dose in Cycle 1), a total of two males and five females were showing similar signs. In addition to these observations, several animals could not be dosed or dosing could not be completed due to difficulties in locating the caudal veins due to the extensive damage to the tails. Females were more affected than males as the changes occurred earlier in females than males.

Due to the severity of the changes in the tails, treatment at 100 mg/kg/day was terminated for main study males after four days of Cycle 2 (i.e. after Day 26) and it was concluded that no further treatment would be given to main study females and satellite males and females.

Following the cessation of treatment, the tail lesions persisted throughout the treatment-free period in the affected animals, but were reduced in severity.

Body weights:
Weight gains during the first dose cycle were lower in males and females receiving 100 mg/kg/day than those of the controls; the inter-group differences were statistically significant ($p < 0.01$). Slightly low weight gains were seen during Cycle 1 in animals receiving 10 or 25 mg/kg/day, although these were not dose-related and did not achieve statistical significance.

Food consumption: Unremarkable

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology:

	Dose (mg/kg/d)	HB (g/L)	Ret (%)	Ret abs (mL/cm)	PLT (10^3)/cm	WBC (10^3)/cm	Lymph (10^3)/cm
Day 8							
M	0	15.4	3.3	0.26	1084	7.2	5.7
	10	1	-12	-8	-7	-13	-12
	25	1	-9	-8	0	-11	-9
	100	-2	3	4	-8	-10	-14
F	0	14.8	3.8	0.3	953	5	3.7
	10	3	0	3	3	-8	-5
	25	3	-3	0	10	-6	3
	100	-5**	29*	23*	22**	-8	-8
Day 29							
M	0	15.6	2.6	0.23	1056	5.3	3.8
	10	-1	12	9	-6	-45**	-39**
	25	-1	-8	-9	-5	43*	34**
	100	-4*	88**	74**	25***	-23	-16
F	0	15.1	3.1	0.25	932	3	2.3
	10	2	-6	-4	3	-33	-35
	25	-1	-3	-4	15	-37	-30
	100	4	-16	-12	10	3	0

p<0.05; ** p<0.01, ***p<0.001

- After the first cycle (females) or second cycle (males), significantly increased reticulocyte and platelet counts were seen in animals treated at 100 mg/kg/day.

- After the first dose cycle, low lymphocyte counts were seen in males receiving 10 or 25 mg/kg/day and in males and females receiving 100 mg/kg/day. Slightly low lymphocyte counts were also seen in treated male groups after the second dose cycle.
- Hemoglobin values were decreased in both males and females during the first cycle.

Clinical chemistry: Unremarkable

Urinalysis: Unremarkable

Gross pathology:
End of treatment:
- At the tail/injection site, sores and areas of discoloration were recorded for both control and high dose animals, generally correlating with treatment-related microscopic findings.
- The overall level of these changes was comparable for both control and treated animals.
- In addition, missing areas of the tail were also recorded for some high dose animals.

14-day recovery period:
- At the injection site, the level of macroscopic findings was reduced when compared with the end of treatment animals.

Organ weights: Unremarkable

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)
End of treatment:
- Treatment- related microscopic findings at the tail/ injection site of both control and high dose animals, see table below.
- The main microscopic changes were phlebitis/periphlebitis and thrombus/intimal proliferation of blood vessels. Phlebitis/periphlebitis was characterized by varying levels of inflammatory cell infiltration, edema and fibrin deposition involving the blood vessel wall and surrounding connective tissue. Thrombus/intimal proliferation was recorded for the majority of animals, with complete occlusion of vessels in many high dose animals. Dermatitis, hemorrhage and myositis were also recorded for some animals from both the control and high dose groups.
- The incidence and severity of the injection site changes in the control group were greater than the normally expected physiological response to the mechanical damage of repeated venipuncture, suggestive of irritation due to the vehicle. The incidence and severity of injection site reactions was greater in the high dose animals, when compared with the controls, suggestive of additional irritation due to PXD101.

	Males				Females			
Tail/injection site	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Level (mg/kg/day)	**0**	**10**	**25**	**100**	**0**	**10**	**25**	**100**
No. examined:	10	0	0	10	10	0	0	10
Phlebitis/periphlebitis	1			0	2			0
Grade-1	8			3	7			6
2	0			5	1			0
3	0			1	0			1
4	1			0	0			3
5	0			1	0			0
Thrombus/intimal proliferation	3	0	0	0	6	0	0	0
Grade -1	5			1	2			1
2	1			2	2			2
3	1			6	0			6
4	0			1	0			1

14-day recovery period

- The level of injection site changes was markedly reduced when compared with end of treatment, suggestive of partial reversal of the- charges recorded at the terminal kill.

	Males		Females	
	1M	**4M**	**1M**	**4M**
Level (mg/kg/d)	**0**	**100**	**0**	**100**
No. examined:	**5**	**5**	**5**	**5**
phlebitis/periphlebitis	5	1	5	4
Grade-1	0	3	0	1
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	1	0	0
thrombus/intimal proliferation	2	0	4	0
Grade -1	2	1	1	1
2	1	2	0	4
3	0	2	0	0

Key :"-" = finding not present, 1= minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5 = severe

Toxicokinetics:

			C_{max} (ng/mL)					T_{max}			
Sex (n=3)	Dose (mg/kg)		Cycle 1			Cycle2		Cycle 1		Cycle 2	
		Ratio	Day 1	Ratio	Day7	Day 22	Day 28	Day 1	Day 7	Day22	Day 28
M	10	1	217.46	1	347.16	286.98	275.24	0.5	0.25	0.25	0.25
	25	2.5	913.17	4	896.34	973.25	1265.22	0.25	0.25	0.25	0.25
	100	10	4917.24	23	3828.71	-	-	0.25	0.25		
F	10	1	291.78	1	355.92	247.73	317.10	0.25	0.25	0.25	0.25
	25	2.5	734.12	3	954.01	794.14	981.76	0.25	0.25	0.25	0.25
	100	10	4246.41	15	4630.41	-	-	0.25	0.25		
			$AUC_{(0-t)}$(ng*h/mL)					$AUC_{(0-inf)}$(ng*h/mL)			
Sex (n=3)	Dose (mg/kg)		Cycle 1			Cycle2		Cycle 1		Cycle 2	
		Ratio	Day 1	Ratio	Day7	Day 22	Day 28	Day 1	Day 7	Day22	Day 28
M	10	1	263.44	1	253.69	298.06	227.34	277.30	314.72	325.28	231.88
	25	2.5	967.21	4	826.69	885.38	969.90	982.84	#	1001.74	1022.71
	100	10	4547.84	17	4074.38	-	-	4964.86	4098.12	-	
F	10	1	237.38	1	224.13	156.90	220.89	#	270.32	187.92	229.19
	25	2.5	565.51	2	780.22	530.78	674.18	576.30	#	540.54	678.97
	100	10	2957.58	12	3201.17	-	-	2999.70	3252.68		

- C_{max} values of PXD101 increased with increasing dose between 10, 25 and 100 mg/kg/day, however the increase was greater than proportional.
- $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values for PXD101 appeared to increase between 10, 25 and 100 mg/kg/day. This increase was greater than proportional.
- The $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values were generally twice higher in males than in females. This may indicate a sex-related difference in absorption and/or clearance of the test article.
- For both sexes and for each dose level, the C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values determined for the first day of each cycle (i.e. Day 1 for Cycle 1 and Day 22 for Cycle 2) were similar to those determined following 7 days repeat dosing (i.e. Day 7 for Cycle 1 and Day 28 for Cycle 2). The values for Cycle 1 were also similar to the corresponding values for Cycle 2.

Study title: **PXD101: Cyclic Intravenous Dosing Study for 28 Days in the Dog**

Key study findings:

- The principal effects of treatment were seen in the lymphoid tissues (spleen, thymus and lymph nodes, bone marrow and peripheral blood). These comprised atrophy of the lymphoid tissues, decreased cellularity in the bone marrow and reduced circulating lymphocyte and, possibly, neutrophil counts.
- Phlebitis and periphlebitis was seen at the dose sites in most animals including controls.
- The Maximum- Tolerated- Dose was approximately 50 mg/kg/day and the No-Observed-Effect-Level was 10 mg/kg/day

Study no.: 1981/008
Volume #4 **and page #**1054
Conducting laboratory and location: (b) (4)

Date of study initiation: 01 November 2001
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Batch number	Purity (%)	Date of receipt at (b) (4)
PR1(1)- 67	96.9	3 August 2001

The control article and the vehicle for the test article were 10% v/ v ethanol; 5% v/v PEG 200 in 0. 1M TRIS buffer (pH 8.5).

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Methods

Doses: Daily for seven days in each cycle with a 14 day recovery period between each cycle.

Group Number	Group Description	Dose (mg/kg/day)	End of treatment		Recovery	
			Males	Females	Males	Females
1	Control	0	3	3	2	2
2	Low	10	3	3		
3	Intermediate	25	3	3		
4	High	50	3	3	2	2

Species/strain: Beagle dogs (b) (4)
Number/sex/group or time point (main study): See table above
Route, formulation, volume, and infusion rate: IV, slow infusion using syring pump; dose volume of 10 mL/kg at a rate of 2.5 mL/ minute
Satellite groups used for toxicokinetics or recovery:
Age: 3 to 5 months old on arrival.
Weight: 5.05 to 8.20 kg

Observation and Times:

Clinical signs: Daily
Body weights: once weekly during the acclimatization, twice weekly thereafter and before necropsy
Food consumption: daily
Ophthalmoscopy: pre- treatment and before the last dose in the second dosing cycle.
EKG: pre- treatment and before the last dose in the second dosing cycle. Readings were taken in the morning pre- treatment or at an 2 hours after dosing. Recordings were taken using the fixed limb leads I, II and III and the augmented leads aVR, aVL and aVF.
Hematology: Pre-treatment, Day 8, and Day 28
Clinical chemistry: Pre-treatment and Day 29
Urinalysis: Pre-treatment and Day 29
Gross pathology: Day 28/29 or Day 43
Organ weights: Adrenals, kidneys, spleen, liver, heart, brain, pituitary, thyroid/parathyroid, prostate, testes/epididymides.
Histopathology
Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)
Toxicokinetics: Before dosing and approximately 10 and 30 minutes after dosing and then approximately 1, 2, 4 and 6 hours after dosing on Days 1 and 7 of the 1st and 2nd dose cycles.
Other: Histone deacetylase activity: Blood samples were collected before dosing and 10 and 30 minutes after dosing and then 1, 2, 4 and 6 hours after dosing on Days 1 and 7 of the 1st dose cycle.

Results:

Mortality: No mortality

Clinical signs:

- In males and females treated at 50 mg/kg/day, clinical signs included tremors, vomiting and retching, salivation, lip licking, liquid feces, head shaking and hypoactivity. Signs continued up to 30 minutes after dosing, but were mostly transient and were only present during or shortly after dosing. Tremors were seen in all animals during the first dose cycle, but not during the second cycle. Other signs were present during both cycles, but were not observed in all animals.
- In animals treated at 25 mg/kg/day, signs included tremors, vomiting and sweating. In general, signs were seen in isolated animals and on infrequent occasions, although tremors were present in all animals during the first dose cycle only.
- Unsteadiness on the feet was seen in controls and animals receiving 10 mg/kg/day and the sponsor considered this finding to be related to treatment with the vehicle. These signs were not present on every day of dosing, but were seen in all animals. This sign was also seen in all animals treated at 25 or 50 mg/kg/day in conjunction with other signs observed.

Body weights: Body weight gains decrease with increasing dosage.

Food consumption: Unremarkable

Ophthalmoscopy: Unremarkable

EKG: Unremarkable

Hematology: Values represent % changed from control group (1)

- After both cycles of treatment, reduced lymphocyte counts were seen in males and females receiving 25 or 50 mg/kg/day. There was a concomitant decrease in total leucocyte counts. The reductions in lymphocyte counts were modest (approximately 50% or less), were dose- related and were more marked in Cycle 1 than in Cycle 2.
- There were modest reductions in neutrophil counts at the HD.
- No effects were seen in respect of changes in erythrocyte, reticulocyte or platelet counts in treated animals.

	Dose (mg/kg/day)	Day 8			Day 29		
		Total WBC 10^3/ml	Neut 10^3/ml	Lymph 10^3/ml	Total WBC 10^3/ml	Neut 10^3/ml	Lymph 10^3/ml
M	0	12.8	6.6	5.1	13.5	7.6	4.8
	10	29	36	20	1	-3	8
	25	13	36	-18	-18	-11	-35
	50	3	29	-33	-21	-13	-33
F	0	12.5	6.4	4.8	11.2	6.2	4.1
	10	16	27	10	30	52	2
	25	10	42	-29	-12	-6	-17
	50	-30	-13	-50	-26	-23	-32

Clinical chemistry: Unremarkable

Urinalysis: Unremarkable

Gross pathology:

- *End of treatment (Day 28):* Red injection sites were seen in several animals, with higher frequency in the high dose group.
- *Recovery (Day 43):* Findings in injection site appear to reverse by the end of 2-weeks.

		Males				Females			
Observations	**Dose (mg/kg/day)**	**0**	**10**	**25**	**50**	**0**	**10**	**25**	**50**
End of treatment	No. examined:	3	3	3	3	3	3	3	3
	Left cephalic- Red	2	0	1	3	0	0	3	3
	Right cephalic- Red	2	0	2	3	1	0	2	3
Recovery	No. examined:	2			2	2			2
	Left cephalic	0			0	0			0
	Right cephalic	0			0	0			0

Organ weights: Unremarkable

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

		Males				Females			
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Tissue and finding	**Dose (mg/kg/day)**	**0**	**10**	**25**	**50**	**0**	**10**	**25**	**50**
Left cephalic	No. examined:	3	3	3	3	3	3	3	3
Phlebitis/ periphlebitis	Grade-	0	0	0	0	0	0	0	0
	1	1	1	1	0	0	0	0	1
	2	1	2	2	1	3	2	1	2
	3	1	0	0	2	0	1	1	0
	4	0	0	0	0	0	0	1	0
Right cephalic	No. examined:	3	3	3	3	3	3	3	3
Phlebitis/ periphlebitis	Grade-	0	0	0	0	0	0	0	0
	1	0	0	0	0	1	0	1	1
	2	2	1	3	0	0	1	0	1
	3	1	2	0	3	2	1	2	1
	4	0	0	0	0	0	1	0	0

End of treatment:

- The reddening seen macroscopically at the injection sites was mainly attributable to perivenous hemorrhage. There was also phlebitis/periphlebitis in most sites characterized by varying degrees of edema, fibrin deposition and acute to chronic inflammatory reactions. Organizing thrombi were occasionally seen in the lumen adjacent to disruptions of the vessel wall, probably due to needle tracks, but there were no massive venocclusive reactions in the sections examined. The extent of phlebitis/periphlebitis varied between different animals of the same group and between different injection sites in the same animal. There was no clear- cut dose-related trend in the extent of inflammatory reactions seen in treated animals compared with the range of responses seen at control injection sites.

		Males				Females			
Tissue and finding	**Dose (mg/kg/day)**	**0**	**10**	**25**	**50**	**0**	**10**	**25**	**50**
Thymus	No. examined:	3	3	3	3	3	3	3	3
atrophy	Incidence:	0	0	2	3	0	0	1	1
Mesenteric LN	No.examined	3	3	3	3	3	2	3	3
atrophy	Incidence:	0	0	1	3	0	0	0	1
Mandibular LN	No. examined:	3	3	3	3	3	3	3	3
atrophy	Incidence:	0	0	1	3	0	0	1	3
Spleen	No. examined:	3	3	3	3	3	3	3	3
Lymphoid atrophy	Incidence:	0	0	0	1	0	0	0	2
Sternum and marrow	No. examined:	3	3	3	3	3	3	3	3
Atrophy	Incidence:	0	0	0	2	0	0	0	0

End of treatment

- Systemically, the main treatment-related effect was in atrophy in lymphoid organs notably in males.
- In the thymus, there was loss of cortical lymphocytes and a blurring of the normal distinct demarcation between the cortex and medulla.
- Germinal centers tended to be indistinctive or reduced in number or size in the mandibular and mesenteric nodes and also in the lymphoid aggregates in the spleen and ileum.
- There was also a decrease in cellularity of sternal marrow in two high dose males.

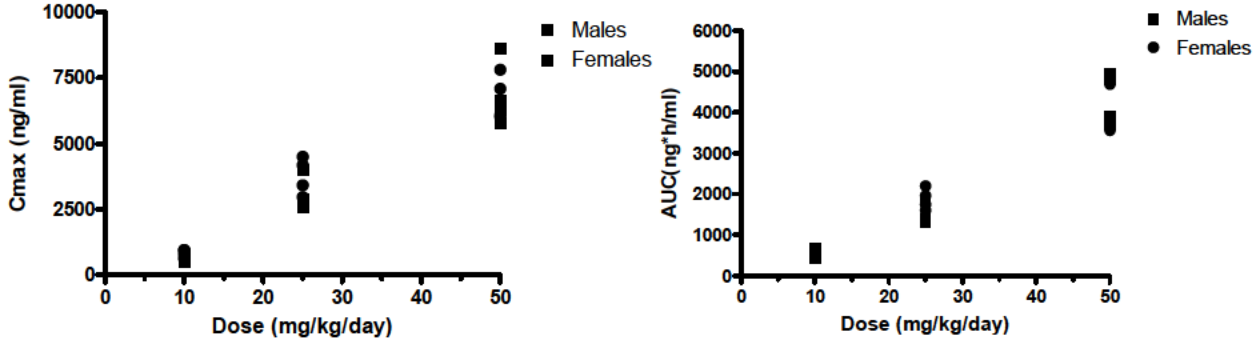
Recovery

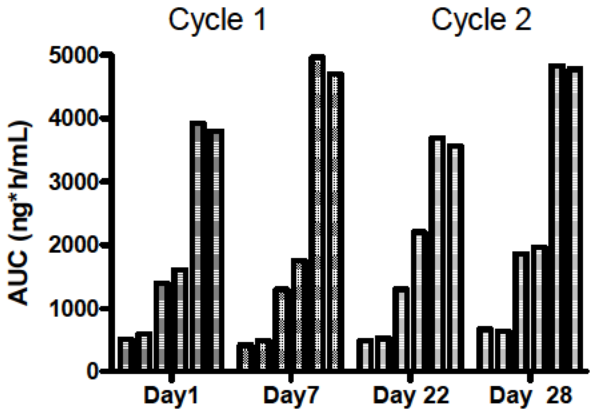
- Microscopic findings appeared to recover by the end of the 2-week period.

Toxicokinetics: (End of treatment)

- C_{max} values increased with increasing dose between 10, 25 and 50 mg/kg/day. However the increase was greater than proportional, particularly between the 10 and 25 mg/kg/day doses.
- No significant differences in the kinetics of PXD101 between male and female dogs were observed.
- AUC(0-t) and AUC(0-inf) values for PXD 101 increased with increasing dose, from 10 to 25 to 50 mg/kg/day. This increase was slightly greater than dose-proportional.
- At the 10 and 25 mg/kg/ day dose levels, there was no apparent pattern of difference between the C_{max}, AUC(0-t) and AUC() values determined for the first day of each cycle (i. e. Day 1 for Cycle 1 and Day 22 for Cycle 2) and those determined following 7 days repeat dosing (i. e. Day 7 for Cycle 1 and Day 28 for Cycle 2). However, at the 50 mg/kg/day dose level, the values measured after 7 days were consistently higher than those determined for the first day of dosing, indicating accumulation of PXD101 at this dose level.
- T_{max} was approximately 10 min.

	C_{max} (ng/ml)				AUC $_{(0-t)}$ (ng*h/mL)				AUC $_{(0-\infty)}$ (ng*h/mL)			
	Cycle 1		Cycle 2		Cycle 1		Cycle 2		Cycle 1		Cycle 2	
	Day1	Day7	Day 22	Day 28	Day 1	Day 7	Day 22	Day 28	Day 1	Day 7	Day 22	Day 28
♂												
10	719.25	481.92	764.5	802.54	507.87	408.23	489.25	655.63	512.15	417.52	493.2	667.01
25	2567.08	3966.9	2603.06	2880.86	1386.64	1279.73	1295.92	1831.16	1390.66	1298.47	1300.61	1855.2
50	5788.19	8600.36	6299.61	6640.74	3903.76	4937.67	3677.94	4791.84	3918.53	4957.77	3684.06	4824.55
♀												
10	949.4	617.53	779.31	925.17	592.3	475.05	522.27	630.34	595.68	485.16	526.15	636.71
25	2962.25	4495.9	4171.39	3412.48	1600.67	1731.19	2197.36	1942.29	1604.57	1749.98	2200.51	1963.5
50	6005.25	7812.38	6077.82	7085.81	3753.65	4672.46	3558.13	4749.21	3788.6	4691.7	3562.86	4774.22





Study title: **PXD101: Intravenous Dose range finding Study for 14 Days in the Rat**

Study no.: 1981/024
Volume #3 and page # 888
Conducting laboratory and location: (b) (4)

Date of study initiation: 17 December 2002
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

	Batch #	Purity	Date of receipt at (b) (4)
Test Article: PXD101	PR1 (1)-67	96.9	3 August 2001
Control Article: L-Arginine	062KOI93	98	3 January 2003

Key study findings:

- The minimum lethal dose was 200 mg/kg following a single administration.
- The MTD is between 100 and 200 mg/kg.
- A dose of 100 mg/kg/day for seven days was well-tolerated with minor changes in body weight and decreased white cell counts. There was no clear evidence of local irritation at the dose sites.

Methods

Doses: Daily x 7 followed by recovery period (4-weeks)

Group number	Group description	Dose level (mg/kg/day)	Animals/group Main study	Recovery
1	Control	0	3	6
2	Low	100	3	6

One week after the completion of treatment at 100 mg/kg/day, additional male rats were assigned to the following dose groups:

Group number	Group description	Dose level (mg/kg/day)	Animals/group Main study
1B	Control	0	6
3	High	250	9

Following treatment at 250 mg/kg/day, four animals died within a few minutes of dosing. Remaining animals in this group showed partially closed eyelids, rapid respiration and were prostrate. These animals were dosed for one day only and consequently, all surviving animals in Group 3 were sent to necropsy immediately after the dose. Control Group 1B animals were retained off-dose for a period of one week then dosed with an additional group of male rats, as follows:

Group number	Group description	Dose level (mg/kg/day)	Animals/group Main study
1B	Control	0	6
4	Intermediate	200	9

These animals were dosed for one day only due to adverse reactions. All surviving animals were then maintained treatment-free for two weeks.

Note:
In study CLE Report 1981/ 007-D6154 (rats) using PXD101 in a vehicle composed of 10% ethanol, 5% PEG200 in a TRIS buffer (pH 7.2), signs of local irritation were seen in animals treated at 100 mg/kg. The maximum concentration of PXD101 in the vehicle was 5 mg/mL, limiting the maximum practicable dose that could be achieved. Therefore, for PXD101 in the ethanol/PEG200/TRIS vehicle, the MTD in the rat was considered to be between 50 and 100 mg/kg/day when given in seven day treatment cycles. For this study, the initial dose of 100 mg/kg/day was selected to confirm if the local irritation seen in the previous study were solely related to the vehicle and to confirm the MTD.

Species/strain: Male Cd:WI (GLx/ BRL/ Han) BR rats
Number/sex/group or time point (main study): See above
Route, formulation, volume, and infusion rate: IV, L-arginine in water for injection, 10 mL/kg administered at a rate of 0.5 mL/minute (to achieve an infusion time of approximately five minutes)
Satellite groups used for toxicokinetics or recovery: None
Age/Weight: At their respective initiations of treatment Group 1 and 2 animals were 8 to 9 weeks old and weighed from 232.2 to 298.8 g, Group IB and 3 animals were 10 to 11 weeks old and weighed between 240.4 and 316.2 g, and Group 4 animals were 11 to 12 weeks old andweighedfrom292.4to 345.9g.

Observation and Times:
Clinical signs: Twice daily (am/ pm)
Body weights: Before treatment on Day 1. Thereafter, twice weekly and before necropsy.
Food consumption: Weekly, calculated as g/animal/week. Food consumption was recorded for Groups 1B and 4, but not reported.
Ophthalmoscopy: Not conducted
EKG: n/a
Hematology: Samples obtained from three animals/group/occasion (where possible) from Groups 1 and 2 on the following occasions:
Main study animals: Days 4 and 8; Recovery animals: Days 12, 16, 20, 24, 28, 32
Bone marrow smears: necropsy
Metabolite profiling: Urine samples collected for eight hours from all Group 2 animals following 7th dose. Samples were not analyzed.
Clinical chemistry: Not conducted
Urinalysis: Not conducted
Gross pathology: On all animals, including decedents. Groups 1 and 2 Main study killed on Day 8. Recovery animals killed on Day 35. Group 3 animals died or were killed in

extremis following dosing on Day 1 only (correlates with Study Day 14). Groups 1B and 4 were killed two weeks after being dosed on Day 1 (correlates with Study Day 21).
Organ weights: Adrenals, kidneys, spleen, liver, heart, brain, pituitary, prostate, and testes/epididymides
Histopathology: Not conducted

Results:
Mortality:

Group #	Dose (mg/kg/d)	(n=)	Mortality	Observations	Clinical Signs
3	250	9	N=4 (Nos. 24, 25, 26 and 27)	died immediately after dosing on Day 1	prostration, uncoordinated movement, rapid breathing and/ or partially closed eyelids were seen shortly after dosing (all animals)
4	200	9	N=1 (No. 33)	found dead immediately after dosing on Day 1	prostration, uncoordinated movement, rapid breathing and/ or partially closed eyelids were seen shortly after dosing (6/9)

Clinical signs:
- In a number of animals, including controls, sores and/ or abrasions on the tails and reddened or bruised tails were observed.
- In two control animals (Nos. 5 and 7), there was some evidence to suggest that some fraction of the dose had been given subcutaneously on one or more occasions. This resulted in more severe tail lesions; animal no 5 was not dosed on two days during the treatment phase of the study. Animal numbers 5 and 7 were terminated prematurely.
- In addition, controls were treated on Day- I with the vehicle (20 mg/mL arginine) where the pH had not been adjusted to pH 9 as the instructions for dose preparation received from the Sponsor did not specify this. Following dosing with a pH 11 formulation, the tails distal to the injection sites were significantly discolored (dark red or black). The following day, the discoloration was not apparent and administration of the control and test formulations at pH 9 commenced.

Body weights:
- During the treatment phase, lower weight gains were observed at 100 mg/kg/day.
- Following end of treatment (i.e. from Day 8), weight gains in control and treated animals were similar.

Food consumption:
- Food intake in treated animals was lower throughout the treatment and recovery periods compared to controls.

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology:
- Low total leukocyte counts were seen on Days 4 (↓~20%) and 8 (↓~36%), i.e. after three and seven doses, in animals treated with 100 mg/kg/d, as compared to controls.
- The reductions in total leukocyte counts were associated with reductions in all sub-populations, but particularly neutrophil counts (↓~30-57%%).
- Leukocyte values were still decreased on Day 12 (↓~30%), Day 20 (↓~34%), Day 24 (↓~16%), Day 28 (↓~29%), and Day 32 (↓~24%).

Clinical chemistry: Not conducted

Urinalysis: Not conducted

Gross pathology: Minor changes in the injection sites were seen in a number of control males; this was probably associated with changes seen in the tails during the dosing period, particularly following the pH 11 L-arginine administration on Day – 1 (see clinical signs). There was no evidence of any similar effect in treated animals.

Dose (mg/kg/d)	**0**	**100**	**250**	**200**
N=	**9**	**9**	**9**	**9**
Liver:				
Large	0	0	2	0
Dark	0	0	1	0
Mottled	0	1	0	0
Stomach:				
Distention	0	0	2	0
Pale	0	1	0	0
Cecum: Injection lesion	1	0	1	1
Kidney: Pelvic dilatation	2	1	0	0
Mandibular LN: red focus	2	1	1	0
Thymus:				
Red	0	0	2	1
Dark	0	0	1	0
Lung:				
Dark	0	0	7	1
Pale Area	0	0	1	0
Inflated	0	0	2	0
Red Focus	0	1	0	0
Tail/Injection Site:				

Dose (mg/kg/d)	0	100	250	200
N=	9	9	9	9
Sore	4	1	0	0
Red	2	0	0	0
Black	1	0	0	0
Raised Area	0	0	1	0

Organ weights: Unremarkable

Histopathology: Not conducted

Toxicokinetics: Not conducted

Study title: **PXD101: Intravenous Dose range finding Study in the Dog**

Key study findings:

- PXD 101 (50 mg/kg/day) for seven days was well tolerated with reduced lymphocytes and lymphoid atrophy.
- There was evidence of local irritation in the dose sites.
- A number of possible metabolites of PXD101 were identified, with some differences in metabolite profiles between urine and fecal routes of excretion.

Study no.: 1981/025
Volume #5 **and page #** 1486
Conducting laboratory and location: (b) (4)
Date of study initiation: 21November 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

	Batch Number	Purity (%)	Date of receipt at (b) (4)
Test Article: PXD101	PR1(1)-67	96.9	3 August 2001
Control Article:	062K0l93	98	3 January 2003

Methods

Doses: Daily x 7 followed by 14-day recovery.
Seven female dogs were used:

Group number	Group description	Dose level (mg/kg/day)	Animal ID #	
			Main study	Recovery
1	Treated	50	1	2
2	TK	50	3-6	

Note:

- Based on the observation following treatment at 50 mg/kg, the sponsor decided that further toxicity investigations were not required and the data obtained thus far in the study can be correlated with that seen in a previous 2 cycle study in the dog (CLE Study 1981/008).
- Following the change in the formulation between previous studies and this study, the study was extended to include toxicokinetic assays and metabolic profiling at a dose level that produced evidence of expected pharmacology (e. g. leukopenia), but was otherwise well tolerated.

Species/strain: Female beagle dogs ((b) (4)
Number/sex/group or time point (main study): See table above
Route, formulation, volume, and infusion rate: IV; 5 mL/kg over a period of 15 minutes.

Satellite groups used for toxicokinetics or recovery: 3 ♀ (TK) and 2 ♀ (recovery)
Age: 20 to 27 weeks old.
Weight: 8.01 to 10.47 kg

Observation and Times:
Clinical signs: Daily

Body weights: Before treatment, twice weekly thereafter and before necropsy.

Food consumption: Weekly

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology: Pre-treatment and on Days 4, 8, 11, 14, 17, 20, 23 and 26.

Clinical chemistry: Not conducted

Metabolic Profiling: Days 1and 7 (Group 2)

Urinalysis: Not conducted

Gross pathology: Group 1: Day 8 or Day 27 following recovery period; (Group 2 toxicokinetic animals killed following final bleed on day 7).

Organ weights: Adrenals, kidneys, spleen, liver, heart, brain, pituitary, thyroids/parathyroids, ovaries, uterus.

Histopathology: Injection sites, duodenum, jejunum, ileum, cecum, colon, thymus and gross lesions from all animals in Group 1 (toxicity phase) were examined microscopically

Toxicokinetics: Day 1 and Day 7 from Group 2 animals.

Results:

Mortality: There were no deaths.

Clinical signs: Vomiting, lip licking and/or retching was seen during the dose infusion in all dogs. These signs were transient and did not persist past the completion of infusion. Swelling in the limbs used for dose infusion was seen in Animals 1 and 2.

Body weights: Unremarkable

Food consumption: Unremarkable

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology: Reduced lymphocyte counts were seen on Days 4 and 8 of treatment (i.e. after three or seven doses at 50 mg/kg). This change was associated with reduced total leukocyte counts in both animals.

Treatment	Pre-treatment*		Day 4**		Day 8**	
	Female 1	Female 2	Female 1	Female 2	Female 1	Female 2
Total WBC (10^3/ml)	10.6	15.5	-21	23	-14	71
Neutrophils (10^3/ml)	6.1	9.9	-53	-23	-20	43
Lymphocytes (10^3/ml)	3.4	4.2	-73	-65	-95	-79

*Raw values; ** % change from pretreatment.

Recovery**	Day 11	Day 14	Day 20	Day 23	Day 26
Total WBC (10^3/ml)	-13	17	-12	-19	-30
Neutrophils (10^3/ml)	-39	33	-11	-24	-30
Lymphocytes (10^3/ml)	5	-18	-18	-13	-34

Clinical chemistry: Not conducted

Urinalysis: Not conducted

Gross pathology:
Main study
- Gelatinous connective tissue was seen at the left shoulder.
- Red and/ or gelatinous injection sites; discolorations of the intestinal tract; and small thymus (correlated with microscopic findings).

Treatment- free: Unremarkable

Organ weights: Unremarkable

Histopathology: Adequate Battery: Yes
Main study
- Injection sites: hemorrhage and edema, with minor phlebitis/periphlebitis and fascitis (correlated with macroscopic findings).
- Thymic atrophy was characterized by loss of cortical lymphocytes and blurring of the normal demarcation between cortex and medulla.
- Mucosal atrophy in the duodenum, jejunum, cecum and colon was characterized by reduction in size of the villi (in the small intestine), reduction in size of the glands, loss of mucosal cells, and infiltration with inflammatory cells, especially plasma cells.
- Lymphoid atrophy in the ileum and cecum was characterized by loss of lymphoid cells from, and hyalinization of, the submucosal lymphoid aggregates.

Treatment- free

- Hemorrhage and edema were not present suggesting reversal of the injection site changes seen at the terminal kill.
- Mucosal atrophy and lymphoid atrophy were not present in the intestines, and the thymus was unremarkable suggesting reversal by the end of the recovery period.

Toxicokinetics:

- C_{max} and $AUC_{(0-t)}$ values were comparable for all animals on Days 1 and 7.
- The maximum plasma concentrations for all animals occurred at the 10 minute (T_{max}) timepoint on both Day 1 and Day 7.
- Clearance values appear similar on both Day 1 and Day 7.

Table 28 Toxicokinetic parameters in Dog 7 Day Study

Animal Number	C_{max} (ng/mL)		T_{max} (h)		$AUC_{(0-t)}$ (ng.h/mL)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
3	16728.54	11703.07	0.167	0.167	10965.04	9603.13
4	11876.26	9853.12	0.167	0.167	8534.69	8054.90
5	13615.91	14832.69	0.167	0.167	10113.27	13534.84
6	11459.62	11071.16	0.167	0.167	6642.21	7625.34

Animal Number	$AUC_{(0-inf)}$ (ng.h/mL)		Clearance CL/F (mL/h/kg)	
	Day 1	Day 7	Day 1	Day 7
3	11182.94	10703.61	4471.10	4671.32
4	8723.69	8825.52	5731.52	5665.39
5	10183.48	14216.04	4909.91	3517.15
6	6689.53	8791.55	7474.37	5687.28

7 Genetic Toxicology

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

Study title: PXD101: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium

Study no.:	1981-018
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 4, 2001
GLP compliance:	OECD
QA statement:	Yes
Drug, lot #, and % purity:	PXD101; Lot # PR1(1)-67; Purity 96.9%

Key Study Findings

- Using a plate incorporation method, PXD101 induced genotoxic responses in bacteria, with or without S9 metabolic activation. This study used the highest concentration level recommended by ICH S2(R1) (5.0 mg/plate). Results are considered valid and adequate.

Methods

Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102)

Concentrations (mcg) in definitive study:

Experiment 1	Experiment 2	Experiment 3
TA98, TA100, TA1535, TA1537, TA102	TA98, TA100, TA1535, TA1537, TA102	TA98, TA100, TA1535, TA1537, TA102
With and Without S9	With and Without S9	With and Without S9
1.6	78.125*	39.0625
8	156.25	78.125
40	312.5	156.25
200	625	312.5
1000	1250	625
5000	2500	1250
--	5000**	--

* TA102 +S9 only. ** All strains except TA102

Basis of concentration selection: A dose range-finding study was conducted on PXD101 using strain TA100 with six doses ranging from 1.6 to 5000 mcg/plate and included negative and positive controls. Toxicity was observed at the 5000 mcg/plate

Negative control: Sterile anhydrous analytical grade dimethyl sulphoxide (DMSO)

Positive control: Table provided in study report:

Chemical	Final concentration (µg/plate)	Use Strain(s)	S-9
2-nitrofluorene (2NF)	5.0	TA98	–
Sodium azide (NaN_3)	2.0	TA100, TA1535	–
9-aminoacridine (AAC)	50.0	TA1537	–
Glutaraldehyde (GLU)	25.0	TA102	–
Benzo[a]pyrene (B[a]P)	10.0	TA98	+
2-aminoanthracene (AAN)	5.0	TA100, TA1535, TA1537	+
	20.0	TA102	+

Formulation/Vehicle: DMSO

Incubation & sampling time: Plates were incubated for 3 days. The metabolic activation system consisted of commercially available S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. Revertant colonies were counted by automated colony counter and/or by hand.

Study Validity

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

Results

A preliminary dose range-finding study was conducted on PXD101 using strain TA100 with six doses ranging from 1.6 to 5000 mcg/plate and included negative and positive controls. Toxicity ranging from slight thinning of the background bacterial lawn to a complete killing of the test bacteria was reported in the presence or absence of metabolic activation. Same doses were used in Experiment 1 which also included strain TA100 for reproducibility purposes. Experiment 3 was added after observing toxicity at various concentrations and no bacterial survival at 5000 mcg/plate in Experiment 2.

Table 29 Average Colony Counts for the Bacterial Mutagenesis Assay (Experiment 1)

Average colony count per plate without S9:

mcg/plate	TA98	TA100	TA1535	TA1537	TA102
1.6	34	98	16	11	383
8	26	96	19	11	328
40	38	98	20	10	348
200	32	104*	17	25***	345
1000	32	130**	26*	35***	280
5000	--	--	12	--	--
-control	32	78	19	9	359
+control	715	555	514	143	740

-- No bacterial survival
Dunnett test * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$

Average colony count per plate with S9:

mcg/plate	TA98	TA100	TA1535	TA1537	TA102
1.6	31	101	21	9	344
8	28	96	15	9	316
40	34	91	18	20***	321
200	39*	118	18	31***	337
1000	44***	135***	27***	33***	327
5000	10	--	25**	--	--
-control	23	106	15	10	351
+control	236	2219	224	282	2172

-- No bacterial survival

Table 30 Average Colony Counts for the Bacterial Mutagenesis Assay (Experiment 2)

Average colony count per plate without S9:

mcg/plate	TA98	TA100	TA1535	TA1537	TA102
156.25	42	159	32***	25***	414
312.5	36	185***	29*	24***	431**
625	29	190***	31*	34***	432**
1250	23	225***	43***	47***	406
2500	16	--	44***	--	391
5000	--	--	20	--	--
-control	70	130	19	15	378
+control	835	637	686	256	695

-- No bacterial survival
Dunnett test * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.005

Average colony count per plate with S9:

mcg/plate	TA98	TA100	TA1535	TA1537	TA102
78.125	NC	NC	NC	NC	465*
156.25	57	191*	36*	29*	475***
312.5	53	165	38*	33***	462*
625	44	192*	28	42***	358
1250	53	200**	42*	54***	173
2500	24	32	42*	7	--
5000	--	--	18	--	NC
-control	45	144	20	22	393
+control	238	1774	132	304	2199

NC= no conducted. -- No bacterial survival

Table 31 Average Colony Counts for the Bacterial Mutagenesis Assay (Experiment 3)

mcg/plate	TA98 Without S9	TA98 With S9	TA102 with S9
19.5312	43	NC	413
39.0625	36	31	317
78.125	33	33	366
156.25	33	43*	352
312.5	47	52***	346
625	57*	61***	389
1250	38	55***	323
-control	45	32	423
+control	1182	281	1938

NC= no conducted.
Dunnett test * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.005;

Conclusion
Revertant frequencies were increased in a PXD101 dose-dependent manner in all strains tested in the absence or presence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: PXD101: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique: Screening study

Study no.:	1981-022
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	No included
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PXD101; Lot # PR1(1)-67; Purity 96.9%

Key Study Findings

- Using the L5178Y$^{TK+/-}$ mouse lymphoma cell mutagenesis system, PXD101 induced mutations in mammalian cells in the absence and presence of metabolic activation. The results are considered valid and adequate.

Methods

Cell line: L5178Y$^{TK+/-}$ mouse lymphoma cells

Concentrations (mcg/mL) in definitive study:

Absence of S9 (24-h treatment)	Presence of S9 (3-h treatment)
mcg/mL	mcg/mL
0	0
0.05	10
0.125	20
0.2	40
0.275	60
0.35	80
0.425	100
0.5	120
0.575	140
0.65	160
0.725	180

Basis of concentration selection: A dose range-finding study was conducted on PXD101 using doses from 0.03125 to 4 mcg/mL for the 24-hour treatment in the absence of S9 and from 26.56 to 850 mcg/mL for the 3-hour treatment in the presence of S9.

Negative control: Sterile anhydrous analytical grade dimethyl sulphoxide (DMSO)

Positive control: 4-nitroquinoline-1-oxide or benzo(a)pyrene

Formulation/Vehicle: DMSO

Incubation & sampling time:	Plates were incubated for 24 hours in the absence of S9 or 3 hours in the presence of S9 and evaluated 2 days later. The metabolic activation system consisted of commercially available S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. Extreme toxicity was defined as <10% relative total growth (RTG).

Study Validity

The study appears valid based on:

- The use of appropriate negative and positive controls
- Duplicate cultures in the main study except for single positive controls at two doses

Results

PXD101 induced mutations at the *tk* locus of L5178Y mouse lymphoma cells in the absence as presence of metabolic activation.

Table 32 Main Study of PXD101 in L5178Y$^{TK+/-}$ mouse lymphoma cells

(Excerpted from Toxicology Tabulated Summary)

Metabolic Actvation	Test Article	Concentration (mcg/ml)	% RTG Relative Total Growth	Mutation Frequency[2]	Proportion of Small Colony Mutants
(-S9), 24h	Vehicle (DMSO)	0	100	104.28	0.58
(-S9), 24h	Belinostat	0.05	(N)	-	-
(-S9), 24h	Belinostat	0.125	(N)	-	-
(-S9), 24h	Belinostat	0.2	(N)	-	-
(-S9), 24h	Belinostat	0.275	28	142.14	-
(-S9), 24h	Belinostat	0.35	22	134.97	-
(-S9), 24h	Belinostat	0.425	18	137.06	-
(-S9), 24h	Belinostat	0.5	14	175.00*	0.57
(-S9), 24h	Belinostat	0.575	10	248.77*	0.65
(-S9), 24h	Belinostat	0.65	9 (X)	201.61	-
(-S9), 24h	Belinostat	0.725	6 (X)	273.11	-
(-S9), 24h	4-nitroquinoline-	0.02	76	389.48	0.55
(-S9), 24h	4-nitroquinoline-	0.04	84	344.43	0.54
(+S9), 3h	Vehicle (DMSO)	0	100	69.07	0.45
(+S9), 3h	Belinostat	10	79	63.06	-
(+S9), 3h	Belinostat	12/20[a]	39	136.36*	0.54
(+S9), 3h	Belinostat	40	23	166.17*	0.56
(+S9), 3h	Belinostat	60	19	168.18*	0.55
(+S9), 3h	Belinostat	80	25	171.54*	0.51
(+S9), 3h	Belinostat	100	17	212.44*	0.66
(+S9), 3h	Belinostat	120	15	281.95*	0.57
(+S9), 3h	Belinostat	140	13	271.38*	0.56
(+S9), 3h	Belinostat	160	13	301.13*	0.72
(+S9), 3h	Belinostat	180	12	324.63*	0.56
(+S9), 3h	Benzo(a)pyrene	2	49	641.35	0.55
(+S9), 3h	Benzo(a)pyrene	3	23	1119.51	0.52

Notes: (N): Not plated for viability / 5-TFT resistance. (X): Treatment excluded from final test statistic due to excessive toxicity. * p< 0.05, Treated vs control Dunnett's (onesided) t-test. aThe report is unclear; states 20 µg/mL on page 5, and 12 µg/mL on page 12-15,
[2] 5-TFT resistant mutant cells pr 106 viable cells.

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

Study title: PXD101: Induction of Micronuclei in the Bone Marrow of Treated Rats

Study no:	2525-005
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 22, 2004
GLP compliance:	OECD
QA statement:	Yes
Drug, lot #, and % purity:	PXD101; Lot # PR1(3)-10 Purity 100.5% and PR1(6)-54 Purity 99.3%;

Key Study Findings

- PXD101 induce a significant increase in micronucleated polychromatic erythrocytes at a non-toxic dose of 260 mg/kg/day.
- PXD101 elicited bone marrow toxicity at 520 mg/kg/day.

Methods

Doses in definitive study:	0, 130, 260, or 520 mg/kg
Frequency of dosing:	Daily for two days
Route of administration:	Tail vein intravenous infusion over 30 minutes
Dose volume:	10 mL/kg
Formulation/Vehicle:	2 Parts of L-Arginine in water / 1 part PXD101
Species/Strain:	Male Han Wistar Crl:WI (Han) rats
Number/Sex/Group:	Six males/group plus 6 male/group in each PDX101 group as vehicle control to test the increasing concentrations of L-Arginine. The decision to use only males in the micronucleus assay was based on results from previously conducted toxicological studies where no relevant differences in toxicity were noted between the sexes.
Satellite groups:	3 males/group; 1 vehicle group, 3 high dose groups
Basis of dose selection:	Dose range-finding study conducted using doses ranging from 150 to 750 mg/kg
Negative control:	Untreated animals
Positive control:	Cyclophosphamide (CPA) 20 mg/kg single intravenous injection on Day 2

Necropsy: Rats were sacrificed 24 hours after the last dose using carbon dioxide followed by cervical dislocation. Sacrifice of rats was conducted in the same order as they were dosed.

Evaluation Criteria
A result was considered positive if:

- A statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCE) occurred at least at one dose.
- The frequency and distribution of micronucleated PCE within the group at such a point exceeded the historical vehicle control data.

Table 33 Experimental Design in Rodent Micronucleus Assay

(Excerpted from Applicant's Submission)

Vehicle Treatment (L-Arginine Concn)	Treatment	Concentration (mg/mL) *	Dose volume (mL/kg)	Test article Dose level (mg/kg/day) [a]	Number of animals treated [b]
-	UTC	-	10	0	5M
26 mg/mL	PXD101	13	10	130.0	6M + 6M (Vehicle)
52 mg/mL	PXD101	26	10	260.0	6M + 6M (Vehicle)
104 mg/mL	PXD101	52	10	520.0	6M + 6M (Vehicle)
-	Positive control, CPA [c]	2	10	20	6M

a Doses administered once daily for two consecutive days, approximately 24 hours apart (except p ositive control)
b Animals sampled 24 hours after final dose administration
c Cyclophosphamide; administered once only
UTC Untreated control group
CPA Cyclophosphamide
M Male
* PXD101 dose levels

Table 34 Satellite Group Dosing and Blood Sampling Times

(Excerpted from Applicant's Submission)

Dose groups (mg/kg/day)	Number of animals	Bleed Time (hours after the administration)					
		0 (pre-dose)	5 min	15 min	30 min	1 hour	2 hour
Vehicle	3M	√			√		
High dose	3M	√			√		
High dose	3M		√			√	
High dose	3M			√			√

Study Validity

- The incidence and distribution of micronucleated PCE in the vehicle control groups was consistent with the historical vehicle control data
- At least five animals out of each group were available for analysis
- The positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE

Results

Dose Formulation Analysis

Analysis of the dose formulations indicated all solutions were within approximately 10% of the expected nominal drug concentrations. Dose formulations for the vehicle control contained PDX101 at 0.01% and 0.02% on Days 1 and 2, respectively. The investigation could not define at what step in the process vehicle control dosing formulations were cross contaminated with PXD101; however, plasma samples from vehicle control animals showed no detectable levels of PXD101. The individual frequencies of micronucleated PCE cells in the control animals were consistent with the historical vehicle data. Based on these results, the presence of PXD101 in the control vehicle dose formulations was considered to have no impact on the interpretation of study results.

PXD101 Plasma Concentrations

Results showed that PXD101 achieved systemic levels following intravenous infusion at 520 mg/kg/day, Table 35.

Table 35 Plasma Concentrations of Belinostat in the Rodent Micronucleus Assay

(Excerpted from Applicant's Submission)

PXD101

Dose Group	Dose (mg/kg)	Sex (n=3)	C_{max} (µg/mL)	$AUC_{(0-t)}$ (µg.hr/mL)	$AUC_{(0-inf)}$ (µg.hr/mL)	T_{max} (min)
High	520	M	45.55	38.79	43.43	15

Micronucleus

Untreated control rats exhibited number and mean percent of micronucleated PCE within the range of historical data. Positive control rats showed increased number and mean percent of micronucleated PCE compared to control. The mean of PCE in the 520 mg/kg/day PXD101 group was lower than the historical data range indicating that PXD101 produces toxicity to the bone marrow. There was no evidence of an increased frequency of micronucleus formation in the L-Arginine-treated (vehicle control) rats. The frequency of MN-PCE in the low dose (130 mg/kg/day) and high dose (520 mg/kg/day) were within historical data and similar to the control group. However, two rats in the 260

mg/kg/day PXD101 group presented elevated MN-PCE frequencies of 4 MN-PCE per 2000 PCE cells. As a result, the mean MN-PCE was significantly higher than the control group.

Table 36 Frequency of Micronucleated PCE in rats

Treatment Group	Number of rats	Mean PCE (%)	Total number of MN-PCE	Mean MN-PCE (%) ± SD
UTC	5	38.22	3	0.03 ± 0.03
Positive Control (CPA 20 mg/kg)	6	38.08	**64**	**0.53 ± 0.22**
Vehicle Control (L-Arginine mg/mL)				
26	6	36.93	4	0.03 ± 0.06
52	6	31.18	2	0.02 ± 0.04
104	5	36.16	3	0.03 ± 0.04
PXD101 (mg/kg/day)				
130	6	37.32	8	0.07 ± 0.05
260	6	30.95	**15**	**0.13 ± 0.06**
520	6	8.56	6	0.06 ± 0.07

UTC = untreated control; CPA = cyclophosphamide; SD = standard deviation
PCE = polychromatic erythrocyte; MN-PCE = micronucleated PCE
Values in **bold** denotes significantly different from control

Conclusion
PXD101 (belinostat) induce a significant increase in micronucleated polychromatic erythrocytes at a non-toxic dose of 260 mg/kg/day.

8 Carcinogenicity

Not conducted or needed for this indication.

9 Reproductive and Developmental Toxicology

Not conducted or needed; refer to Section 10.

10 Integrated Summary and Safety Evaluation

The nonclinical safety evaluation of belinostat produced findings that were consistent with the anticipated toxicity of a HDAC inhibitor: reduced body weights and feed consumption, localized injection site toxicity that was severe in rats, lymphoid depletion, decreased white blood cell counts, local toxicity in the gastrointestinal tract, and in dogs, vomiting and soft/loose feces. There were also findings indicating potential belinostat-mediated cardiovascular toxicities in both rats (cardiomyopathy) and dogs (increased heart weight and heart rate).

Belinostat demonstrated genotoxicity in all 3 assays used (Ames assay, in vitro mouse lymphoma and in vivo mouse micronucleus assay). Embryo-fetal development toxicology studies were not conducted because of belinostat is genotoxic and is cytotoxic towards rapidly dividing cells both in vitro and in vivo (peripheral blood lymphocytes and gut mucosal epithelium) and therefore, it is expected to cause teratogenicity and/or embryofetal lethality. Belinostat elicited toxicity towards male reproductive organs in the dog and included both delayed testicular maturation and exfoliated spermatid cells in the epididymis.

Doses administered to rats and dogs for the 24-week repeat dose toxicology studies were less than or equivalent to the recommended human dose for patients with PTCL. Systemic exposures (AUC_{0-t}) in the high dose groups for both rats and dogs exceeded that measured clinically.

Table 37 Comparison of Rat, Dog and Human AUC Values Following IV Administration of PXD101

Daily Dose (mg/m^2)	Rat Daily Dose (mg/kg)	Dog Daily Dose (mg/kg)	Steady State AUC (ng*hr/ml)				
			Rats (AUC_{0-24h}) Day 151[a]		Dogs (AUC_{0-24h}) Day 151[a]		Humans (AUC_{0-t})[b]
			M	F	M	F	
60	10		710	530	-	-	-
150	25		2220	1760	-	-	1216±329
200		10	-	-	2650	2210	-
300			-	-	-	-	3461±1249
500		25	-	-	6100	5820	-
600	100		18450	12090	-	-	9619±3024
1000		50	-	-	12480	13780	10664±4694

a: IV TK values from Rat: Study 2525-001 and Dog: Study 2525-013
b TT20 data: FR 160 – TT20 PK Report

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/s/

PEDRO L DEL VALLE
04/29/2014

M S RICCI
04/29/2014

HALEH SABER
04/30/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206256 **Applicant: Spectrum Pharmaceuticals** **Stamp Date: December 9, 2013**

Drug Name: BELEODAQ™ (belinostat) **NDA Type: Original NDA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		Reproductive toxicology studies were not conducted. However, these studies may not be needed for this drug per ICH S9 (belinostat is genotoxic and targets rapidly dividing cells)
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		Formulation using L-Arginine and water for injection was similar in clinical and animal toxicology studies.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	√		Belinostat was administered intravenously to animals in toxicology studies, same route intended for administration in patients.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		This is a review issue. It appears that organic impurities levels will be either below the threshold described in ICH guidelines or are qualified by toxicological studies
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

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

NA

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

None

Pedro L. Del Valle and M. Stacey Ricci — January 15, 2014
Reviewing Pharmacologist — Date

Haleh Saber — January 15, 2014
Team Leader/Supervisor — Date

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

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/s/

PEDRO L DEL VALLE
01/16/2014

HALEH SABER
01/16/2014