PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 18951-86719

STUDY TITLE:

PLASMA, TUMOR, AND TUMOR EXTRACELLULAR FLUID PHARMACOKINETICS OF BUPARLISIB (BKM120) IN MICE BEARING NEUROBLASTOMA (MAST 3) ORTHOTOPIC XENOGRAFTS

SHORT TITLE:	**Buparlisib (BKM120) Plasma Tumor ECF PK**
TEST ARTICLE:	Buparlisib (BKM120)
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) Studies

Pharmacokinetic (PK) studies were performed following single oral doses of buparlisib in both non-tumor bearing NOD SCID mice and CD1 nu/nu mice bearing MAST 3 neuroblastoma orthotopic xenografts. Additional details regarding in vivo PK studies are presented in **Table 1.1.** Briefly, plasma samples were obtained at various times up to 24 hours after dosing, with 1 to 3 samples acquired per mouse via retro-orbital bleeds with heparinized pipettes. Tumor bearing mice were sacrificed at selected post-dose time points, with paired plasma and tumor samples. Tumors were harvested, extracted, and rinsed with PBS. Tumor extracellular fluid (ECF) was sampled for compound concentrations in separate groups of tumor bearing mice using microdialysis. Microdialysis probes (BASi; 1 mm membrane) were introduced into tumors through cannulae inserted during tumor cell implantation. The probes were allowed to equilibrate prior to dosing, and recovery was estimated for each probe using retrodialysis techniques. The dialysate solution consisted of Lactated Ringers equivalent with 10% hydroxypropyl-β-cyclodextrin to improve recovery of the hydrophobic compounds. Dialysate fractions were collected periodically for up to 14 hours after dosing. At the end of collection, plasma, tumor, and dialysate samples were immediately placed on dry ice and stored at -80°C until analysis.

Table 1.1 Summary of Buparlisib PK Studies in Mice

PK Study Name	Mouse Strain	Dose/Formulation	Matrix	Sample Times
Plasma #1	NOD SCID	30 mg/kg in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma	3 mice; 0.25, 2, 8 hr 3 mice; 0.5, 4, 12 hr 3 mice; 1, 6, 24 hr 3 mice; 0.75, 10, 24 hr 4 mice; 6, 24 hr
Tumor	CD1 nu/nu (MAST3)	40 mg/kg in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma, Tumor	18 mice; 0.083, 0.25, 1.25, 8, 16, 24 hr
Microdialysis – ECF	CD1 nu/nu (MAST3)	40 mg/kg in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma, ECF	3 mice; 0.083, 2.5, 16 hr & every 1 hr for 10 hr

1.2 Bioanalysis

Compound concentrations in mouse plasma, tumor, and dialysate samples were assessed using a sensitive and specific LC-MS/MS assay. The method used NVP-BAG956 as the internal standard, and demonstrated a linear response over the range of 1 to 500 ng/mL (R=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 ≤ 5.31% CV and 93.6% to 98.5%, respectively Samples were prepared as follows: tumor was homogenized after dilution with purified water using a Fast-Prep 24 bead homogenizer system following the methods of Liang [1]. Plasma and tumor homogenates were protein precipitated with methanol and injected onto the LC-MS/MS system. Dialysate samples underwent a liquid-liquid extraction procedure using MTBE, were dried under vacuum and heat, and reconstituted with mobile phase for injection.

1.3 Pharmacokinetic (PK) Analysis

Resultant concentration-time (Ct) data for the compound were analyzed using a non-linear mixed effects population approach as implemented in ADAPT 5 using the MLEM algorithm [2]. A variety of models, parameterized using either inter-compartmental rate constants or clearances, were tested and assessed for goodness of fit using the -2 log likelihood value, visual predictive checks, plots of model individual and population predicted vs. observed data, and residual plots. A log-normal inter-individual parameter distribution was assumed, with only diagonal elements of parameter covariance matrices estimated. Additive residual error was fixed to the lower limit of quantitation (LLOQ) value of the corresponding

compound assay, while proportional residual error was either estimated or fixed to the assay's observed precision. Beal's M3 method was used to handle data that were below the LLOQ [3].

Secondary PK parameters were derived from the population and post-hoc individual subject model parameter estimates using standard formulae [4] and included the following: Apparent oral plasma clearance (CL/F, calculated as K_e x V_c/F), area under the plasma concentration-time curve (AUC_p, calculated as Dose*F/CL), and the unbound plasma AUC ($AUC_{u,p}$, calculated as AUC_p*F_u). The plasma F_u ratio values for buparlisib was obtained from literature [5]. To assess compound distribution into the MAST 3 neuroblastoma orthotopic xenografts, a tumor or ECF to unbound plasma partition coefficient ($K_{P,tumor}$, $K_{P,ECF}$) was estimated as either a primary model parameter or was calculated as the tumor or ECF AUC:$AUC_{u,p}$ ratio. Additionally, Monte Carlo simulations (n=1000 individual mice) with the model parameter estimates were used to generate 90% prediction intervals (90% PIs) for each compound's parameters.

The resultant PK data and estimates were then used to synthesize clinically reasonable dosing regimens for mouse efficacy studies. A clinically relevant dose (CRD) was calculated as the oral dose of compound achieving the same calculated $AUC_{u,p}$ estimated at the single agent human recommended Phase 2 dose (RP2D) or the maximally tolerated dose (MTD) reported in the literature. Median plasma, tumor, and tumor ECF Ct profiles were also simulated for each compound using the dosing regimens applied in the preclinical mouse efficacy studies. Then, these median Ct profiles were graphically compared with 72-hour median effective concentration (EC_{50}) estimate for compound activity in CellTiter Glo (CTG) ATP assays as a surrogate for in vitro and in vivo cytotoxic pharmacodynamic effect for the MAST 3 line.

2.0 RESULTS

Multi-compartmental models were simultaneously fit to each matrix for buparlisib. The plasma data were adequately described using a linear one-compartment model with first order oral absorption. Tumor and ECF models were driven by unbound plasma concentrations in a manner similar to a forcing function [6]. Total tumor concentrations were well described using an apparent perfusion-limited component, whereas a perfusion-limited model best described buparlisib extracellular fluid (ECF) concentrations. **Figure 2.1** describes the final structural model and the parameter estimates and precision are presented in **Table 2.1**, along with parameter abbreviation descriptions.

Figure 2.1 Buparlisib PK Model for Plasma, Tumor, and Tumor ECF in Mice

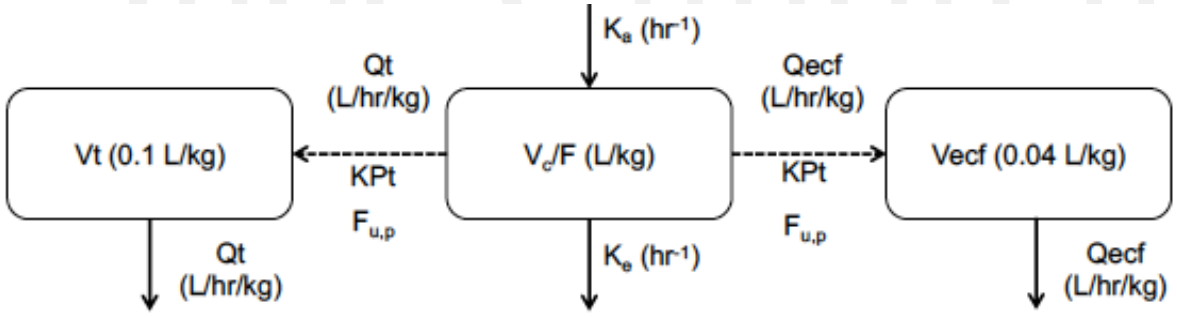

Table 2.1 Buparlisib PK Model Parameter Estimates

Matrix	Parameter	Units	Estimate	%RSE	IIV (%CV)	%RSE
Plasma	K_e	hr^{-1}	0.264	12.9	31.7	33.3
	V_c/F	L/kg	2.87	39.2	79.4	34.9
	K_a	hr^{-1}	5	FIXED		
	$F_{u,p}$	-	0.05	FIXED		
	σ add	µg/L	1	FIXED		
	σ prop	%	47.3	39.9		
Tumor	Qt	L/hr/kg	0.341	36.5	45.2	85
	KPt	-	2.08	16.2	20.1	116
	Vt	L/kg	0.1	FIXED		
	σ add	µg/L	1	FIXED		
	σ prop	%	4.35	434		
ECF	Qecf	L/hr/kg	6.23E-03	61.8	33.9	194
	KPecf	-	1.91	146	63.9	191
	Vecf	L/kg	4.00E-02	FIXED		
	σ add	µg/L	1	FIXED		
	σ prop	%	26.4	71.5		

Abbreviations – %RSE, percent relative standard error; IIV, inter-individual variability; K_e, elimination rate constant; V_c/F, apparent oral volume of distribution of central compartment; K_a, oral absorption rate constant; $F_{u,p}$, fraction unbound in plasma; Qt, tumor apparent perfusional flow; KPt, tumor partition coefficient; Vt, tumor volume (assuming specific density of 1.0); VMAXecf, maximum velocity of elimination in extracellular fluid; KMecf, concentration of half-maximal velocity of elimination in extracellular fluid; QDecf, extracellular fluid apparent distributional flow; QEecf, extracellular fluid clearance; Vecf, estimated volume of the extracellular fluid; Qecf, extracellular fluid apparent perfusional flow; KPecf, extracellular fluid partition coefficient; σ add, additive residual error as a standard deviation; σ prop, proportional residual error as a percentage.

Buparlisib plasma PK parameters were well estimated as indicated by the small to modest %RSE values, all being < 50%. Tumor and ECF parameters were not as precise, most likely due to the limited data and inter-animal variability. Despite this relative imprecision, the models demonstrated adequate goodness of fit upon visual inspection with some bias (data not shown). The joint model tended to underpredict total tumor homogenate values at later time points, and globally underpredicted ECF concentrations. See **Section 4.0** for figures. However, the modeled partition coefficients compared well with noncompartmental methods. The PK parameters displayed appreciable inter-animal and residual variability, likely resulting from variances in gavage administration between studies, husbandry conditions (i.e. ad libitum food), and model misspecification. However, such PK variability can be anticipated in any longer-term preclinical efficacy or clinical studies.

Our plasma PK results compare well with the limited published mouse PK for buparlisib. Using buparlisib mouse IV clearance and oral bioavailability values reported by Burger et al. [5] and assuming linear PK, we calculated a plasma AUC of 48500 hr-ng/mL for a 40 mg/kg PO buparlisib dosage – this agrees with our experimentally simulated median AUC of 52284 hr-ng/mL (90% PI: 11800 – 213359).

We derived a clinically relevant dose (CRD) for buparlisib based upon the Monte Carlo simulated $AUC_{u,p}$ values in mice and the observed clinical $AUC_{u,p}$ at the single agent MTD or RP2D from literature. At the human RP2D for buparlisib of 100 mg PO QD, the unbound plasma AUC was estimated to be 3330 hr-ng/L [7]. Assuming linear PK across species and with dose, and similar plasma protein binding, a dosage of 17 mg/kg PO QD in mice would be equivalent. However, buparlisib tumor and tumor ECF concentration predictions failed to meet the target in vitro EC_{50} values even at the 40 mg/kg dose level in mice (**Attached File 5.1**).

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean Population Predicted Plasma Ct Profile of Buparlisib 40 mg/kg PO in Mice

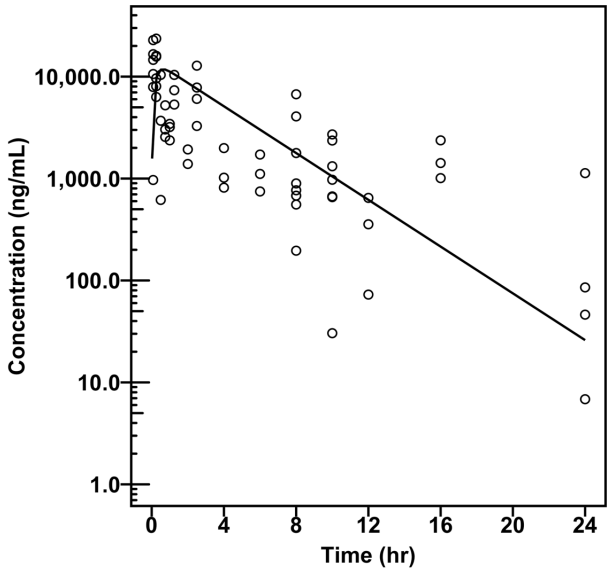

Figure 4.2: Mean Population Predicted Tumor Homogenate Ct Profile of Buparlisib 40 mg/kg PO in Mice

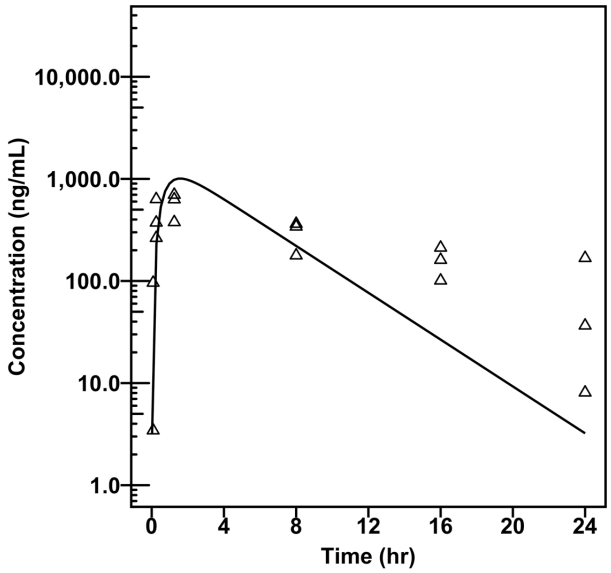

Figure 4.3: Mean Population Predicted Tumor ECF Ct Profile of Buparlisib 40 mg/kg PO in Mice

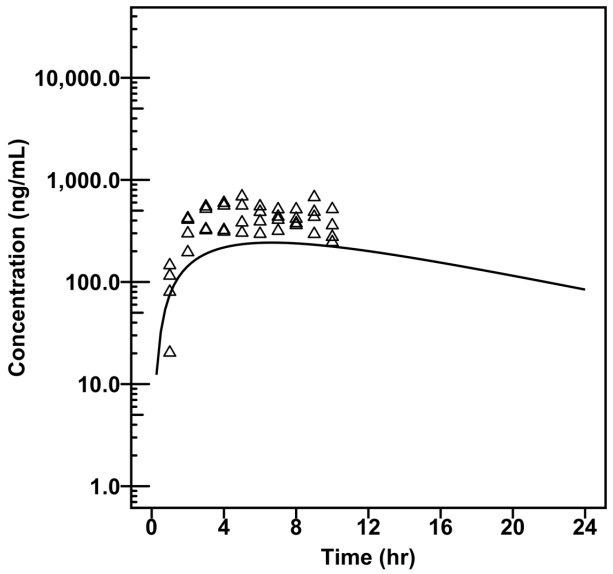

Table 4.1: Listing of Observed Plasma, Tumor Homogenate, and Tumor Ct Data for Buparlisib PK Modeling

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
BKM_pPK_1151	0.25	8050			Plasma #1	30
BKM_pPK_1151	2	1390			Plasma #1	30
BKM_pPK_1151	8	556			Plasma #1	30
BKM_pPK_1152	0.25	9620			Plasma #1	30
BKM_pPK_1152	8	679			Plasma #1	30
BKM_pPK_1153	0.25	6310			Plasma #1	30
BKM_pPK_1153	2	1930			Plasma #1	30
BKM_pPK_1153	8	767			Plasma #1	30
BKM_pPK_1154	0.5	10400			Plasma #1	30
BKM_pPK_1154	4	1020			Plasma #1	30
BKM_pPK_1154	12	356			Plasma #1	30
BKM_pPK_1155	0.5	3690			Plasma #1	30
BKM_pPK_1155	4	1990			Plasma #1	30
BKM_pPK_1155	12	646			Plasma #1	30
BKM_pPK_1156	0.5	617			Plasma #1	30
BKM_pPK_1156	4	814			Plasma #1	30
BKM_pPK_1156	12	72.7			Plasma #1	30

Buparlisib (BKM120) Plasma Tumor ECF PK

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
BKM_pPK_1157	1	3200			Plasma #1	30
BKM_pPK_1157	6	1720			Plasma #1	30
BKM_pPK_1157	24	6.84			Plasma #1	30
BKM_pPK_1158	1	2370			Plasma #1	30
BKM_pPK_1158	6	747			Plasma #1	30
BKM_pPK_1158	24	BLOQ			Plasma #1	30
BKM_pPK_1159	1	3440			Plasma #1	30
BKM_pPK_1159	6	1110			Plasma #1	30
BKM_pPK_1159	24	BLOQ			Plasma #1	30
BKM_pPK_1160	0.75	3030			Plasma #1	30
BKM_pPK_1160	10	668			Plasma #1	30
BKM_pPK_1160	24	BLOQ			Plasma #1	30
BKM_pPK_1161	0.75	2560			Plasma #1	30
BKM_pPK_1161	10	30.4			Plasma #1	30
BKM_pPK_1161	24	BLOQ			Plasma #1	30
BKM_pPK_1162	0.75	5230			Plasma #1	30
BKM_pPK_1162	10	973			Plasma #1	30
BKM_pPK_1162	24	BLOQ			Plasma #1	30
BKM_pPK_1163	8	196			Plasma #1	30
BKM_pPK_1163	24	BLOQ			Plasma #1	30
BKM_pPK_1164	8	896			Plasma #1	30
BKM_pPK_1164	24	BLOQ			Plasma #1	30
BKM_TH_1	0.08	968	3.44		Tumor	40
BKM_TH_10	8	4070	363		Tumor	40
BKM_TH_11	8	1780	178		Tumor	40
BKM_TH_12	16	1420	161		Tumor	40
BKM_TH_13	16	1010	101		Tumor	40
BKM_TH_14	16	2370	212		Tumor	40
BKM_TH_15	24	46.2	8.08		Tumor	40
BKM_TH_16	24	1130	168		Tumor	40
BKM_TH_17	24	85.5	36.6		Tumor	40
BKM_TH_2	0.08	7910	96.1		Tumor	40
BKM_TH_3	0.25	16000	633		Tumor	40
BKM_TH_4	0.25	23600	373		Tumor	40
BKM_TH_5	0.25	15700	264		Tumor	40

Buparlisib (BKM120) Plasma Tumor ECF PK

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
BKM_TH_6	1.25	5320	377		Tumor	40
BKM_TH_7	1.25	10400	699		Tumor	40
BKM_TH_8	1.25	7350	630		Tumor	40
BKM_TH_9	8	6710	341		Tumor	40
BKM_uD_5015-1	0.083	22800			Microdialysis - ECF	40
BKM_uD_5015-1	1			145.908	Microdialysis - ECF	40
BKM_uD_5015-1	2			407.186	Microdialysis - ECF	40
BKM_uD_5015-1	2.5	12800			Microdialysis - ECF	40
BKM_uD_5015-1	3			548.902	Microdialysis - ECF	40
BKM_uD_5015-1	4			594.81	Microdialysis - ECF	40
BKM_uD_5015-1	5			688.623	Microdialysis - ECF	40
BKM_uD_5015-1	6			550.898	Microdialysis - ECF	40
BKM_uD_5015-1	7			514.97	Microdialysis - ECF	40
BKM_uD_5015-1	8			514.97	Microdialysis - ECF	40
BKM_uD_5015-1	9			487.026	Microdialysis - ECF	40
BKM_uD_5015-1	10	2710		245.509	Microdialysis - ECF	40
BKM_uD_5015-2	0.083	14600			Microdialysis - ECF	40
BKM_uD_5015-2	1			115.19	Microdialysis - ECF	40
BKM_uD_5015-2	2			299.051	Microdialysis - ECF	40
BKM_uD_5015-2	2.5	6050			Microdialysis - ECF	40
BKM_uD_5015-2	3			321.203	Microdialysis - ECF	40
BKM_uD_5015-2	4			322.785	Microdialysis - ECF	40
BKM_uD_5015-2	5			303.797	Microdialysis - ECF	40
BKM_uD_5015-2	6			295.886	Microdialysis - ECF	40
BKM_uD_5015-2	7			316.456	Microdialysis - ECF	40
BKM_uD_5015-2	8			360.759	Microdialysis - ECF	40
BKM_uD_5015-2	9			677.215	Microdialysis - ECF	40
BKM_uD_5015-2	10			359.177	Microdialysis - ECF	40
BKM_uD_5015-3	0.083	10600			Microdialysis - ECF	40
BKM_uD_5015-3	1			20.3177	Microdialysis - ECF	40
BKM_uD_5015-3	2			195.569	Microdialysis - ECF	40
BKM_uD_5015-3	2.5	3280			Microdialysis - ECF	40
BKM_uD_5015-3	3			328.933	Microdialysis - ECF	40
BKM_uD_5015-3	4			311.032	Microdialysis - ECF	40
BKM_uD_5015-3	5			382.636	Microdialysis - ECF	40

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
BKM_uD_5015-3	6			391.586	Microdialysis - ECF	40
BKM_uD_5015-3	7			436.339	Microdialysis - ECF	40
BKM_uD_5015-3	8			416.2	Microdialysis - ECF	40
BKM_uD_5015-3	9			434.102	Microdialysis - ECF	40
BKM_uD_5015-3	10	657		516.894	Microdialysis - ECF	40
BKM_uD_5015-4	0.083	16600			Microdialysis - ECF	40
BKM_uD_5015-4	1			80.1766	Microdialysis - ECF	40
BKM_uD_5015-4	2			420.637	Microdialysis - ECF	40
BKM_uD_5015-4	2.5	7810			Microdialysis - ECF	40
BKM_uD_5015-4	3			520.567	Microdialysis - ECF	40
BKM_uD_5015-4	4			560.074	Microdialysis - ECF	40
BKM_uD_5015-4	5			560.074	Microdialysis - ECF	40
BKM_uD_5015-4	6			485.708	Microdialysis - ECF	40
BKM_uD_5015-4	7			406.693	Microdialysis - ECF	40
BKM_uD_5015-4	8			376.482	Microdialysis - ECF	40
BKM_uD_5015-4	9			295.143	Microdialysis - ECF	40
BKM_uD_5015-4	10	1320		276.551	Microdialysis - ECF	40

5.0 ATTACHED FILES

Attached File 5.1 buparlisib_ct_sim.pdf – *Day 15 population mean predicted Ct profiles in plasma, tumor, and tumor ECF for buparlisib (BKM120) 40 mg/kg PO QD in mice.*

Attached File 5.2 Buparlisib BKM120 CtData Listing.xlsx – *Table 4.1 in Excel file format. Listing of observed buparlisib concentrations in plasma, tumor homogenate, and tumor ECF used for modeling*

Attached File 5.3 Buparlisib (BKM120) Plasma Tumor ECF PK ADAPT5 Files.zip – *ADAPT5 modeling files and results used for analysis and reporting*