PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 24315-117369

STUDY TITLE:

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

SHORT TITLE:	**Talazoparib Screening Plasma Tumor PK (SPTPK)**	
TEST ARTICLE:	Talazoparib	
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 talazoparib was evaluate in Female CD-1 nude mice (The Jackson Laboratories), approximately 12 weeks in age, bearing Ewing sarcoma EW-8 orthotopic xenografts. Talazoparib free base (LC Laboratories, Lot # PAR-105, Purity 99%) was dissolved in 10% *n,n*-dimethylacetamide (DMAc) /5% Solutol HS15 /85% PBS, at a concentration of 0.0125 mg/mL as a 10 mL/kg oral gavage, for a 0.125 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 talazoparib (Abmole, Lot # NA, 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, 100 µL each, were protein precipitated with 100 µL of 1 ng/mL phthalazinone pyrazole (Cayman Chemical Co., Lot # 0430632-13, Purity ≥98%) in methanol as an internal standard. The resulting supernatants (100 µL each) were transferred to high recovery HPLC vials and were evaporated in a Centrivap Mobile evaporation system (Labconco, Kansas City, MO) for 30 minutes at 60ºC followed by 30 minutes at 100ºC. The sample solids were reconstituted with 25 µL of 0.1% formic acid in methanol. 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 0.4 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 45% B and was followed by a linear increase to 75% B in 2.0 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 5.0 min. Under these conditions, the analyte and IS eluted at 1.29 and 1.83 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: talazoparib 381.12 → 285.10, and phthalazinone pyrazole 318.04 → 102.00.

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 0.1 to 50 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 0.1 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 13.5% CV and 88.8% to 113%, 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 talazoparib plasma and tumor Ct data demonstrated moderate variability between mice, with coefficients of variation ranging from 9.67% to 70.8%. The absorption rate of talazoparib was rapid, with the plasma Tmax occurring at 30 minutes post-dose. After Cmax, plasma concentrations diminished in a nearly mono-exponential manner, with a suggestion of a slight distribution phase to 2 hours post-dose. The apparent plasma terminal half-life of talazoparib was 3.49 hours.

The apparent plasma clearance (CL/F) of talazoparib was low at 16.7 mL/min/kg, or approximately 19% of murine hepatic blood flow. The apparent terminal volume of distribution (Vz/F) for talazoparib in plasma was high at 5.03 L/kg. The oral bioavailability of talazoparib was unknown in the current study, but has been previously reported to be 43-73% in rats [1].

Tumor penetration of talazoparib was moderate, with a Kp,inf of 1.864. The tumor terminal half-life of talazoparib appeared longer than plasma, suggesting a high affinity of talazoparib for the tumor tissue.

In clinical studies, the total plasma AUC of talazoparib at steady state was reported as 170 hr-ng/mL with the dose of 1.1 mg PO QD [2]. The fraction unbound in plasma (Fu,p) for talazoparib has been estimated as 0.261 and 0.0445 for humans and mice, respectively [1]. Therefore, the precise CRD for mice calculated by unbound AUCs is talazoparib 0.5 mg/kg PO BID. Due to the vast difference in human and mouse half-life, a twice daily regimen was suggested to maintain consistent peak-to-trough plasma concentrations and pharmacodynamic coverage. Considering the data available at the time of this work in 2014, and the low tolerability of talazoparib in mice, a dose of 0.125 mg/kg PO BID was instead suggested.

An alternate version of these PK results, using different PK analysis methods, was published in Stewart et al. 2014 [4].

3.0 REFERENCES

1. FDA. Drug Approval Package: Talzenna (talazoparib) NDA # 211651 Multi-Discipline Review/Summary, Clinical, Non-Clinical [Internet]. Drugs@FDA. 2018 [cited 2020 Apr 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/211651Orig1s000MultidisciplineR.pdf

2. Bono JSD, Mina LA, Gonzalez M, Curtin NJ, Wang E, Henshaw JW, Chadha M, Sachdev JC, Matei D, Jameson GS, Ong M, Basu B, Wainberg ZA, Byers LA, Chugh R, Dorr A, Kaye SB, Ramanathan

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of Talazoparib by Group

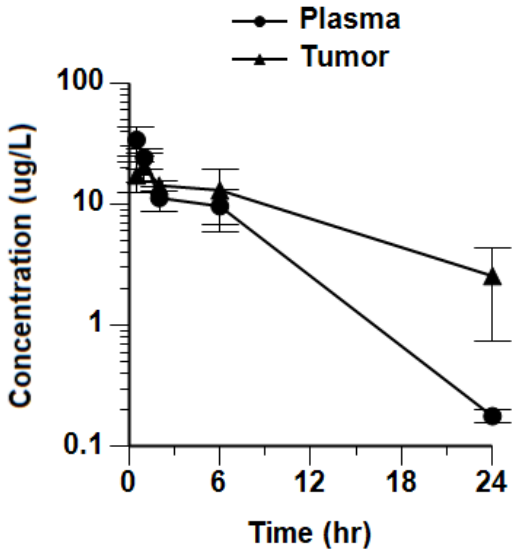

Table 4.1: NCA PK Parameter Estimates of Talazoparib by Group

		Analyte	
		Talazoparib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	34.0	20.5
Tmax	hr	0.500	1.00
AUClast	hr*ug/L	124	202
AUCinf	hr*ug/L	125	233
Kel	1/hr	0.199	0.0819
T1/2	hr	3.49	8.46
CL/F	L/hr/kg	1.00	0.535
Vz/F	L/kg	5.03	6.53
Clast	ug/L	0.178	2.56
Tlast	hr	24.0	24.0
Kp,inf		-	1.864
Kp,last		-	1.629

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

		Analyte	
		Talazoparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	34.0	17.3
	SD	9.06	4.77
	Min	26.2	11.9
	Median	31.7	19.5
	Max	43.9	20.6
	CV%	26.7	27.5
	Geometric Mean	33.2	16.8
	CV% Geometric Mean	26.6	31.2
1.000	N	3	3
	Mean	24.1	20.5
	SD	4.83	5.49
	Min	20.0	16.0
	Median	23.0	18.9
	Max	29.4	26.6
	CV%	20.0	26.8
	Geometric Mean	23.8	20.1
	CV% Geometric Mean	19.8	26.4
2.000	N	3	3
	Mean	11.3	14.3
	SD	2.52	1.38
	Min	9.57	12.7
	Median	10.0	14.7
	Max	14.2	15.4
	CV%	22.4	9.67
	Geometric Mean	11.1	14.2
	CV% Geometric Mean	21.6	9.92
6.000	N	3	3
	Mean	9.62	13.1
	SD	3.72	6.37
	Min	5.81	8.56
	Median	9.83	10.3
	Max	13.2	20.3
	CV%	38.6	48.8
	Geometric Mean	9.11	12.1
	CV% Geometric Mean	43.6	48.2
24.000	N	3	3
	Mean	0.178	2.56

		Analyte	
		Talazoparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	SD	0.0225	1.81
	Min	0.154	1.18
	Median	0.181	1.88
	Max	0.198	4.61
	CV%	12.7	70.8
	Geometric Mean	0.177	2.17
	CV% Geometric Mean	13.0	78.2

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Talazoparib	Plasma	0.50	26.20
M1	Talazoparib	Tumor	0.50	11.85
M2	Talazoparib	Plasma	0.50	43.92
M2	Talazoparib	Tumor	0.50	20.62
M3	Talazoparib	Plasma	0.50	31.74
M3	Talazoparib	Tumor	0.50	19.49
M4	Talazoparib	Plasma	1.00	22.99
M4	Talazoparib	Tumor	1.00	16.02
M5	Talazoparib	Plasma	1.00	19.96
M5	Talazoparib	Tumor	1.00	26.65
M6	Talazoparib	Plasma	1.00	29.41
M6	Talazoparib	Tumor	1.00	18.92
M7	Talazoparib	Plasma	2.00	10.05
M7	Talazoparib	Tumor	2.00	12.73
M8	Talazoparib	Plasma	2.00	14.15
M8	Talazoparib	Tumor	2.00	14.67
M9	Talazoparib	Plasma	2.00	9.57
M9	Talazoparib	Tumor	2.00	15.40
M10	Talazoparib	Plasma	6.00	13.24
M10	Talazoparib	Tumor	6.00	20.34
M11	Talazoparib	Plasma	6.00	9.83
M11	Talazoparib	Tumor	6.00	8.56
M12	Talazoparib	Plasma	6.00	5.81
M12	Talazoparib	Tumor	6.00	10.26
M13	Talazoparib	Plasma	24.00	0.18
M13	Talazoparib	Tumor	24.00	1.88
M14	Talazoparib	Plasma	24.00	0.20

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M14	Talazoparib	Tumor	24.00	1.18
M15	Talazoparib	Plasma	24.00	0.15
M15	Talazoparib	Tumor	24.00	4.61

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Talazoparib	Plasma	0.50	33.95	9.06	3.00
Concentration	ug/L	Talazoparib	Plasma	1.00	24.12	4.83	3.00
Concentration	ug/L	Talazoparib	Plasma	2.00	11.25	2.52	3.00
Concentration	ug/L	Talazoparib	Plasma	6.00	9.62	3.72	3.00
Concentration	ug/L	Talazoparib	Plasma	24.00	0.18	0.02	3.00
Concentration	ug/L	Talazoparib	Tumor	0.50	17.32	4.77	3.00
Concentration	ug/L	Talazoparib	Tumor	1.00	20.53	5.49	3.00
Concentration	ug/L	Talazoparib	Tumor	2.00	14.27	1.38	3.00
Concentration	ug/L	Talazoparib	Tumor	6.00	13.05	6.37	3.00
Concentration	ug/L	Talazoparib	Tumor	24.00	2.56	1.81	3.00

5.0 ATTACHED FILES

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