PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 74806-737948

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF ABEMACICLIB IN FEMALE CD1 NU/NU MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Abemaciclib Screening Plasma and Tumor PK (SPTPK)**
TEST ARTICLE:	Abemaciclib mesylate
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	74806-737948 Preclinical Pharmacokinetic Shared Resource
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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 total plasma and tumor PK profiles of abemaciclib in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) bearing MAST 95 rhabdomyosarcoma xenografts in the quadriceps were assessed after a single oral gavage of 50 mg/kg of abemaciclib free base equivalents. Abemaciclib mesylate (LY2835219, Abmole, M2112, Lot 2, purity >98%) was suspended in 1% hydroxyethylcellulose (HEC), 0.25% Tween 80, and ~0.05% simethicone in ultrapure water at a final nominal concentration of 5 mg/mL free base equivalents for a 10 mL/kg gavage volume. Mice were sacrificed using an IACUC-approved method at 10 min, 1, 4, 8, and 24 hr post-dose, with 3 mice per time point. Whole blood by cardiac puncture was collected into Sarstedt Microvette® 500 µl K3 EDTA microcentrifuge tubes, vortexed to mix anticoagulant, immediately centrifuged to plasma, and stored on dry ice for remainder of study. Mice were then perfused with PBS via the aorta, the orthotopic xenografts excised, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

1.2 Bioanalysis

Total plasma and tumor homogenate abemaciclib concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. First, tumor samples were diluted with a 5:1 volume of ultrapure water, and homogenized with a bead-based technique [1] on a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). 1.4 mm ceramic spheres (MP Biomedicals, Lysing matrix D, 10 mg per mg of tumor) were added to the microcentrifuge tubes containing samples. The samples were then subjected to three 60 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.

Abemaciclib mesylate (LY2835219, Abmole, M2112, Lot 2, purity >98%) stock solutions corrected for salt content were prepared in acetonitrile and used to spike matrix calibrators and quality controls. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of 150 ng/mL palbociclib (LC Labs, P-7788, Lot PLH-103, Purity >99%) in acetonitrile 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 CTC PAL autosampler. The LC separation was performed using a Phenomenex Kinetex 2.6 µm EVO C18 (100 Å, 50 x 2.1 mm) maintained at 50 °C with gradient elution at a flow rate of 0.5 mL/min. The binary mobile phase consisted of 20 mM ammonium acetate in H_2O in reservoir A and methanol: acetonitrile: 2-propanol (40:30:30 v/v) in reservoir B. The initial mobile phase was maintained at 25% B for 0.5 minutes followed by a linear increase to 100% B in 2.5 minutes. The column was then rinsed for 2.0 minutes at 100% B and then equilibrated at the initial conditions for two minutes for a total run time of seven minutes. Under these conditions, the analyte and IS eluted at 1.37 and 0.98 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode with monitoring of the following mass transitions: abemaciclib 507.28 -> 393.20, palbociclib 448.25 -> 380.10.

The experimental bioanalytical runs were all found to be acceptable for the purpose of a singlicate non-GLP, preclinical PK assessment. A linear model ($1/X^2$ weighting) fit the calibrators across the 5.00 to 500 ng/mL range, with a correlation coefficient (R) of ≥0.9967. 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 5.00 ng/mL. The intra-run precision and accuracy was < 15.6% CV and 90.7% to 112%, respectively, across the matrices.

1.3 Pharmacokinetic (PK) Analysis

The resultant abemaciclib concentration-time (Ct) data were grouped by matrix and time point, and 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. Then, using Phoenix WinNonlin 6.4 (Certara USA, Inc., Princeton, NJ), Ct data summary statistics (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the abemaciclib arithmetic mean Ct data for each matrix was subjected to noncompartmental pharmacokinetic analysis (NCA). The extravascular model (Model 202) was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" trapezoidal rule. The terminal phase was defined as the three time points at the end of the Ct profile, and the elimination rate constant (Ke) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Ke, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + predicted Clast/Ke.

Other NCA 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 average concentration over a dosing interval (Cavg) was estimated as AUCinf / dosing interval in hours. The apparent partition coefficient of abemaciclib from the plasma to the tissue of interest (Kp,*tissue*) was estimated as the ratio of the AUCinf, tissue to AUCinf plasma when available. To estimate a clinically relevant mouse dosage, the resultant mouse plasma AUCinf was compared with the reported human plasma PK values at the putative single agent abemaciclib maximum tolerated dose (MTD) of 200 mg PO BID [2]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Abemaciclib concentrations showed low variability in the mouse plasma, demonstrating coefficients of variation of 4.10% to 40.1% across the sampling time points, with the highest variability noted at the 0.167 hr time point in plasma and tumor. All plasma and tumor results were above the LLOQ. Tumor penetration appeared to be relatively rapid and extensive, with a Kp,tumor value of 6.20 based on AUCinf. The T1/2 of abemaciclib in the plasma was 5.20 hr with a similar half-life observed in the tumor (5.98 hr). Notably, our abemaciclib plasma PK at 50 mg/kg PO differed from that reported by Tate [3]. Our mice showed a similar plasma Ct profile up to 4 hours post-dose, but then concentrations declined rapidly resulting in a ~3.8-fold lower AUC. Our mice did not exhibit the prolonged absorption and low CL/F as described by these investigators, despite the use of the mesylate salt form and the same suspension formulation.

In a Phase 1 study of single agent abemaciclib administered continuously, the MTD was 200 mg PO BID [2]. The geometric mean human total plasma AUCtau at steady state was 3000 hr-ng/mL, with a corresponding geometric mean trough concentration of 197 ng/mL. Assuming dose proportionality and linear PK processes in mice, a murine equivalent dose (MED) providing a similar total plasma AUC would be 14.4 mg/kg. The mean in vitro fraction unbound in the plasma (Fu,p) was determined for mice and humans, yielding values of 0.054 and 0.027, respectively [4]. As these values are at the two-fold threshold defining an appreciable difference for preclinical studies, it is unclear whether abemaciclib doses should be adjusted for plasma protein binding between the two species. Therefore, the rounded and recommended MED ranges from 7.5 to 15 mg/kg PO BID, based upon unbound and total plasma AUCs respectively. Doses in this range should also provide unbound steady state trough plasma concentrations similar to humans at 200 mg PO BID. More PK studies may be necessary to determine the source of the discrepancy between our plasma PK and that reported by Tate, or to further confirm our PK findings.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

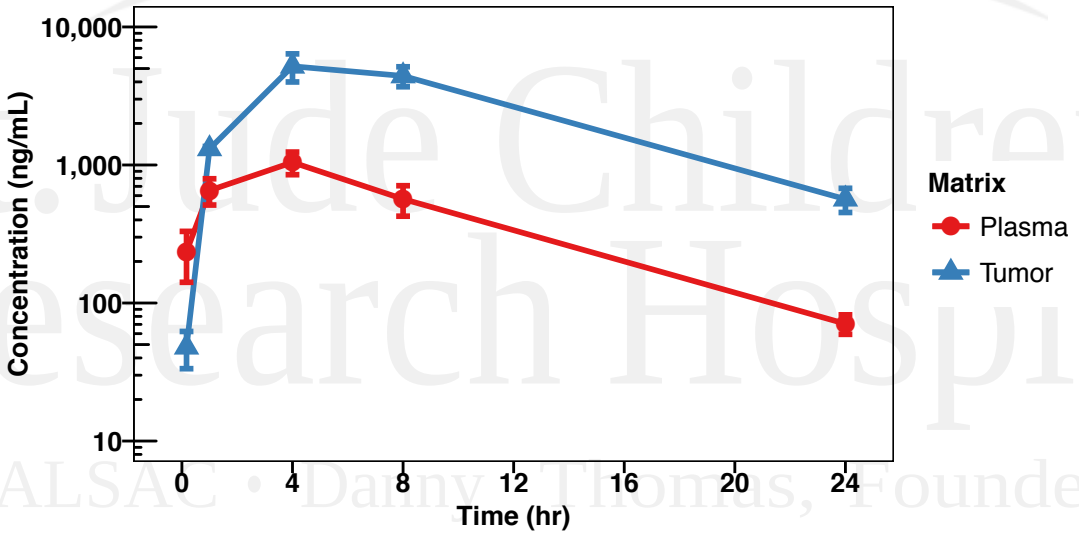

Table 4.1: NCA PK Parameter Estimates of Abemaciclib by Group

		Analyte Abemaciclib Group Plasma	Analyte Abemaciclib Group Tumor
Parameter	**Units**	**Estimate**	**Estimate**
Cmax	ug/L	1050	5190
Tmax	hr	4	4
AUClast	hr*ug/L	9880	59500
AUCinf	hr*ug/L	10400	64500
Kel	1/hr	0.133	0.116
T1/2	hr	5.20	5.98
CL/F	L/hr/kg	4.81	0.775
Vz/F	L/kg	36	6.69
Clast	ug/L	70.7	565
Tlast	hr	24.0	24
Kp,tumor		-	6.20

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

		Analyte Abemaciclib Group Plasma	Analyte Abemaciclib Group Tumor
Time (hr)		**Concentration (ug/L)**	**Concentration (ug/L)**
0.167	N	3	3
	Mean	236	47.8
	SD	94.6	14.4
	Min	132	33.6
	Median	258	47.6
	Max	317	62.3
	CV%	40.1	30.1
	Geometric Mean	221	46.4
	CV% Geometric Mean	48.3	31.8
1	N	3	3
	Mean	654	1309
	SD	141	53.7
	Min	514	1250
	Median	651	1330
	Max	796	1350
	CV%	21.6	4.10
	Geometric Mean	644	1310
	CV% Geometric Mean	22.1	4.15
4	N	3	3

		Analyte Abemaciclib Group Plasma	Analyte Abemaciclib Group Tumor
Time (hr)		Concentration (ug/L)	Concentration (ug/L)
	Mean	1050	5190
	SD	195	1200
	Min	853	4460
	Median	1040	4540
	Max	1240	6570
	CV%	18.7	23.0
	Geometric Mean	1030	5100
	CV% Geometric Mean	19.0	22.1
8	N	3	3
	Mean	567	4420
	SD	141	728
	Min	464	3960
	Median	509	4030
	Max	727	5260
	CV%	24.9	16.5
	Geometric Mean	556	4380
	CV% Geometric Mean	24.1	15.9
24	N	3	3
	Mean	70.7	565
	SD	11.4	112
	Min	59.1	440
	Median	71.2	598
	Max	81.8	658
	CV%	16.1	19.9
	Geometric Mean	70.1	557
	CV% Geometric Mean	16.5	21.2

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Abemaciclib	Plasma	0.167	257.978513
M2	Abemaciclib	Plasma	0.167	317.417275
M3	Abemaciclib	Plasma	0.167	132.241487
M4	Abemaciclib	Plasma	1	796.284951
M5	Abemaciclib	Plasma	1	514.328724
M6	Abemaciclib	Plasma	1	650.658732
M7	Abemaciclib	Plasma	4	853.271513
M8	Abemaciclib	Plasma	4	1243.15888
M9	Abemaciclib	Plasma	4	1040.17243

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M10	Abemaciclib	Plasma	8	508.63762
M11	Abemaciclib	Plasma	8	727.368713
M12	Abemaciclib	Plasma	8	464.126712
M13	Abemaciclib	Plasma	24	71.2167731
M14	Abemaciclib	Plasma	24	59.0715501
M15	Abemaciclib	Plasma	24	81.8372425
M1	Abemaciclib	Tumor	0.167	62.3417364
M2	Abemaciclib	Tumor	0.167	33.5551641
M3	Abemaciclib	Tumor	0.167	47.6051645
M4	Abemaciclib	Tumor	1	1332.73534
M5	Abemaciclib	Tumor	1	1247.74262
M6	Abemaciclib	Tumor	1	1346.9993
M13	Abemaciclib	Tumor	24	657.979516
M14	Abemaciclib	Tumor	24	440.318061
M15	Abemaciclib	Tumor	24	597.647703
M7	Abemaciclib	Tumor	4	4539.23566
M8	Abemaciclib	Tumor	4	4461.2745
M9	Abemaciclib	Tumor	4	6569.56168
M10	Abemaciclib	Tumor	8	3964.34381
M11	Abemaciclib	Tumor	8	5256.99107
M12	Abemaciclib	Tumor	8	4029.21095

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Abemaciclib	Plasma	0.167	235.88	94.55	3.00
Concentration	ug/L	Abemaciclib	Plasma	1	653.76	141.00	3.00
Concentration	ug/L	Abemaciclib	Plasma	4	1045.53	195.00	3.00
Concentration	ug/L	Abemaciclib	Plasma	8	566.71	140.90	3.00
Concentration	ug/L	Abemaciclib	Plasma	24	70.71	11.39	3.00
Concentration	ug/L	Abemaciclib	Tumor	0.167	47.83	14.39	3.00
Concentration	ug/L	Abemaciclib	Tumor	1	1309.16	53.66	3.00
Concentration	ug/L	Abemaciclib	Tumor	4	5190.02	1195.35	3.00
Concentration	ug/L	Abemaciclib	Tumor	8	4416.85	728.31	3.00
Concentration	ug/L	Abemaciclib	Tumor	24	565.32	112.38	3.00

5.0 ATTACHED FILES

Attached File 5.1 Abemaciclib Prelim PK.docx – *Final in vivo study plan*
Attached File 5.2 Abemaciclib Prelim PK_updated.docx – *Submitted in vivo study data collection form (DCF)*
Attached File 5.3 Abemaciclib CtData FINAL 20161031.csv – *Final abemaciclib Ct data listings for plasma and tumor in .csv format*
Attached File 5.4 Abemaciclib CtStats FINAL 20161031.csv – *Final abemaciclib Ct data summary statistics for plasma and tumor in .csv format*

Attached File 5.5 Abemaciclib Supplemtary Materials FINAL 20161031.docx – *Final abemaciclib study summary in manuscript supplementary materials format*