PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 19519-89517

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF VELIPARIB IN FEMALE CD-1 NUDE MICE BEARING EWING SARCOMA EW-8 ORTHOTOPIC XENOGRAFTS AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Veliparib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Veliparib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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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 plasma and tumor pharmacokinetic (PK) profile of the PARP inhibitor veliparib was evaluate in Female CD-1 nude mice (The Jackson Laboratories), approximately 12 weeks in age, bearing Ewing sarcoma EW-8 orthotopic xenografts. Veliparib free base (SelleckChem, Purity >95%) was dissolved in acidified 0.9% normal saline for injection (pH 2-3), at a concentration of 1.25 mg/mL as a 10 mL/kg oral gavage, for a 12.5 mg/kg oral dose. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. Following terminal bleeds, animals were perfused with PBS to flush blood from the vasculature. Tumors were then extracted, rinsed with PBS as necessary, and then placed in appropriately labeled microcentrifuge tubes in a cooler on dry ice. Tissue samples were then transferred to a -80°C freezer as soon as possible.

1.2 Bioanalysis

Frozen tumor samples were weighed in tared 15 mL Lysing Matrix D (MP Biomedical, Santa Ana, CA) tubes and diluted with a 5:1 volume of ultrapure water. The tissue samples were then homogenized with a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). The homogenization consisted of three 6.0 M/S vibratory cycles of 1 min each on the FastPrep-24 system. To prevent over-heating due to friction, samples were placed on wet ice for 5 min between each cycle. The homogenates were then stored at -80°C until analysis.

Plasma and tumor samples were analyzed for veliparib (SelleckChem, Purity >95%) with a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Matrix calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in methanol. Plasma and tissue samples, 25 µL each, were protein precipitated with 100 µL of 20 ng/mL niraparib (CHEMIETEK, Lot # 01A, purity > 99%) in 0.1% formic acid 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 PAL HTS-xt autosampler.

The LC separation was performed using a Waters XBridge BEH-XP C18 (2.5µm, 2.1 mm x 75 mm) column maintained at room temperature with gradient elution at a flow rate of 0.35 mL/min. The binary mobile phase consisted of Water – Methanol – 100 mM ammonium formate (pH=3.0) (85:10:5) in reservoir A and Acetonitrile – Methanol – 100 mM ammonium formate (pH=3.0) (47.5:47.5:5.0) in reservoir B. The initial mobile phase consisted of 15% B and was followed by a linear increase to 100% B in 3.0 min. The column was then rinsed for 1 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 6.0 min. Under these conditions, the analyte and IS eluted at 1.10 and 2.07 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a SCIEX 5500 QTRAP in the positive ESI mode with monitoring of the following mass transitions: veliparib 245.10 → 84.10, and niraparib 321.17 → 304.20.

The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 1.0 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.998. 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 1.0 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 3.44% CV and 103% to 110%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Talazoparib plasma and tumor Ct data were grouped by matrix and nominal time point. 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. Summary statistics were calculated and the

arithmetic mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular model was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" method. The terminal phase was defined as at least three time points at the end of the Ct profile, and the elimination rate constant (Kel) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Kel, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Kel. Other 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 apparent plasma-to-tumor partition coefficient (Kp,inf) was estimated as the ratio of the AUCinf in tissue to AUCinf plasma, whereas Kp,last was similarly estimated using AUClast values.

2.0 RESULTS

The veliparib plasma and tumor Ct data demonstrated moderate-to-high variability between mice, with coefficients of variation ranging from 8.82% to 94.7%. The absorption rate of veliparib was rapid, with the plasma Tmax occurring at 15 minutes post-dose. After Cmax, plasma concentrations diminished in a mono-exponential manner. The apparent plasma terminal half-life of veliparib was approximately 1 hour. The apparent plasma clearance (CL/F) of veliparib was high at 123 mL/min/kg, in excess of murine hepatic blood flow. The apparent terminal volume of distribution (Vz/F) for veliparib in plasma was high at 10.4 L/kg. The oral bioavailability of veliparib was unknown in the current study, but has been previously reported to be 92% in mice [1]. Tumor penetration of veliparib was moderate-to-high, with a Kp,inf of 3.018. The tumor terminal half-life of veliparib appeared longer than plasma, suggesting a high affinity of veliparib for the tumor tissue.

In clinical studies, the total plasma AUC of veliparib at steady state was reported as 1530 hr-ng/mL with the dose of 25 mg/m^2 PO BID in combination with temozolomide children with brain tumors [2]. The fraction unbound in plasma (Fu,p) for veliparib is high and assumed to be similar for humans and mice [3]. Therefore, the precise CRD for mice calculated by unbound AUCs is veliparib 11.25 mg/kg PO BID.

Considering the data available at the time of this work in 2014, namely a misidentified target clinical dose underrepresenting veliparib MTD exposure, a dose of 12.5 mg/kg PO BID was instead suggested. Later data from 2018 identified a single agent recommended phase 2 dose (RP2D) of veliparib 400 mg PO BID in adult cancer patients [4]. This higher dose and human exposure would equate to veliparib ~100 mg/kg PO BID in mice by plasma AUC.

An alternate version of these PK results, using different PK analysis methods, was published in Stewart et al. 2014 [5].

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Veliparib Ct Summary (Mean, SD, N) by Group

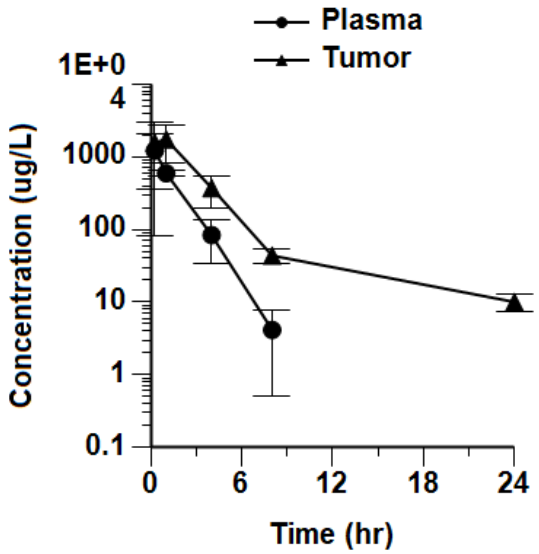

Table 4.1: NCA PK Parameter Estimates of Veliparib by Group

		Analyte	
		Veliparib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	1210	1740
Tmax	hr	0.250	1.00
AUClast	hr*ug/L	1690	5080
AUCinf	hr*ug/L	1700	5130
Kel	1/hr	0.712	0.155

		Analyte	
		Veliparib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
T1/2	hr	0.974	4.46
CL/F	L/hr/kg	7.37	2.44
Vz/F	L/kg	10.4	15.7
Clast	ug/L	4.14	10.1
Tlast	hr	8.00	24.0
Kp,inf		-	3.018
Kp,last		-	3.006

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

		Analyte	
		Veliparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.250	N	3	3
	Mean	1210	1560
	SD	851	1480
	Min	231	15.7
	Median	1670	1710
	Max	1740	2970
	CV%	70.2	94.7
	Geometric Mean	875	431
	CV% Geometric Mean	167	6300
1.000	N	3	3
	Mean	594	1740
	SD	52.4	936
	Min	550	1190
	Median	580	1210
	Max	652	2820
	CV%	8.82	53.9
	Geometric Mean	593	1590
	CV% Geometric Mean	8.71	52.6
4.000	N	3	3
	Mean	83.7	373
	SD	49.7	177
	Min	29.5	174
	Median	94.6	435
	Max	127	511
	CV%	59.3	47.4

		Analyte	
		Veliparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	Geometric Mean	70.7	338
	CV% Geometric Mean	90.4	63.5
8.000	N	3	3
	Mean	4.14	43.7
	SD	3.65	9.55
	Min	0.500	32.7
	Median	4.14	48.4
	Max	7.79	50.0
	CV%	88.0	21.8
	Geometric Mean	2.53	42.9
	CV% Geometric Mean	263	23.9
24.000	N	0	3
	Mean	BLOQ	10.1
	SD		2.83
	Min		8.07
	Median		8.81
	Max		13.3
	CV%		28.1
	Geometric Mean		9.81
	CV% Geometric Mean		27.1

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Veliparib	Plasma	0.25	1736.54
M1	Veliparib	Tumor	0.25	1709.07
M2	Veliparib	Plasma	0.25	230.80
M2	Veliparib	Tumor	0.25	15.74
M3	Veliparib	Plasma	0.25	1672.44
M3	Veliparib	Tumor	0.25	2967.26
M4	Veliparib	Plasma	1.00	579.80
M4	Veliparib	Tumor	1.00	1187.32
M5	Veliparib	Plasma	1.00	652.23
M5	Veliparib	Tumor	1.00	2818.50
M6	Veliparib	Plasma	1.00	550.46
M6	Veliparib	Tumor	1.00	1206.49
M7	Veliparib	Plasma	4.00	126.93
M7	Veliparib	Tumor	4.00	510.62
M8	Veliparib	Plasma	4.00	29.45

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M8	Veliparib	Tumor	4.00	173.76
M9	Veliparib	Plasma	4.00	94.61
M9	Veliparib	Tumor	4.00	435.17
M10	Veliparib	Plasma	8.00	0.50
M10	Veliparib	Tumor	8.00	32.72
M11	Veliparib	Plasma	8.00	7.79
M11	Veliparib	Tumor	8.00	48.40
M12	Veliparib	Plasma	8.00	4.14
M12	Veliparib	Tumor	8.00	50.00
M13	Veliparib	Plasma	24.00	BLOQ
M13	Veliparib	Tumor	24.00	8.07
M14	Veliparib	Plasma	24.00	BLOQ
M14	Veliparib	Tumor	24.00	13.29
M15	Veliparib	Plasma	24.00	BLOQ
M15	Veliparib	Tumor	24.00	8.81

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Veliparib	Plasma	0.25	1213.26	851.44	3.00
Concentration	ug/L	Veliparib	Plasma	1.00	594.16	52.39	3.00
Concentration	ug/L	Veliparib	Plasma	4.00	83.66	49.65	3.00
Concentration	ug/L	Veliparib	Plasma	8.00	4.14	3.65	3.00
Concentration	ug/L	Veliparib	Plasma	24.00	BLOQ		0.00
Concentration	ug/L	Veliparib	Tumor	0.25	1564.02	1481.10	3.00
Concentration	ug/L	Veliparib	Tumor	1.00	1737.44	936.28	3.00
Concentration	ug/L	Veliparib	Tumor	4.00	373.18	176.78	3.00
Concentration	ug/L	Veliparib	Tumor	8.00	43.70	9.55	3.00
Concentration	ug/L	Veliparib	Tumor	24.00	10.06	2.83	3.00

5.0 ATTACHED FILES

Attached File 5.1 Veliparib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*