PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 18423-83442

STUDY TITLE:

PLASMA, TUMOR, AND TUMOR EXTRACELLULAR FLUID PHARMACOKINETICS OF DACTOLISIB (BEZ235) IN MICE BEARING NEUROBLASTOMA (MAST 3) ORTHOTOPIC XENOGRAFTS

SHORT TITLE:	**Dactolisib (BE235) Plasma Tumor ECF PK**
TEST ARTICLE:	Dactolisib (BEZ235)
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 dactolisib 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 Dactolisib PK Studies in Mice

PK Study Name	Mouse Strain	Dose/Formulation	Matrix	Sample Times
Plasma #1	NOD SCID	50 mg/kg PO in 0.5% MC (400 cPs)	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
Plasma #2	NOD SCID	50 mg/kg PO in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma	6 mice; 0.083, 1, 5.2 hr 6 mice; 1, 1.4, 22.5 hr
Tumor	CD1 nu/nu (MAST3)	50 mg/kg PO in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma, Tumor	15 mice; 0.083, 0.75, 3.5, 7.5, 18 hr
Microdialysis – ECF	CD1 nu/nu (MAST3)	50 mg/kg PO in 0.5% MC (400 cPs) & 0.5% Tween 80	Plasma, ECF	3 mice; 0.117, 7.5, 10 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>0.997). 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 ≤ 11.7% CV and 88.0% to 108%, 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 dactolisib 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 dactolisib. The plasma data were adequately described using a linear one-compartment model with first order oral absorption. The tumor model was 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. A vast majority of ECF concentrations for BEZ235 were below the LLOQ, which precluded any model fitting or analysis. The physicochemical characteristics of dactolisib suggest it to be highly non-specifically bound to plasma proteins and tumor tissues, resulting in very low unbound 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 Dactolisib PK Model for Plasma and Tumor in Mice

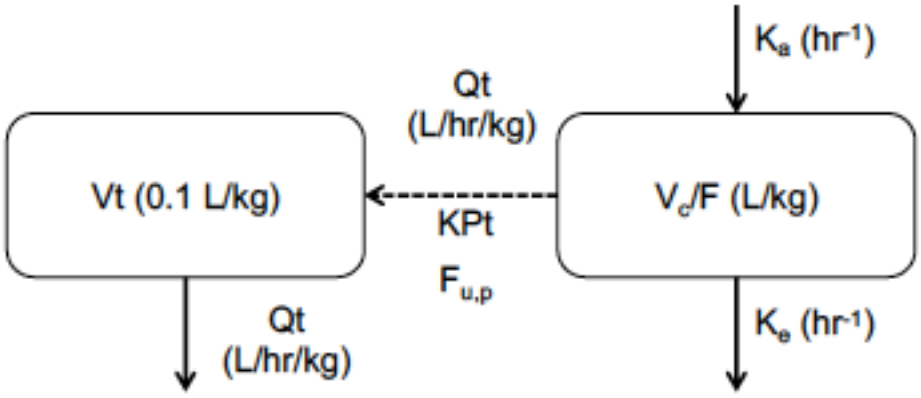

Table 2.1 Dactolisib PK Model Parameter Estimates

Matrix	Parameter	Units	Estimate	%RSE	IIV (%CV)	%RSE
Plasma	K_e	hr^{-1}	0.175	17	45	46
	V_c/F	L/kg	83.4	15.3	47.9	44.8
	K_a	hr^{-1}	1.13	20.7	63.6	44.4
	$F_{u,p}$	-	0.02	FIXED		
	σ add	µg/L	1	FIXED		
	σ prop	%	37.4	22.4		
Tumor	Qt	L/hr/kg	4.07	45.9	65.4	181
	KPt	-	19.2	33.2	69.1	56.9
	Vt	L/kg	0.1	FIXED		
	σ add	µg/L	2.5	FIXED		
	σ prop	%	4.33	193		
ECF	*Insufficient quantifiable data for modeling					

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.

Dactolisib plasma PK parameters were well estimated as indicated by the small to modest %RSE values, all being < 50%. Tumor inter-animal variability parameters were not as precise, most likely due to the limited data. Despite this relative imprecision, the models demonstrated adequate goodness of fit upon visual inspection with little bias (data not shown). See **Section 4.0** for figures. 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 dactolisib, with a difference in AUC of only ~30% noted. Using graphical extrapolation of data presented by Maira et al. [5], we estimated a plasma AUC of 4500 hr-ng/mL for dactolisib at 50 mg/kg PO in mice. Similarly, our PK analysis showed a median dactolisib plasma AUC of 3460 hr-ng/mL (90% PI: 1139 – 9754).

We derived a clinically relevant dose (CRD) for dactolisib 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 MTD for dactolisib of 1600 mg PO QD, the unbound plasma AUC was estimated to be 74.6 hr-ng/L [5]. Assuming linear PK across species and with dose, and similar plasma protein binding, a dosage of 50-70 mg/kg PO QD in mice would be equivalent. The model predicted dactolisib tumor and plasma concentrations exceeded the target in vitro EC_{50} values for nearly an entire dosing interval at the 70 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 Dactolisib 50 mg/kg PO in Mice

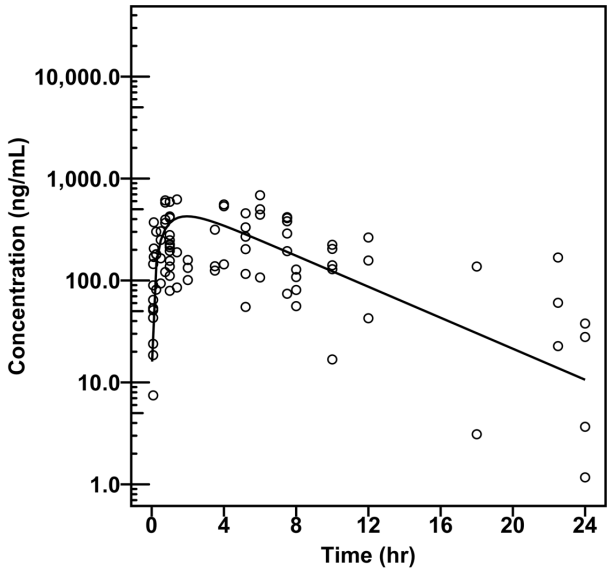

Figure 4.2: Mean Population Predicted Tumor Homogenate Ct Profile of Dactolisib 50 mg/kg PO in Mice

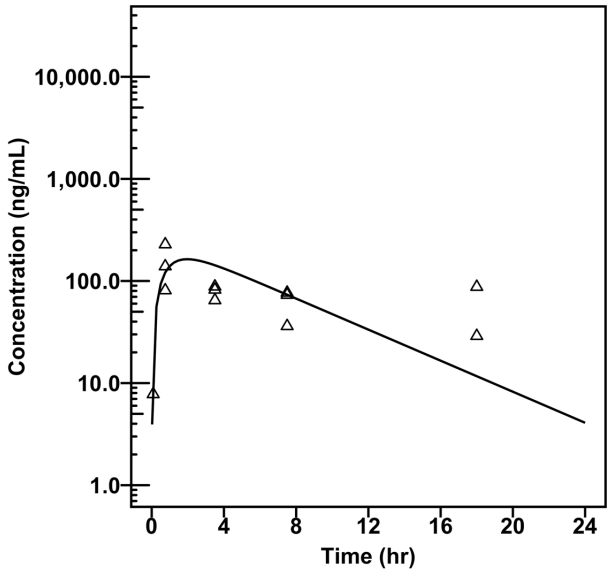

Table 4.1: Listing of Observed Plasma, Tumor Homogenate, and Tumor ECF Ct Data for Dactolisib PK Modeling

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
M10	0.75	611	81.01		Tumor	50
M11	0.75	396	139.22		Tumor	50
M12	0.75	581	228.42		Tumor	50
M13	0.083	89.6	7.79		Tumor	50
M14	0.083	7.46	BLOQ		Tumor	50
M15	0.083	145	BLOQ		Tumor	50
M2	18	3.1	28.96		Tumor	50
m2151	0.25	81.2			Plasma #1	50
m2151	2	133			Plasma #1	50
m2151	8	80.7			Plasma #1	50
m2152	0.25	181			Plasma #1	50
m2152	2	159			Plasma #1	50
m2152	8	56			Plasma #1	50
m2153	0.25	180			Plasma #1	50
m2153	2	101			Plasma #1	50
m2153	8	128			Plasma #1	50
m2154	0.5	250			Plasma #1	50
m2154	4	535			Plasma #1	50
m2154	12	157			Plasma #1	50
m2155	0.5	304			Plasma #1	50
m2155	4	557			Plasma #1	50
m2155	12	264			Plasma #1	50
m2156	0.5	165			Plasma #1	50
m2156	4	144			Plasma #1	50
m2156	12	42.6			Plasma #1	50
m2157	1	212			Plasma #1	50
m2157	6	500			Plasma #1	50
m2157	24	1.17			Plasma #1	50
m2158	1	111			Plasma #1	50
m2158	6	107			Plasma #1	50
m2158	24	BLOQ			Plasma #1	50
m2159	1	425			Plasma #1	50
m2159	6	442			Plasma #1	50

Dactolisib (BEZ235) Plasma Tumor ECF PK

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
m2159	24	27.9			Plasma #1	50
m2160	0.75	367			Plasma #1	50
m2160	10	141			Plasma #1	50
m2160	24	BLOQ			Plasma #1	50
m2161	0.75	121			Plasma #1	50
m2161	10	129			Plasma #1	50
m2161	24	BLOQ			Plasma #1	50
m2162	1	411			Plasma #1	50
m2162	6	686			Plasma #1	50
m2162	24	3.67			Plasma #1	50
m2163	0.5	93.5			Plasma #1	50
m2163	8	108			Plasma #1	50
m2163	24	BLOQ			Plasma #1	50
m2164	0.25	301			Plasma #1	50
m2164	24	37.8			Plasma #1	50
M3	18	137	87.6		Tumor	50
M4	7.5	381	36.16		Tumor	50
M5	7.5	418	76.8		Tumor	50
M54	0.1167	170			Microdialysis - ECF	50
M54	0.5			BLOQ	Microdialysis - ECF	50
M54	1.5			BLOQ	Microdialysis - ECF	50
M54	2.5			BLOQ	Microdialysis - ECF	50
M54	3.5			BLOQ	Microdialysis - ECF	50
M54	4.5			4.05	Microdialysis - ECF	50
M54	5.5			4.36	Microdialysis - ECF	50
M54	6.5			5.85	Microdialysis - ECF	50
M54	7.5	409		9.44	Microdialysis - ECF	50
M54	8.5			5.93	Microdialysis - ECF	50
M54	9.5			6.34	Microdialysis - ECF	50
M54	10	224			Microdialysis - ECF	50
M57	0.1167	372			Microdialysis - ECF	50
M57	0.5			BLOQ	Microdialysis - ECF	50
M57	1.5			BLOQ	Microdialysis - ECF	50
M57	2.5			BLOQ	Microdialysis - ECF	50
M57	3.5			BLOQ	Microdialysis - ECF	50

Dactolisib (BEZ235) Plasma Tumor ECF PK

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
M57	4.5			BLOQ	Microdialysis - ECF	50
M57	5.5			BLOQ	Microdialysis - ECF	50
M57	6.5			BLOQ	Microdialysis - ECF	50
M57	7.5	74.1		BLOQ	Microdialysis - ECF	50
M57	8.5			BLOQ	Microdialysis - ECF	50
M57	9.5			BLOQ	Microdialysis - ECF	50
M57	10	16.8			Microdialysis - ECF	50
M58	0.1167	206			Microdialysis - ECF	50
M58	0.5				Microdialysis - ECF	50
M58	1.5				Microdialysis - ECF	50
M58	2.5				Microdialysis - ECF	50
M58	3.5				Microdialysis - ECF	50
M58	4.5			3.83	Microdialysis - ECF	50
M58	5.5			BLOQ	Microdialysis - ECF	50
M58	6.5			BLOQ	Microdialysis - ECF	50
M58	7.5	194		2.72	Microdialysis - ECF	50
M58	8.5			3.45	Microdialysis - ECF	50
M58	9.5				Microdialysis - ECF	50
M58	10	204			Microdialysis - ECF	50
M6	7.5	289	73.17		Tumor	50
M7	3.5	315	88.19		Tumor	50
M8	3.5	125	64.76		Tumor	50
M9	3.5	138	82.21		Tumor	50
m951	0.08	53.5			Plasma #2	50
m951	1	227			Plasma #2	50
m951	5.2	332			Plasma #2	50
m952	0.08	43			Plasma #2	50
m952	1	195			Plasma #2	50
m952	5.2	116			Plasma #2	50
m953	0.08	64.5			Plasma #2	50
m953	1	222			Plasma #2	50
m953	5.2	456			Plasma #2	50
m954	0.08	51.1			Plasma #2	50
m954	1	156			Plasma #2	50
m954	5.2	55			Plasma #2	50

IndividID	Time (hr)	Plasma (ng/mL)	Tumor Homogenate (ng/mL)	Tumor ECF (ng/mL)	Study	Dosage (mg/kg)
m955	0.08	18.5			Plasma #2	50
m955	1	279			Plasma #2	50
m955	5.2	203			Plasma #2	50
m956	0.08	23.9			Plasma #2	50
m956	1	79.2			Plasma #2	50
m956	5.2	269			Plasma #2	50
m957	1	591			Plasma #2	50
m957	1.4	624			Plasma #2	50
m957	22.5	168			Plasma #2	50
m958	1	137			Plasma #2	50
m958	1.4	85.3			Plasma #2	50
m958	22.5	22.7			Plasma #2	50
m959	1	248			Plasma #2	50
m959	1.4	189			Plasma #2	50
m959	22.5	60.4			Plasma #2	50

5.0 ATTACHED FILES

Attached File 5.1 dactolisib_ct_sim.pdf – *Day 15 population mean predicted Ct profiles in plasma and tumor for dactolisib (BEZ235) 70 mg/kg PO QD in mice.*

Attached File 5.2 Dactolisib BEZ235 CtData Listing.xlsx – *Table 4.1 in Excel file format. Listing of observed dactolisib concentrations in plasma, tumor homogenate, and tumor ECF used for modeling*

Attached File 5.3 Dactolisib (BEZ235) Plasma Tumor ECF PK ADAPT5 Files.zip – *ADAPT5 modeling files and results used for analysis and reporting*