St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

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 and St. Jude Children's Research Hospital, 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, 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.

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 157127-1630753

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF ALISERTIB IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Alisertib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Alisertib (free base)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>
SJCRH SRM2 O/R:	157127-1630753 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA CIVIT
IN VIVO SCIENTIST(S)	Writt, Haley <Haley.Writt@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>
REPORT AUTHOR(S):	Wang, Lindsey Lindsey.Wang@STJUDE.ORG; Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2019-05-29

FOR P-PKSR APPROVED USE AND DISTRIBUTION

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 alisertib as free base was evaluate female Athymic nude mice (Charles River), bearing orthotopic rhabdomyosarcoma xenografts (MAST39, TB-12-1442, SJHBBBD73HK, SJRHB000026_X1) approximately 12 weeks in age. Alisertib (SJ000784293-10, Abmole, M1752, Lot # NA, purity 98.74%) was dissolved in 10% hydroxypropyl beta cyclodextrin (HPBCD, Accela ChemBio) and 1% $NaHCO_3$ in ultrapure water, for a 2 mg/mL free base equivalents dose as a 5 mL/kg oral gavage, for an 10 mg/kg oral dose. Terminal samples were collected over a 16 hour post-dose period by cardiac puncture using a 1 mL syringe, and the blood placed in a Sarstedt Microvette K3EDTA 500 µL tube, and immediately separated to plamsa. The carcass was then perfused with PBS, the tumor extracted, rinsed, and placed in a microcentrifuge tube. All samples were immediately stored on dry ice and transferred to -80 °C until analysis. Remaining dosing solution was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Tumor samples were weighed in 15 mL Lysing matrix D (MP Biomedicals, Santa Ana, CA), diluted with a 1:5 volume of ultrapure water, and homogenized using a FastPrep-24 system (MP Biomedicals, Santa Ana, CA) for five cycles of 1 min vibration at 6.5 M/S speed, with 5 min in ice bath between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor samples were analyzed for alisertib (SJ000784293-10, Abmole, M1752, purity 98.74%) with a qualified LC MS/MS assay. Calibrators and quality controls in plasma were spiked with solutions prepared in methanol. Matrix samples, 25 µL each, were protein precipitated with 100 µL of 0.1% formic acid in acetonitrile and 25 µL of 30 ng/mL CX-5461 (MedChemExpress, HY-13323, purity 98.39%) in methanol as an internal standard. A 5 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu SIL-20AC XR autosampler. The LC separation was performed using a Phenomenex Kinetex C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 40 °C with gradient elution at a flow rate of 0.25 mL/min. The binary mobile phase consisted of 0.1% formic acid in water-acetonitrile (90:10 v/v) in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 10% B for 0.5 min with a linear increase to 80% B in 2 min. The column was then rinsed for 1 min at 80% B and then equilibrated at the initial conditions for 1.5 min for a total run time of 5 min. Under these conditions, the analyte and IS eluted at 3.68 and 2.71 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode and the following mass transitions were monitored: alisertib 519.2 -> 311.2, CX-5461 514.2 -> 391.2. 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 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9966. 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 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 11.11% CV and 85.1% to 112%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Alisertib plasma Ct data were grouped by matrix and nominal time point, and the 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

Alisertib at 10 mg/kg PO showed rapid absorption, with the Tmax occurring at the first measured time point of 0.5 hr. The plasma exposure by dose adjusted AUCinf at 10 mg/kg PO was moderately lower than what was expected from the first study at 80 mg/kg PO, by about 60-70%. Apparent oral clearance was moderate at 1.71 L/hr/kg or 28.5 mL/min/kg, and was within 2-fold of the previous plasma only study at 80 mg/kg PO (1.08 L/hr/kg). The oral apparent terminal volume of distribution was high at 5.81 L/kg, and alisertib demonstrated a short terminal T1/2 of 2.36 hr in the current study. The tumor penetration was moderate, with a Kp,inf of 0.246, with later time points showing slightly higher penetration.

Compared with previously published mouse PK in literature [1], our CL/F value in this tumor bearing study was higher (1.71 vs 0.980 L/hr/kg in [1]), but still within 2-fold. Our mice demonstrated a lower Vz/F value (5.81 vs 9.1 L/kg), with a proportionally shorter terminal half-life (2.36 vs 6.4 hours). Alisertib's PK profile my lack dose proportionality and linearity between 10 mg/kg and 80 mg/kg PO with this formulation in hands. The formulation met specification (1.77 ± 0.0373 mg/mL) and was stable over the usage period of 7 days at room temperature.

The fraction unbound in plasma (Fu,p) of alisertib in mice and humans has been reported as 0.042 and 0.025, respectively by Huck [2]. In-house plasma protein binding using rapid equilibrium dialysis (RED) showed a Fu,p of 0.0201 and 0.0188 respectively for mice and humans. The human value was adequately similar to the previously reported, yet the mouse values exhibited a >2-fold difference. As such, the literature values from Huck were used in derivation of a revised clinically relevant dose (CRD)

Using the mean observed CL/F from our two mouse PK studies (1.40 L/hr/kg), the Fu,p values from Huck, and the AUCtau at the single agent recommended phase 2 dose (RP2D) of 50 mg PO BID for 7 of 21 days [3], a CRD was estimated using the anticipated unbound plasma AUC or Cavg at steady state in mice and humans [4]. A CRD of 10 mg/kg PO BID fo 7 of 21 days in mice is recommended.

3.0 REFERENCES

1. Palani S, Patel M, Huck J, Zhang M, Balani SK, Yang J, Chen S, Mettetal J, Manfredi M, Shyu WC, Ecsedy JA, Chakravarty A. Preclinical pharmacokinetic/pharmacodynamic/efficacy relationships for alisertib, an investigational small-molecule inhibitor of Aurora A kinase. Cancer Chemother Pharmacol. 2013 Dec 1;72(6):1255–64.

2. Huck JJ, Zhang M, Mettetal J, Chakravarty A, Venkatakrishnan K, Zhou X, Kleinfield R, Hyer ML, Kannan K, Shinde V, Dorner A, Manfredi MG, Shyu WC, Ecsedy JA. Translational Exposure–Efficacy Modeling to Optimize the Dose and Schedule of Taxanes Combined with the Investigational Aurora A Kinase Inhibitor MLN8237 (Alisertib). Mol Cancer Ther. 2014 Sep 1;13(9):2170–83.

3. Zhou X, Mould DR, Takubo T, Sheldon-Waniga E, Huebner D, Milton A, Venkatakrishnan K. Global population pharmacokinetics of the investigational Aurora A kinase inhibitor alisertib in cancer patients: rationale for lower dosage in Asia. Br J Clin Pharmacol. 2018;84(1):35–51.

4. Spilker ME, Chen X, Visswanathan R, Vage C, Yamazaki S, Li G, Lucas J, Bradshaw-Pierce EL, Vicini P. Found in Translation: Maximizing the Clinical Relevance of Nonclinical Oncology Studies. Clin Cancer Res. 2017 Feb 15;23(4):1080–90.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

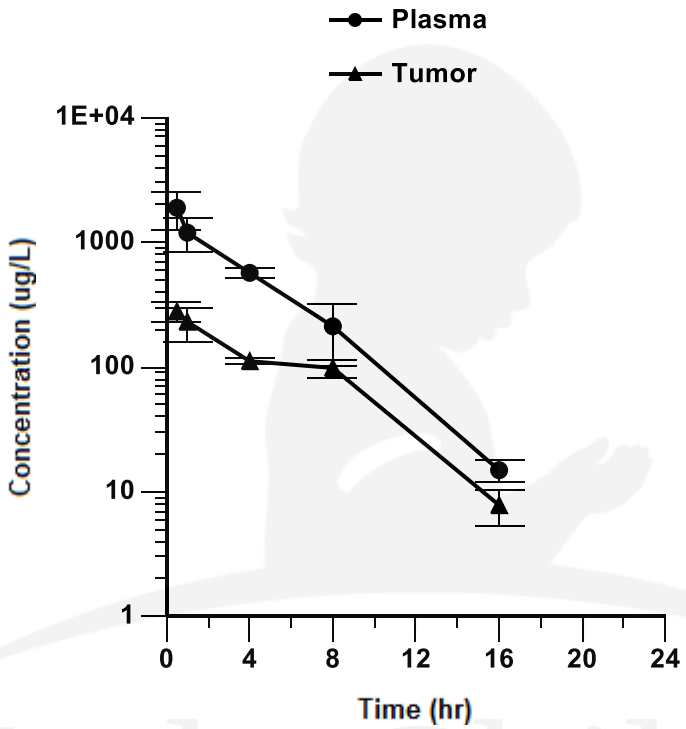

Table 4.1: NCA PK Parameter Estimates of Alisertib by Group

		Analyte	
		Alisertib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	1890	279
Tmax	hr	0.500	0.500
AUClast	hr*ug/L	5800	1390
AUCinf	hr*ug/L	5850	1440
Kel	1/hr	0.294	0.220

		Analyte	
		Alisertib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
T1/2	hr	2.36	3.15
CL/F	L/hr/kg	1.71	6.97
Vz/F	L/kg	5.81	31.6
Clast	ug/L	14.9	7.80
Tlast	hr	16.0	16.0
Kp,inf	-	-	0.246
Kp,last	-	-	0.240

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

		Analyte	
		Alisertib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	1890	279
	SD	656	47.9
	Min	1210	226
	Median	1940	293
	Max	2520	318
	CV%	34.7	17.2
	Geometric Mean	1810	276
	CV% Geometric Mean	38.4	18.1
1.000	N	3	3
	Mean	1190	230
	SD	372	69.1
	Min	797	181
	Median	1250	202
	Max	1540	309

Alisertib Screening Plasma Tumor PK (SPTPK)

		Analyte	
		Alisertib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	CV%	31.2	30.0
	Geometric Mean	1150	224
	CV% Geometric Mean	34.5	29.0
4.000	N	3	3
	Mean	568	111
	SD	58.5	7.15
	Min	528	103
	Median	542	113
	Max	635	117
	CV%	10.3	6.43
	Geometric Mean	566	111
	CV% Geometric Mean	10.1	6.52
8.000	N	3	3
	Mean	212	98.2
	SD	109	17.4
	Min	129	86.3
	Median	172	90.1
	Max	335	118
	CV%	51.4	17.7
	Geometric Mean	195	97.2
	CV% Geometric Mean	52.2	17.2
16.000	N	3	3
	Mean	14.9	7.80
	SD	3.08	2.57
	Min	12.3	6.18
	Median	14.0	6.46
	Max	18.3	10.8
	CV%	20.7	32.9
	Geometric Mean	14.7	7.55
	CV% Geometric Mean	20.4	31.6

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Alisertib	Plasma	0.50	1936.40
M1	Alisertib	Tumor	0.50	318.23
M2	Alisertib	Plasma	0.50	1212.60
M2	Alisertib	Tumor	0.50	225.60
M3	Alisertib	Plasma	0.50	2522.60
M3	Alisertib	Tumor	0.50	292.74
M4	Alisertib	Plasma	1.00	796.76
M4	Alisertib	Tumor	1.00	180.57
M5	Alisertib	Plasma	1.00	1535.60
M5	Alisertib	Tumor	1.00	309.29
M6	Alisertib	Plasma	1.00	1247.10
M6	Alisertib	Tumor	1.00	201.54
M7	Alisertib	Plasma	4.00	635.41
M7	Alisertib	Tumor	4.00	113.14
M8	Alisertib	Plasma	4.00	542.17
M8	Alisertib	Tumor	4.00	117.24
M9	Alisertib	Plasma	4.00	527.72
M9	Alisertib	Tumor	4.00	103.32
M10	Alisertib	Plasma	8.00	128.78
M10	Alisertib	Tumor	8.00	86.33
M11	Alisertib	Plasma	8.00	171.59
M11	Alisertib	Tumor	8.00	90.08
M12	Alisertib	Plasma	8.00	335.20
M12	Alisertib	Tumor	8.00	118.17
M13	Alisertib	Plasma	16.00	14.03
M13	Alisertib	Tumor	16.00	6.18
M14	Alisertib	Plasma	16.00	18.29
M14	Alisertib	Tumor	16.00	6.46
M15	Alisertib	Plasma	16.00	12.31
M15	Alisertib	Tumor	16.00	10.77

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Alisertib	Plasma	0.50	1890.53	656.20	3.00
Concentration	ug/L	Alisertib	Plasma	1.00	1193.15	372.36	3.00
Concentration	ug/L	Alisertib	Plasma	4.00	568.43	58.45	3.00
Concentration	ug/L	Alisertib	Plasma	8.00	211.86	108.94	3.00
Concentration	ug/L	Alisertib	Plasma	16.00	14.88	3.08	3.00
Concentration	ug/L	Alisertib	Tumor	0.50	278.86	47.85	3.00
Concentration	ug/L	Alisertib	Tumor	1.00	230.47	69.06	3.00
Concentration	ug/L	Alisertib	Tumor	4.00	111.23	7.15	3.00
Concentration	ug/L	Alisertib	Tumor	8.00	98.19	17.40	3.00
Concentration	ug/L	Alisertib	Tumor	16.00	7.80	2.57	3.00

5.0 ATTACHED FILES

Attached File 5.1 Alisertib Screening Plasma PK V1.0.docx – *Final in vivo study plan as executed*
Attached File 5.2 157127-1630753_ALI_SPTPK_2019-04-10 FINAL.xlsx – *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 AlisertibTBPK.jpg – *Submitted in vivo study worksheet #1*
Attached File 5.4 Alisertib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*