PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 58190-533791

STUDY TITLE:

SCREENING PLASMA TUMOR PHARMACOKINETICS (SPTPK) OF GEDATOLISIB IN FEMALE CD-1 NUDE MICE BEARING OSTEOSARCOMA 143B ORTHOTOPIC XENOGRAFTS AFTER A SINGLE INTRAVENOUS DOSE

SHORT TITLE:	**Gedatolisib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Gedatolisib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>
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REFERENCE STUDY NUMBERS:	NA CIVIT
IN VIVO SCIENTIST(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>; Woods, Jessica; Ocarz, Monica; Honnell, Victoria <Victoria.Honnell@STJUDE.ORG>; Gordon, Brittney <Brittney.Gordon@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Caufield, William <William.Caufield@STJUDE.ORG>
REPORT AUTHOR(S):	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>; Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
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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 plasma pharmacokinetic (PK) profile of gedatolisib was evaluate in Female CD-1 nude mice (The Jackson Laboratories) bearing osteosarcoma 143B orthotopic xenografts, approximately 12 weeks in age. Gedatolisib free base (MedKoo, Lot # CRB50506, Purity >98%) was dissolved in 5% dextrose for injection (D5W) / 0.3% lactic acid (pH 3.5), at a concentration of 2 mg/mL as a 5 mL/kg intravenous tail vein injection, for a 10 mg/kg intravenous dose. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 16 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 vasculature. Tumors were then extracted, rinsed with PBS as necessary, and then placed in appropriately labeled microcentrifuge tubes in a cooler on dry ice. Tissue samples were then transferred to a -80°C freezer as soon as possible.

1.2 Bioanalysis

Frozen tumor samples were weighed in tared 15 mL Lysing Matrix D (MP Biomedical, Santa Ana, CA) tubes and diluted with a 5:1 volume of ultra pure water. The tissue samples were then homogenized with a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). The homogenization consisted of three 6.0 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.

Plasma and tumor samples were analyzed for gedatolisib (MedKoo, Lot # CRB50506, Purity >98%) with a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Matrix calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in methanol. Plasma and tissue samples, 25 µL each, were protein precipitated with 100 µL of 50 ng/mL PKI-402 (Selleckchem, Lot # S273901, purity 99%) 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 Leap PAL HTS-xt autosampler.

The LC separation was performed using a Phenomenex Gemini (3 µm, 110 Å, 30 x 2 mm) column maintained at 40°C with gradient elution at a flow rate of 0.6 mL/min. The binary mobile phase consisted of H2O - 200 mM ammonium acetate pH 6.0 (110:10 v/v) in reservoir A and acetonitrile - H2O - 200 mM ammonium acetate pH 6.0 (90:10:10 v/v/v) in reservoir B. The initial mobile phase consisted of 30% B and was followed by a linear increase to 60% B in 3.0 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 7.0 min. Under these conditions, the analyte and IS eluted at 1.45 and 1.90 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: gedatolisib 616.30 → 488.20, and PKI-402 571.17 → 120.00.

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 ≥ 0.993. 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 ≤ 8.59% CV and 91.0% to 106%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The resultant gedatolisib 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 8.1 (Certara USA, Inc., Princeton, NJ), Ct data summary statistics (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the gedatolisib arithmetic mean Ct data for each

matrix was subjected to noncompartmental pharmacokinetic analysis (NCA). The IV bolus model (Model 201) 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 the back-extrapolated initial concentration (C0), observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), clearance (CL = Dose/AUCinf), and steady state volume of distribution (Vss). The apparent partition coefficient of gedatolisib 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 gedatolisib maximum tolerated dose of 154 mg IV QWK [1]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Gedatolisib plasma and tumor concentrations showed moderate-to-high variability between mice, with CVs ranging from 13.8 to 81.1%. Plasma concentrations tended to decay in a bi- or tri-phasic manner through 16 hours post-dose. The plasma clearance of gedatolisib was moderate at 26 mL/min/kg, or approximately 29% of murine hepatic blood flow. The volume of distribution at steady state for gedatolisib was high at 5.38 L/kg. The plasma terminal half-life of gedatolisib was 3.62 hours. Tumor penetration was relatively rapid and modest-to-high, with a Kp,tumor of 1.81. The terminal half-life in the tumor was prolonged compared with plasma, suggesting a high affinity of gedatolisib for tumor tissue. Brain homogenates from these mice were opportunistically analyzed, and showed a low total brain penetration with a Kp,brain of 0.245 for gedatolisib (data not shown).

The PK results from this study differed appreciably from those previously reported for IV gedatolisib in mice [2]. The clearance in this current study was 1.4-1.9 fold higher than previously reported for 3 mg/kg IV, under the assumption of dose-proportional PK. However, the PK from 3 to 25 mg/kg IV in referenced study was super-proportional. This published data suggests nonlinear PK, or saturable clearance / distribution of gedatolisib in mice. Additionally, gedatolisib is poorly soluble, and in vivo scientists reported that our dosing solution was homogenously hazy. The lower exposures in our study could have also been due to an inadequate formulation.

The plasma protein binding for gedatolisb has not been reported for either mice or humans. Therefore, only total plasma AUCs can be used for a clinically relevant dose (CRD) derivation. The total plasma AUC of gedatolisib in humans was 16250 hr-ng/mL at the MTD of 154 mg IV QWK [1]. A firm CRD for gedatolisib cannot be recommended, given potential nonlinear mouse PK and lack of protein binding information. Gedatolisib dosages between 3 and 25 mg/kg IV may be clinically relevant in mice.

3.0 REFERENCES

1. Shapiro GI, Bell-McGuinn KM, Molina JR, Bendell J, Spicer J, Kwak EL, Pandya SS, Millham R, Borzillo G, Pierce KJ, Han L, Houk BE, Gallo JD, Alsina M, Braña I, Tabernero J. First-in-Human Study of PF-05212384 (PKI-587), a Small-Molecule, Intravenous, Dual Inhibitor of PI3K and mTOR in Patients with Advanced Cancer. Clin Cancer Res. 2015 Apr 15;21(8):1888–95.

2. Mallon R, Feldberg LR, Lucas J, Chaudhary I, Dehnhardt C, Santos ED, Chen Z, dos Santos O, Ayral-Kaloustian S, Venkatesan A, Hollander I. Antitumor efficacy of PKI-587, a highly potent dual PI3K/mTOR kinase inhibitor. Clin Cancer Res Off J Am Assoc Cancer Res. 2011 May 15;17(10):3193–203.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

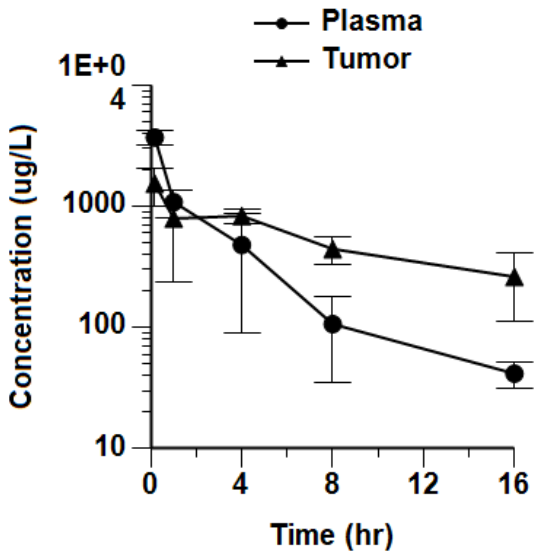

Table 4.1: NCA PK Parameter Estimates of Gedatolisib by Group

		Analyte	
		Gedatolisib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
C0	ug/L	4730	1760
Cmax	ug/L	3690	1540
Tmax	hr	0.167	0.167
AUClast	hr*ug/L	6230	8870
AUCinf	hr*ug/L	6420	11600
Kel	1/hr	0.191	0.0921
T1/2	hr	3.62	7.53
CL	L/hr/kg	1.56	0.864
Vss	L/kg	5.38	9.43
Clast	ug/L	41.5	262
Tlast	hr	16.0	16.0
Kp,tumor	-	-	1.81

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

		Analyte	
		Gedatolisib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.167	N	3	3
	Mean	3690	1540
	SD	509	535
	Min	3110	924
	Median	3980	1800
	Max	4000	1900
	CV%	13.8	34.8
	Geometric Mean	3670	1470
	CV% Geometric Mean	14.5	41.7
1.000	N	3	3
	Mean	1080	792
	SD	280	557
	Min	913	185
	Median	928	910
	Max	1400	1280
	CV%	25.9	70.3
	Geometric Mean	1060	600
	CV% Geometric Mean	24.8	138
4.000	N	3	3
	Mean	478	832
	SD	388	125
	Min	224	689
	Median	287	886
	Max	924	921
	CV%	81.1	15.0
	Geometric Mean	390	825
	CV% Geometric Mean	88.1	15.9
8.000	N	3	3
	Mean	106	443
	SD	71.7	116
	Min	39.3	310
	Median	98.1	503
	Max	182	517
	CV%	67.3	26.1
	Geometric Mean	88.9	432
	CV% Geometric Mean	90.0	29.4
16.000	N	3	3
	Mean	41.5	262
	SD	9.89	149

		Analyte	
		Gedatolisib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	Min	30.1	135
	Median	47.0	224
	Max	47.4	426
	CV%	23.8	57.1
	Geometric Mean	40.6	234
	CV% Geometric Mean	26.5	62.8

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Gedatolisib	Plasma	0.17	3998.82
M1	Gedatolisib	Tumor	0.17	1895.15
M2	Gedatolisib	Plasma	0.17	3106.85
M2	Gedatolisib	Tumor	0.17	1798.61
M3	Gedatolisib	Plasma	0.17	3978.18
M3	Gedatolisib	Tumor	0.17	924.08
M4	Gedatolisib	Plasma	1.00	1404.86
M4	Gedatolisib	Tumor	1.00	185.48
M5	Gedatolisib	Plasma	1.00	912.59
M5	Gedatolisib	Tumor	1.00	1280.39
M6	Gedatolisib	Plasma	1.00	928.49
M6	Gedatolisib	Tumor	1.00	909.93
M7	Gedatolisib	Plasma	4.00	924.31
M7	Gedatolisib	Tumor	4.00	688.60
M8	Gedatolisib	Plasma	4.00	286.68
M8	Gedatolisib	Tumor	4.00	885.87
M9	Gedatolisib	Plasma	4.00	223.65
M9	Gedatolisib	Tumor	4.00	920.55
M10	Gedatolisib	Plasma	8.00	39.34
M10	Gedatolisib	Tumor	8.00	503.12
M11	Gedatolisib	Plasma	8.00	98.11
M11	Gedatolisib	Tumor	8.00	517.35
M12	Gedatolisib	Plasma	8.00	181.95
M12	Gedatolisib	Tumor	8.00	310.01
M13	Gedatolisib	Plasma	16.00	47.42
M13	Gedatolisib	Tumor	16.00	426.07
M14	Gedatolisib	Plasma	16.00	30.10
M14	Gedatolisib	Tumor	16.00	134.77
M15	Gedatolisib	Plasma	16.00	47.05

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M15	Gedatolisib	Tumor	16.00	223.89

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Gedatolisib	Plasma	0.17	3694.61	509.13	3.00
Concentration	ug/L	Gedatolisib	Plasma	1.00	1081.98	279.74	3.00
Concentration	ug/L	Gedatolisib	Plasma	4.00	478.21	387.61	3.00
Concentration	ug/L	Gedatolisib	Plasma	8.00	106.47	71.68	3.00
Concentration	ug/L	Gedatolisib	Plasma	16.00	41.52	9.89	3.00
Concentration	ug/L	Gedatolisib	Tumor	0.17	1539.28	534.96	3.00
Concentration	ug/L	Gedatolisib	Tumor	1.00	791.93	556.91	3.00
Concentration	ug/L	Gedatolisib	Tumor	4.00	831.68	125.11	3.00
Concentration	ug/L	Gedatolisib	Tumor	8.00	443.49	115.82	3.00
Concentration	ug/L	Gedatolisib	Tumor	16.00	261.58	149.26	3.00

5.0 ATTACHED FILES

Attached File 5.1 Gedatolisib PKI587 Prelim PK.docx – *Final in vivo study plan as executed*
Attached File 5.2 Gedatolisib PKI587.docx – *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 Gedatolisib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*