PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 24490-119478

STUDY TITLE:

SCREENING PLASMA PHARMACOKINETICS (SPPK) OF PANOBINOSTAT (D5W, 10 MM LACTIC ACID) IN FEMALE CD1 NU/NU MICE AFTER A SINGLE INTRAPERITONEAL DOSE

SHORT TITLE:	**Panobinostat (Lactic Acid) Screening Plasma PK**
TEST ARTICLE:	Panobinostat
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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FOR P-PKSR APPROVED USE AND DISTRIBUTION

Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The total plasma PK of panobinostat in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single intraperitoneal (IP) injection of 20 mg/kg as an acidified solution. It was hypothesized that this solution would result in better solubility, absorption, and an improved PK profile compared with the previous suspension IP study (RPT.19516-89512).

Panobinostat (LC Labs, Lot PNB-101, purity >99%, MolWt 349.43) was dissolved as the free base form in dextrose 5% for injection, USP (D5W, Baxter) with 10 mM lactic acid at a nominal concentration of 2 mg/mL for a 10 mL/kg injection volume. The IP route was chosen given the low oral bioavailability reported in rodents [1,2], and as it appeared to be the preferred route in reviewed mouse studies [3–5].

Survival blood samples were obtained from each mouse via retro-orbital plexus using glass microhematocrit capillaries, and final terminal samples by cardiac puncture. Mice were sampled using an IACUC-approved methods at 15 min, 40 min, 2.25, 8, and 24 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for remainder of study. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

1.2 Bioanalysis

Total plasma panobinostat concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. Panobinostat (LC Labs, Lot PNB-101, purity >99%) stock solutions were prepared in methanol and used to spike matrix calibrators and quality controls. Plasma samples, 25 µL each, were protein precipitated with 100 µL of 240 ng/mL panobinostat-d8 hydrochloride salt (Toronto Research Chemicals, Inc., P180502, Lot 5-KSS-175-5, purity 96%) in methanol as an internal standard. A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a LEAP CTC PAL autosampler. The LC separation was performed using a Waters XBridge BEH C18 LC column (2.5 µm, 75 mm x 2.1 mm) maintained at 60 °C with gradient elution at a flow rate of 0.35 mL/min. The binary mobile phase consisted of ultra-pure water - 100 mM ammonium formate, pH=3.0 – methanol (850:50:100 v/v) in reservoir A and methanol – acetonitrile – 100 mM ammonium formate, pH=3.0 (475:475:50 v/v) in reservoir B. The initial mobile phase consisted of 42.5% B and was maintained for 1.6 minutes. The column was then rinsed for 1.4 minutes at 100% B and then equilibrated at the initial conditions for 2 minutes for a total run time of 5 minutes. Under these conditions, the analyte and IS eluted at 0.85 and 0.83 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode with monitoring of the following mass transitions: panobinostat 350.18 -> 158.18, panobinostat-d8 357.91 -> 147.19.

The experimental bioanalytical runs were all found to be acceptable for the purpose of a singlicate non-GLP, preclinical PK assessment. A linear model ($1/X^2$ weighting) fit the calibrators across the 5 to 1000 ng/mL range, with a correlation coefficient (R) of ≥0.99. The lower limit of quantitation (LLOQ), defined as a peak area signal-to-noise ratio of 5 or greater verses a matrix blank with IS, was 5 ng/mL for plasma. The intra-run precision and accuracy was < 0.312% CV and 99.4% to 113%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The resultant panobinostat concentration-time (Ct) data were grouped by matrix and time point, and manual imputation of data below the lower limit of quantitation (BLOQ) was as follows: IF at any time point ≥ 2/3rds of the Ct results were above the LLOQ, the BLOQ data were replaced with a value of ½ LLOQ, ELSE the entire time point's data were treated as missing. Then, using Phoenix WinNonlin 6.4

(Certara USA, Inc., Princeton, NJ), Ct data summary statistics (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the panobinostat arithmetic mean Ct data for each matrix was subjected to noncompartmental pharmacokinetic analysis (NCA). The extravascular model (Model 202) was applied, and area under the Ct curve (AUC) values were estimated using the linear trapezoidal method and the sparse sampling option. The terminal phase was defined as the three time points at the end of the Ct profile, and the elimination rate constant (Ke) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Ke, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast/Ke.

Other NCA parameters estimated included observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The average concentration over a dosing interval (Cavg) was estimated as AUCinf / dosing interval in hours. The plasma PK results were qualitatively compared with the previous findings from RPT.19516-89512.

2.0 RESULTS

Panobinostat concentrations resulting from the lactic acid formulation showed low variability, demonstrating average coefficients of variation of 0.730% to 49.2% for plasma across the sampling time points. The plasma Ct profile showed a rapid absorption phase and an initial distribution phase ending at about 4 hours. The apparent terminal half-life for panobinostat in plasma was 9.86 hours. The apparent clearance was very high at 212 mL/min/kg, several fold higher than murine hepatic blood flow. The apparent terminal volume of distribution was also large at 181 L/kg. The intraperitoneal bioavailability of panobinostat in this study was unknown. Overall, the plasma PK of panobinostat with the lactic acid formulation appeared similar to other published studies [1,6,7].

Compared with the plasma PK from the previous study, panobinostat displayed a vastly different Ct profile, with an almost 50-fold higher Cmax and 4.5-fold lower Vz/F. The apparent clearance and AUC values were similar however, as was the Cavg over a proposed 48-hour dosing interval.

The D5W 10 mM lactic acid vehicle was not well tolerated by the IP route. A number of mice treated only with the vehicle died shortly after IP injection, and this was attributed to the formulation. The solution may have been overly acidic, or caused a metabolic catastrophe from an abundance of lactate being introduced to the liver. Therefore, despite the more favorable PK profile from this formulation, it was not further pursued, and the D5W suspension was utilized.

3.0 REFERENCES

1. Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): Successes and challenges. Cancer Lett. 2009 Aug 8;280(2):233–41.

2. Drug Approval Package: Farydak (panobinostat) NDA # 205353 Pharmacology Review [Internet]. Drugs@FDA. [cited 2016 Jun 9]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/205353Orig1s000TOC.cfm

3. Anne M, Sammartino D de, Barginear M, Budman D. Profile of panobinostat and its potential for treatment in solid tumors: an update. OncoTargets Ther [Internet]. 2013 Nov 15 [cited 2016 Jun 9]; Available from: https://www.dovepress.com/profile-of-panobinostat-and-its-potential-for-treatment-in-solid-tumor-peer-reviewed-article-OTT

4. Xie C, Drenberg C, Edwards H, Caldwell JT, Chen W, Inaba H, Xu X, Buck SA, Taub JW, Baker SD, Ge Y. Panobinostat Enhances Cytarabine and Daunorubicin Sensitivities in AML Cells through Suppressing the Expression of BRCA1, CHK1, and Rad51. PLOS ONE. 2013 Nov 11;8(11):e79106.

5. Ocio EM, Vilanova D, Atadja P, Maiso P, Crusoe E, Fernández-Lázaro D, Garayoa M, San-Segundo L, Hernández-Iglesias T, Álava E de, Shao W, Yao Y-M, Pandiella A, San-Miguel JF. In vitro and in vivo rationale for the triple combination of panobinostat (LBH589) and dexamethasone with either bortezomib or lenalidomide in multiple myeloma. Haematologica. 2010 May 1;95(5):794–803.

6. Estella-Hermoso de Mendoza A, Imbuluzqueta I, Campanero MA, Gonzalez D, Vilas-Zornoza A, Agirre X, Lana H, Abizanda G, Prosper F, Blanco-Prieto MJ. Development and validation of ultra high performance liquid chromatography–mass spectrometry method for LBH589 in mouse plasma and tissues. J Chromatogr B. 2011 Nov 15;879(30):3490–6.

7. Chopra V, Quinti L, Khanna P, Paganetti P, Kuhn R, Young AB, Kazantsev AG, Hersch S. LBH589, A Hydroxamic Acid-Derived HDAC Inhibitor, is Neuroprotective in Mouse Models of Huntington's Disease. J Huntingt Dis. 2016 Jan 1;5(4):347–55.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Plasma Ct Profile of Panobinostat

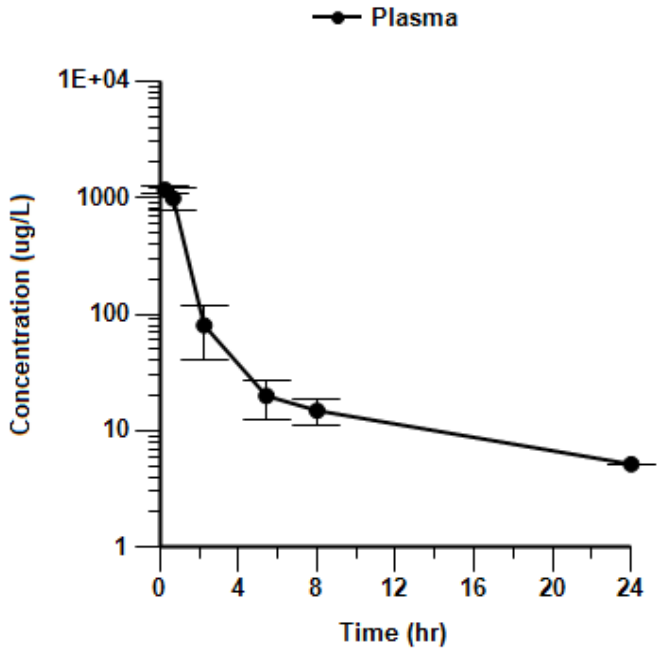

Table 4.1: Plasma NCA PK Parameter Estimates of Panobinostat

		Analyte
		Panobinostat
		Group
		Plasma
Parameter	**Units**	**Estimate**
Cmax	ug/L	1170
Tmax	hr	0.250
AUClast	hr*ug/L	1500
AUCinf	hr*ug/L	1570
Kel	1/hr	0.0703
T1/2	hr	9.86
CL/F	L/hr/kg	12.7
Vz/F	L/kg	181
Clast	ug/L	5.17
Tlast	hr	24.0
Cavg, 48hr	ug/L	32.7

Table 4.2: Full Summary Statistics of Panobinostat Ct Data by Group

		Analyte
		Panobinostat
		Group
		Plasma
Time (hr)		**Concentration (ug/L)**
0.250	N	3
	Mean	1170
	SD	84.6
	Min	1100
	Median	1140
	Max	1260
	CV%	7.24
	Geometric Mean	1170
	CV% Geometric Mean	7.16
0.670	N	3
	Mean	986
	SD	217
	Min	861
	Median	861
	Max	1240

Panobinostat (Lactic Acid) Screening Plasma PK

		Analyte
		Panobinostat
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	CV%	22.0
	Geometric Mean	971
	CV% Geometric Mean	21.2
2.250	N	3
	Mean	80.3
	SD	39.5
	Min	57.5
	Median	57.5
	Max	126
	CV%	49.2
	Geometric Mean	74.7
	CV% Geometric Mean	47.6
5.420	N	3
	Mean	19.9
	SD	7.49
	Min	12.3
	Median	20.3
	Max	27.2
	CV%	37.6
	Geometric Mean	18.9
	CV% Geometric Mean	42.0
8.000	N	3
	Mean	14.9
	SD	3.71
	Min	12.8
	Median	12.8
	Max	19.2
	CV%	24.9
	Geometric Mean	14.6
	CV% Geometric Mean	23.9
24.000	N	2
	Mean	5.17
	SD	0.0378
	Min	5.14

		Analyte
		Panobinostat
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	Median	5.17
	Max	5.20
	CV%	0.730
	Geometric Mean	5.17
	CV% Geometric Mean	0.730

Table 4.3: Panobinostat Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Panobinostat	Plasma	0.25	1263.09
M1	Panobinostat	Plasma	5.42	20.29
M1	Panobinostat	Plasma	24.00	5.14
M2	Panobinostat	Plasma	0.25	1099.20
M2	Panobinostat	Plasma	5.42	12.28
M2	Panobinostat	Plasma	24.00	5.20
M3	Panobinostat	Plasma	0.25	1144.38
M3	Panobinostat	Plasma	5.42	27.25
M4	Panobinostat	Plasma	0.67	860.63
M4	Panobinostat	Plasma	2.25	57.50
M4	Panobinostat	Plasma	8.00	12.75
M5	Panobinostat	Plasma	0.67	860.63
M5	Panobinostat	Plasma	2.25	57.50
M5	Panobinostat	Plasma	8.00	12.75
M6	Panobinostat	Plasma	0.67	1237.06
M6	Panobinostat	Plasma	2.25	125.86
M6	Panobinostat	Plasma	8.00	19.18

Table 4.4: Panobinostat Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Panobinostat	Plasma	0.25	1168.89	84.65	3.00
Concentration	ug/L	Panobinostat	Plasma	0.67	986.11	217.33	3.00
Concentration	ug/L	Panobinostat	Plasma	2.25	80.29	39.47	3.00
Concentration	ug/L	Panobinostat	Plasma	5.42	19.94	7.49	3.00
Concentration	ug/L	Panobinostat	Plasma	8.00	14.89	3.71	3.00

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Panobinostat	Plasma	24.00	5.17	0.04	2.00

5.0 ATTACHED FILES

Attached File 5.1 Panobinostat Plasma PK Study #2.docx– *Final in vivo study plan as executed*
Attached File 5.2 Pano_PK_1_23_14.xlsx– *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 Panobinostat Lactic Acid Plasma PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*