1.0 METHODS

In Vivo Pharmacokinetic (PK) Study

The plasma pharmacokinetic (PK) profile of the PARP inhibitor olaparib was evaluate in Female CD-1 nude mice (The Jackson Laboratories) approximately 12 weeks in age, bearing Ewing sarcoma EW-8 orthotopic xenografts. Olaparib free base (LC Laboratories, Lot # PAR-105, Purity 99%) was dissolved in 10% DMSO /10% hydroxypropyl beta cyclodextrin (HPBCD) / 80% PBS, at a concentration of 5 mg/mL as a 10 mL/kg oral gavage, for a 50 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 [illegible] tumors were then extracted, rinsed with PBS as necessary, and then placed in appropriate [illegible] centrifuge tubes in a cooler on dry ice. Tissue samples were then transferred to a -80 [illegible] possible.

1.2 Bioanalysis

Frozen tumor samples were weighed in [illegible] Matrix D (MP Biomedical, Santa Ana, CA) tubes and diluted with a 5:1 volume of 5 mM am[illegible]ate pH 7.0 in ultrapure water. The tissue samples were then homogenized with a FastPre[illegible] Biomedicals, Santa Ana, CA). The homogenization consisted of three 6.0 M/S vibratory [illegible] on each on the FastPrep-24 system. To prevent over-heating due to friction, samples were [illegible] ice for 5 min between each cycle. The homogenates were then stored at -80°C until analy[illegible]

Plasma and tumor samples were an[illegible] fo[illegible] LC L[illegible]ries, Lot # PAR-105, Purity 99%) with a qualified liquid chromatography – t[illegible] (LC-MS/MS) assay. Matrix calibrators and quality controls were spiked with so[illegible] content, prepared in methanol. Plasma and tissue samples, 25 µL each, were p[illegible]00 µL of 20 ng/mL erlotinib hydrochloride (LC Laboratories, Lot # BBE-10[illegible]y > 99%) in 0.1% formic acid in [illegible]ol as an internal standard. A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20AD[illegible] high performance liquid chromatography system via a Leap PAL HTS-xt autosampler.

The LC separation was performed using a Phenomenex Synergi Hydro-RP C18 (4.0 µm, 2.0 mm x 30 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.6 mL/min. The binary mobile phase consisted of Water-Methanol-Formic Acid (95:10:0.1) in reservoir A and Methanol-Formic Acid (100:0.1) in reservoir B. The initial mobile phase consisted of 40% B and was followed by a linear increase to 75% B in 1.5 min. The column was then rinsed for 2 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 5.5 min. Under these conditions, the analyte and IS eluted at 0.88 and 0.64 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: olaparib 435.18 → 281.10, and erlotinib 394.17 → 336.05.

The method qualification and bioanalytical runs all passed P-PKSR's 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 ≥ 0.999. 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 ≤ 6.4% CV and 93.8% to 104% respectively.

1.3 Pharmacokinetic (PK) Analysis

Olaparib 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

Due to low tumor engraftment rates, a smaller number of mice than planned were studied, with only 2 mice per time point through 16 hours. The plasma and tumor Ct data demonstrated moderate-to-high variability between and within mice, with coefficients of variation ranging from 10.3% to 84.8%.

The absorption rate of olaparib was rapid, with the plasma Tmax occurring at 1 hour post-dose. After Cmax, plasma concentrations diminished in an apparent biphasic manner, with a notable number of BLOQ observations starting at 16 hours post-dose. The apparent terminal half-life of olaparib in plasma was 1.58 hours, which may be biased due to the BLOQ data.

The apparent plasma clearance (CL/F) of olaparib was very high at 208 mL/min/kg, or approximately 2.31-fold higher than murine hepatic blood flow. The apparent terminal volume of distribution (Vz/F) for olaparib in plasma was also high at 28.6 L/kg, in excess of total body water. The oral bioavailability of olaparib was unknown in the current study, but has been previously reported to be 55-60% in mice and highly variable due to low solubility [1].

Tumor penetration of olaparib was modest, with a Kp,inf of 0.782. The tumor terminal half-life of olaparib appeared longer than plasma, suggesting a high affinity of olaparib for the tumor tissue.

In clinical studies, the total plasma AUC of olaparib at steady state was reported as 20500 hr-ng/mL with the dose of 100 mg PO BID [2]. The fraction unbound in plasma (Fu,p) for olaparib has been estimated as 0.112 and 0.296 in humans and mice, respectively [3]. Therefore, the precise CRD for mice calculated by unbound AUCs is olaparib 100 mg/kg PO BID. Considering the literature and tolerability of olaparib in mice, a dose of 50 mg/kg PO BID was suggested and is within 2-fold of the precise CRD.

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

3.0 REFERENCES

1. EMA. Lynparza (olaparib) : EPAR - Public assessment report [Internet]. 2015 [cited 2020 Apr 24]. Available from: https://www.ema.europa.eu/documents/assessment-report/lynparza-epar-public-assessment-report_en.pdf

2. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JHM, de Bono JS. Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers. N Engl J Med. 2009;361(2):123–34.

3. FDA. Drug Approval Package: Lynparza (olaparib) NDA # 206162 Pharmacology Review [Internet]. Drugs@FDA. 2014 [cited 2020 Apr 24]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/206162Orig1s000PharmR.pdf

4. Stewart E, Goshorn R, Bradley C, Griffiths LM, Benavente C, Twarog NR, Miller GM, Caufield W, Freeman BB, Bahrami A, Pappo A, Wu J, Loh A, Karlström Å, Calabrese C, Gordon B, Tsurkan L, Hatfield MJ, Potter PM, Snyder SE, Thiagarajan S, Shirinifard A, Sablauer A, Shelat AA, Dyer MA. Targeting the DNA Repair Pathway in Ewing Sarcoma. Cell Rep. 2014 Nov 6;9(3):829–40.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

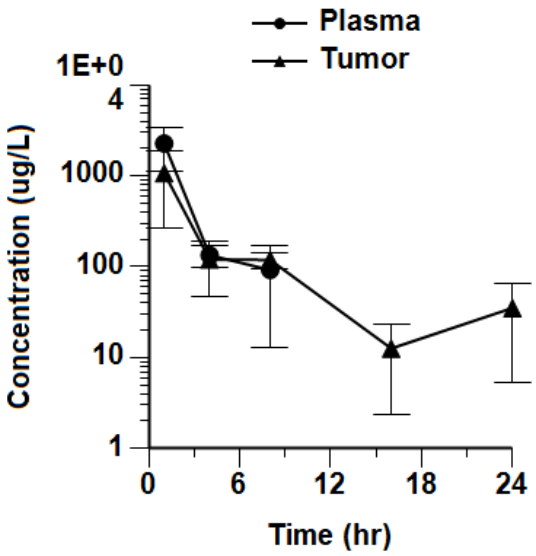

Table 4.1: NCA PK Parameter Estimates of Olaparib by Group

		Analyte	
		Olaparib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	2280	1080
Tmax	hr	1.00	1.00
AUClast	hr*ug/L	3860	2900
AUCinf	hr*ug/L	3990	3120
Kel	1/hr	0.438	0.0858
T1/2	hr	1.58	8.08
CL/F	L/hr/kg	12.5	16.0
Vz/F	L/kg	28.6	187
Clast	ug/L	92.3	35.2
Tlast	hr	8.00	24.0
Kp,inf		-	0.782

		Analyte	
		Olaparib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Kp,last		-	0.751

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

		Analyte	
		Olaparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
1.000	N	2	2
	Mean	2280	1080
	SD	1140	815
	Min	1470	500
	Median	2280	1080
	Max	3080	1650
	CV%	50.1	75.7
	Geometric Mean	2130	909
	CV% Geometric Mean	56.2	102
4.000	N	2	2
	Mean	134	120
	SD	35.8	73.4
	Min	109	68.5
	Median	134	120
	Max	160	172
	CV%	26.7	60.9
	Geometric Mean	132	109
	CV% Geometric Mean	27.5	72.8
8.000	N	2	2
	Mean	92.3	119
	SD	79.5	23.5
	Min	36.1	102
	Median	92.3	119
	Max	149	136
	CV%	86.1	19.8
	Geometric Mean	73.2	118
	CV% Geometric Mean	131	20.1
16.000	N	0	2
	Mean	BLOQ	12.6
	SD		10.2
	Min		5.37

		Analyte	
		Olaparib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	Median		12.6
	Max		19.9
	CV%		81.2
	Geometric Mean		10.3
	CV% Geometric Mean		116
24.000	N	0	3
	Mean	BLOQ	35.2
	SD		29.8
	Min		17.4
	Median		18.5
	Max		69.6
	CV%		84.8
	Geometric Mean		28.2
	CV% Geometric Mean		92.0

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Olaparib	Plasma	24.00	BLOQ
M1	Olaparib	Tumor	24.00	69.61
M2	Olaparib	Plasma	24.00	BLOQ
M2	Olaparib	Tumor	24.00	17.43
M3	Olaparib	Plasma	24.00	BLOQ
M3	Olaparib	Tumor	24.00	18.49
M4	Olaparib	Plasma	16.00	BLOQ
M4	Olaparib	Tumor	16.00	19.86
M5	Olaparib	Plasma	16.00	BLOQ
M5	Olaparib	Tumor	16.00	5.37
M6	Olaparib	Plasma	8.00	148.52
M6	Olaparib	Tumor	8.00	135.62
M7	Olaparib	Plasma	8.00	36.12
M7	Olaparib	Tumor	8.00	102.31
M8	Olaparib	Plasma	4.00	108.93
M8	Olaparib	Tumor	4.00	172.27
M9	Olaparib	Plasma	4.00	159.55
M9	Olaparib	Tumor	4.00	68.53
M10	Olaparib	Plasma	1.00	3081.38
M10	Olaparib	Tumor	1.00	1652.97

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M11	Olaparib	Plasma	1.00	1469.18
M11	Olaparib	Tumor	1.00	500.21

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Olaparib	Plasma	1.00	2275.28	1140.00	2.00
Concentration	ug/L	Olaparib	Plasma	4.00	134.24	35.79	2.00
Concentration	ug/L	Olaparib	Plasma	8.00	92.32	79.48	2.00
Concentration	ug/L	Olaparib	Plasma	16.00	BLOQ		0.00
Concentration	ug/L	Olaparib	Plasma	24.00	BLOQ		0.00
Concentration	ug/L	Olaparib	Tumor	1.00	1076.59	815.12	2.00
Concentration	ug/L	Olaparib	Tumor	4.00	120.40	73.35	2.00
Concentration	ug/L	Olaparib	Tumor	8.00	118.97	23.55	2.00
Concentration	ug/L	Olaparib	Tumor	16.00	12.62	10.25	2.00
Concentration	ug/L	Olaparib	Tumor	24.00	35.17	29.82	3.00

5.0 ATTACHED FILES

Attached File 5.1 Olaparib Plasma Tumor PK Study– *Final in vivo study plan*
Attached File 5.2 Olaparib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*