PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 51521-448163

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF GANETESPIB IN FEMALE CD1 NU MICE AFTER A SINGLE INTRAVENOUS DOSE

SHORT TITLE:	**Ganetespib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Ganetespib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Dyer, Michael A <Michael.Dyer@STJUDE.ORG>
SJCRH SRM2 O/R:	51521-448163 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA NA
IN VIVO SCIENTIST(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>; Bradley, Cori
BIOANALYTICAL SCIENTIST:	Vuppala, Pradeep K
REPORT AUTHOR(S):	Vuppala, Pradeep K; Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2020-04-08

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 total plasma and tumor PK of ganetespib in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single, slow intravenous (IV) injection of 150 mg/kg via the tail vein. Ganetespib (MedChem Express, HY-15205, Lot 08249, purity >98%, MolWt 364.40) was dissolved in DMSO and further diluted with Kolliphor RH 40 (Sigma) in 5% dextrose for injection, USP (D5W, Baxter) for a final nominal concentration of 15 mg/mL in 10% DMSO / 18% Kolliphor RH 40 / 72% D5W and a 10 mL/kg injection volume. Mice were sacrificed using an IACUC-approved method at 10 min, 1, 4, 8, and 16 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, 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. As two of the mice died at the 10 min time point, most likely due to a thromboembolism for precipitated intravenous drug, three additional mice were evaluated separately.

1.2 Bioanalysis

Total plasma and tumor homogenate ganetespib concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry (LC-MS/MS) assay. First, tissue samples were macerated, 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). Ceramic lysing matrix beads (MP Biomedicals, Ceramic Bead Lysing Matrix, 3 mg per mg of tissue) were added to the microcentrifuge tubes containing tumor samples. 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.

Ganetespib stock solutions were prepared in 100% methanol and used to spike matrix calibrators and quality controls. Plasma and tissue homogenate samples, 50 µL each, were treated with 10 µL of internal standard (SLV320, Tocris Bioscience, purity >99%) 100 ng/mL spiking solution and then subjected to a liquid-liquid extraction procedure using methyl tert-butyl ether, vortexed and centrifuged at 4 °C, with the supernatant evaporated to dryness in a CentriVap centrifugal vacuum concentrator (Labconco). The extracts were then reconstituted with 100 µL of 100% acetonitrile, and a 5 µL aliquot of the reconstituted extract was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu SIL-20ACXR autosampler. The LC separation was performed using a Phenomenex Luna C8 80Å LC column (4.0 µm, 30 mm x 2.0 mm) maintained at ambient temperature with gradient elution at a flow rate of 0.25 mL/min. The binary mobile phase consisted of 0.1% formic acid in ultra-pure water in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 5% B with an increase to 90% B over 2.5 minutes. After maintenance at 90% B for 4 minutes, the column was then rinsed for 1 minute at 100% B and then equilibrated at the initial conditions for 1.5 minutes for a total run time of 9 minutes. Under these conditions, the analyte and IS eluted at 3.52 and 2.86 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode with monitoring of the following mass transitions: ganetespib 365.2 -> 131.1, SLV320 309.2 -> 211.1.

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 to 200 ng/mL range, with a correlation coefficient (R) of ≥0.99. Above the calibration range quality control samples were diluted with adequate precision and accuracy. 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 ng/mL for plasma and 30 ng/mL for tissues due to the dilution factor. The intra-run precision and accuracy was ≤ 9.62% CV and 98.5% to 111%, respectively across the matrices.

1.3 Pharmacokinetic (PK) Analysis

The resultant ganetespib 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 ganetespib 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 ganetespib 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 ganetespib at the recommended Phase 2 dose (RP2D) of 200 mg/m^2 [2]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Ganetespib concentrations showed moderate variability in the plasma, demonstrating coefficients of variation of 2.90% to 65.6% across the sampling time points. All plasma and tumor results were above the LLOQ. Tumor penetration appeared to be rapid yet modest, with a Kp,tumor value of 0.639 based on AUCinf. The T1/2 of ganetespib in the plasma was 2.03 hr. The apparent tumor half-life was longer than that observed in plasma (8.42 hr), suggesting a high affinity of ganetespib for the orthotopic tumor. The large apparent Vss for tumor also suggests that ganetespib has high orthotopic tumor retention. Notably, our plasma and tumor PK was in line with that published by Shimamura et al. at 125 mg/kg IV in mice [3].

In a Phase 1 study of single agent ganetespib administered IV once weekly for 3 weeks of a 4-week cycle, the RP2D was 200 mg/m^2 [2]. The major adverse events with ganetespib are diarrhea and fatigue; however, retinal toxicities have been of concern. Cmax and AUC values appear to increase in a dose proportional manner over the dose range studied in humans (7 to 259 mg/m^2). The estimated human total plasma AUCinf at 200 mg/m^2 was approximately 7000 hr-ng/mL. Assuming dose proportionality and linear PK processes in mice, a murine equivalent dose (MED) providing a similar total plasma AUCinf would be 12.8 mg/kg. The plasma protein binding of ganetespib has only been reported as "high," and data regarding differences in the extent between species are lacking [4]. Therefore a MED can only be derived using total plasma concentrations, under the assumption of similar plasma protein binding between mice and humans.

It bears noting that there are data suggesting that high affinity HSP90 inhibitors such as ganetespib may not demonstrate dose proportional PK, particularly in mice [5]. This is thought to result from a target mediated drug disposition, where the compound's affinity for a highly abundant and specific target drives a nonlinear tissue distribution. We have generated limited plasma PK following ganetespib 50 mg/kg IV in mice indicating that this might be the case (data not shown). In this instance, a 3-fold increase in dose from 50 to 150 mg/kg results in an approximate 17-fold increase in plasma Cmax at 10 minutes after injection. Therefore, it is difficult for us to assume proportionality when back-extrapolating a MED based on the AUC from the 150 mg/kg dose. It was thus decided to evaluate two dose levels of ganetespib in

our mouse efficacy model: 10 mg/kg and 50 mg/kg. Additional plasma and tumor PK may be conducted in the future to assess the clinical relevance of these doses with respect to PK.

3.0 REFERENCES

1. Liang X, Ubhayakar S, Liederer BM, Dean B, Ran-Ran Qin A, Shahidi-Latham S, Deng Y. Evaluation of homogenization techniques for the preparation of mouse tissue samples to support drug discovery. Bioanalysis. 2011 Sep;3(17):1923–33.

2. Goldman JW, Raju RN, Gordon GA, El-Hariry I, Teofilivici F, Vukovic VM, Bradley R, Karol MD, Chen Y, Guo W, Inoue T, Rosen LS. A first in human, safety, pharmacokinetics, and clinical activity phase I study of once weekly administration of the Hsp90 inhibitor ganetespib (STA-9090) in patients with solid malignancies. BMC Cancer. 2013;13:152.

3. Shimamura T, Perera SA, Foley KP, Sang J, Rodig SJ, Inoue T, Chen L, Li D, Carretero J, Li Y-C, Sinha P, Carey CD, Borgman CL, Jimenez J-P, Meyerson M, Ying W, Barsoum J, Wong K-K, Shapiro GI. Ganetespib (STA-9090), a Nongeldanamycin HSP90 Inhibitor, Has Potent Antitumor Activity in In Vitro and In Vivo Models of Non–Small Cell Lung Cancer. Clin Cancer Res. 2012 Sep 15;18(18):4973–85.

4. Synta Pharmaceuticals Corporation. Ganetespib [Internet]. Meeting of the Pediatric Oncology Subcommittee of the Oncologic Drugs Advisory Committee; 2014 Dec 11 [cited 2016 Sep 19]; Silver Spring, Maryland. Available from: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM430870.pdf

5. Yamazaki S, Shen Z, Jiang Y, Smith BJ, Vicini P. Application of Target-Mediated Drug Disposition Model to Small Molecule Heat Shock Protein 90 Inhibitors. Drug Metab Dispos. 2013 Jun 1;41(6):1285–94.

THIS SPACE INTENTIONALLY LEFT BLANK

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

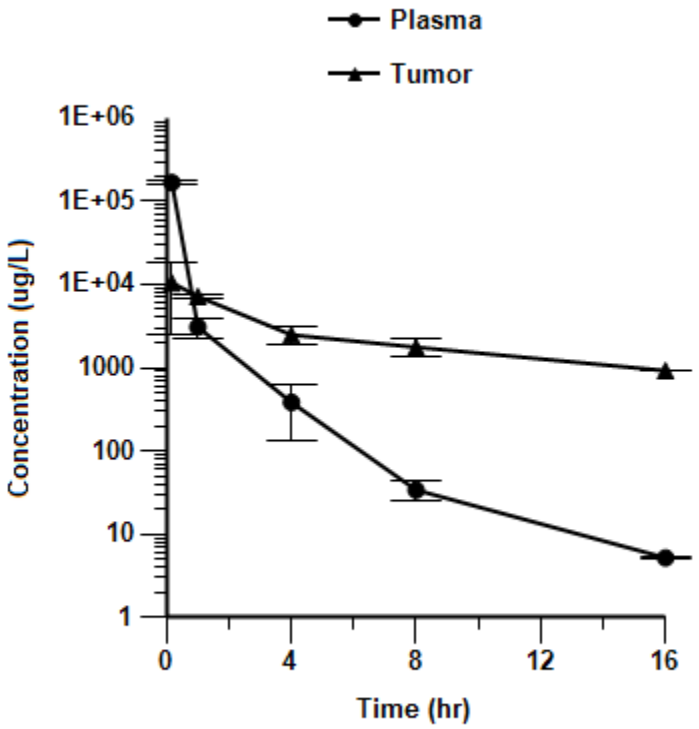

Table 4.1: NCA PK Parameter Estimates of Ganetespib by Group

		Analyte	
		Ganetespib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
C0	Ug/L	374000	11300
Cmax	ug/L	168000	10500
Tmax	hr	0.167	0.167
AUClast	hr*ug/L	81800	41200
AUCinf	hr*ug/L	81900	52300
Kel	1/hr	0.341	0.0823
T1/2	hr	2.03	*8.42*
CL	L/hr/kg	1.83	2.87
Vss	L/kg	0.624	28.4

		Analyte	
		Ganetespib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Clast	ug/L	5.20	922
Tlast	hr	16	16
Kp,tumor	-	-	0.639

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

		Analyte	
		Ganetespib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.167	N	4	4
	Mean	168000	10500
	SD	10300	7940
	Min	159000	1610
	Median	166000	9820
	Max	181000	20800
	CV%	6.12	75.6
	Geometric Mean	167000	7490
	CV% Geometric Mean	6.06	152
1.000	N	3	3
	Mean	3080	7180
	SD	854	358
	Min	2100	6790
	Median	3510	7260
	Max	3640	7490
	CV%	27.7	4.99
	Geometric Mean	2990	7170
	CV% Geometric Mean	31.5	5.04
4.000	N	3	3
	Mean	384	2480
	SD	251	600
	Min	96.1	2000
	Median	491	2300
	Max	563	3150

		Analyte	
		Ganetespib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	CV%	65.6	24.1
	Geometric Mean	298	2440
	CV% Geometric Mean	128	23.7
8.000	N	3	3
	Mean	33.9	1760
	SD	9.45	422
	Min	24.2	1450
	Median	34.6	1600
	Max	43.0	2240
	CV%	27.8	23.9
	Geometric Mean	33.0	1730
	CV% Geometric Mean	29.7	23.2
16.000	N	3	3
	Mean	5.20	922
	SD	0.151	9.10
	Min	5.06	914
	Median	5.18	922
	Max	5.36	932
	CV%	2.89	0.987
	Geometric Mean	5.20	922
	CV% Geometric Mean	2.89	0.986

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Ganetespib	Plasma	0.17	180918.41
M1	Ganetespib	Tumor	0.17	20775.00
M2	Ganetespib	Plasma	0.17	158779.74
M2	Ganetespib	Tumor	0.17	8460.43
M3	Ganetespib	Plasma	0.17	160454.01
M3	Ganetespib	Tumor	0.17	11176.22
M4	Ganetespib	Plasma	0.17	170720.29
M4	Ganetespib	Tumor	0.17	1605.94
M5	Ganetespib	Plasma	1.00	2098.27

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M5	Ganetespib	Tumor	1.00	6786.25
M6	Ganetespib	Plasma	1.00	3641.57
M6	Ganetespib	Tumor	1.00	7262.13
M7	Ganetespib	Plasma	1.00	3505.53
M7	Ganetespib	Tumor	1.00	7488.64
M8	Ganetespib	Plasma	4.00	491.34
M8	Ganetespib	Tumor	4.00	3153.74
M9	Ganetespib	Plasma	4.00	563.10
M9	Ganetespib	Tumor	4.00	1997.38
M10	Ganetespib	Plasma	4.00	96.11
M10	Ganetespib	Tumor	4.00	2300.62
M11	Ganetespib	Plasma	8.00	43.03
M11	Ganetespib	Tumor	8.00	2243.93
M12	Ganetespib	Plasma	8.00	34.63
M12	Ganetespib	Tumor	8.00	1596.41
M13	Ganetespib	Plasma	8.00	24.17
M13	Ganetespib	Tumor	8.00	1450.67
M14	Ganetespib	Plasma	16.00	5.06
M14	Ganetespib	Tumor	16.00	921.72
M15	Ganetespib	Plasma	16.00	5.36
M15	Ganetespib	Tumor	16.00	913.63
M16	Ganetespib	Plasma	16.00	5.18
M16	Ganetespib	Tumor	16.00	931.80

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Ganetespib	Plasma	0.17	167718.11	10261.95	4.00
Concentration	ug/L	Ganetespib	Plasma	1.00	3081.79	854.47	3.00
Concentration	ug/L	Ganetespib	Plasma	4.00	383.52	251.47	3.00
Concentration	ug/L	Ganetespib	Plasma	8.00	33.94	9.45	3.00
Concentration	ug/L	Ganetespib	Plasma	16.00	5.20	0.15	3.00
Concentration	ug/L	Ganetespib	Tumor	0.17	10504.40	7943.49	4.00
Concentration	ug/L	Ganetespib	Tumor	1.00	7179.01	358.49	3.00
Concentration	ug/L	Ganetespib	Tumor	4.00	2483.91	599.57	3.00
Concentration	ug/L	Ganetespib	Tumor	8.00	1763.67	422.25	3.00
Concentration	ug/L	Ganetespib	Tumor	16.00	922.38	9.10	3.00

5.0 ATTACHED FILES

Attached File 5.1 Ganetespib Prelim PK.docx – *Final in vivo study plan as executed*

Attached File 5.2 Genetispib_PK_5_29_15.xlsx– *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 Ganetespib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*