PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 17856-81205

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF PAZOPANIB IN FEMALE CD1 NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Pazopanib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Pazopanib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Dyer, Michael A <Michael.Dyer@STJUDE.ORG>
SJCRH SRM2 O/R:	17856-81205 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA NA
IN VIVO SCIENTIST(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>; Bradley, Cori
BIOANALYTICAL SCIENTIST:	Li, Lie <Lie.Li@STJUDE.ORG>
REPORT AUTHOR(S):	Jogiraju, Harini <Harini.Jogiraju@STJUDE.ORG>; <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2020-04-20

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 pazopanib in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single oral dose of 100 mg/kg. Pazopanib (free base) was suspended in 0.5% methylcellulose (type 400 cPs) / 0.1% polysorbate 80 at 10 mg/mL for a 10 mL/kg gavage. Mice were sacrificed using an IACUC-approved method at 5 min, 30 min 1.5, 5, 12, and 24 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 MAST 39 rhabdomyosarcoma 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

Frozen tumor samples were homogenized using a bead-based technique in a ~10-fold volume of ultrapure water then stored at -80°C until analysis. Plasma and tumor samples were analyzed for pazopanib (LC-Labs, Lot # PZP-104) using respective tumor and plasma calibration curves and quality controls with a validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. This assay was executed by Dr. Lie Li, SJCRH Department of Pharmaceutical Sciences, and was based upon the method of

Both plasma and tumor calibrators and quality controls were spiked with solutions, prepared in 80% acetonitrile. Tumor homogenate samples, 100 µL each, were protein precipitated with 400 µL of 100 ng/mL dasatinib (LC-Labs, Lot # BDS-102) in acetonitrile-methanol (6:4 v/v) as an internal standard, whereas 10 µL of plasma samples were precipitated with 60 µL of dasatinib IS solution. A 1 µL aliquot of the extracted supernatant was injected onto a Waters UPLC H-class high performance liquid chromatography system via a Quaternary sample manager with a flow-through needle (SM-FTN).

The LC separation was performed using an ACQUITY UPLC BEH C18 (1.7 µm, 2.1 × 50 mm) column maintained at 40°C ± 5 °C with gradient elution at a flow rate of 0.7 mL/min. The binary mobile phase consisted of water-formic acid (100:0.1 v/v) in reservoir A and acetonitrile-formic acid (100:0.1 v/v) in reservoir B. The initial mobile phase consisted of 25% B with a linear increase to 80% B in 1.0 min. The column was then equilibrated at the initial conditions for 2.0 min for a total run time of 3 min. Under these conditions, the analyte and IS eluted at 0.54 and 0.7 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a Waters TQD mass spectrometer in the positive ESI mode with the following mass transitions were monitored: 438.064 -> 357.131 for pazopanib and 488.064 -> 401.028 for dasatinib.

The method validation and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A quadratic regression with $1/X^2$ weighing model fits the calibrators across the 5 to 1000 ng/mL range with a correlation coefficient (R) of 0.99. The lower limit of quantitation (LLOQ), defined as a peak area signal- to-noise ratio of 3 or greater verses a matrix blank with IS, was 5 ng/mL. QC dilution integrity was confirmed. For the plasma matrix, the within day precision was ≤ 10% CV and the average accuracy (3-days) was within the range of 92.88% - 101.104%.

1.3 Pharmacokinetic (PK) Analysis

The resultant pazopanib 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.

Pazopanib plasma and tumor 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 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 the observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent oral clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The apparent partition coefficient of the compound 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 dosage (CRD) for mice, the resultant mouse plasma unbound AUCinf was compared with the reported unbound plasma PK value at the commonly applied dose of pazopanib of 800 mg PO QD, or approximately 450 mg/m^2 for children [1]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Pazopanib plasma and tumor Ct data demonstrated moderate-to-high variability between mice, particularly at early time points after dosing, with CVs ranging from 1.09 to 74.5%. Tumor concentrations were below the limit of quantitation at the 5 min time point. Absorption of pazopanib appeared to be slow, with the Tmax observed at 5 hours post-dose. Penetration to the tumor was delayed and limited, but generally mirrored the plasma Ct profile with a Kp,tumor value of 0.191. The apparent oral plasma clearance was low at 5.43 mL/min/kg, or 6% of murine hepatic blood flow. The apparent plasma terminal volume of distribution was large at 2.31 L/kg. The apparent plasma terminal half-life of pazopanib was 4.9 hours. The oral bioavailability of pazopanib was unknown in this study, but has been reported to be 61% in rats [2].

A population PK analysis of pazopanib in adult patients with cancer showed nonlinear pharmacokinetics, with relative bioavailability decreasing with dose and time [3]. The steady state plasma AUC was estimated at 665000 hr-ng/mL for an 800 mg PO QD regimen in a typical patient. The plasma protein binding of pazopanib is high, with fraction unbound in plasma (Fu,p) values of 0.0004 and 0.0012 for humans and mice, respectively [2]. Considering the plasma protein binding and our mouse PK findings, a CRD of pazopanib 75 mg/kg PO QD in mice is suggested, based on unbound plasma AUCs.

3.0 REFERENCES

1. Glade Bender JL, Lee A, Reid JM, Baruchel S, Roberts T, Voss SD, Wu B, Ahern CH, Ingle AM, Harris P, Weigel BJ, Blaney SM. Phase I Pharmacokinetic and Pharmacodynamic Study of Pazopanib in Children With Soft Tissue Sarcoma and Other Refractory Solid Tumors: A Children's Oncology Group Phase I Consortium Report. J Clin Oncol. 2013 Aug 20;31(24):3034–43.

2. FDA. Drug Approval Package: Votrient (pazopanib hydrochloride) NDA # 022465 Pharmacology Review [Internet]. Drugs@FDA. 2009 [cited 2020 Apr 20]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022465s000TOC.cfm

3. Yu H, van Erp N, Bins S, Mathijssen RHJ, Schellens JHM, Beijnen JH, Steeghs N, Huitema ADR. Development of a Pharmacokinetic Model to Describe the Complex Pharmacokinetics of Pazopanib in Cancer Patients. Clin Pharmacokinet. 2017 Mar 1;56(3):293–303.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

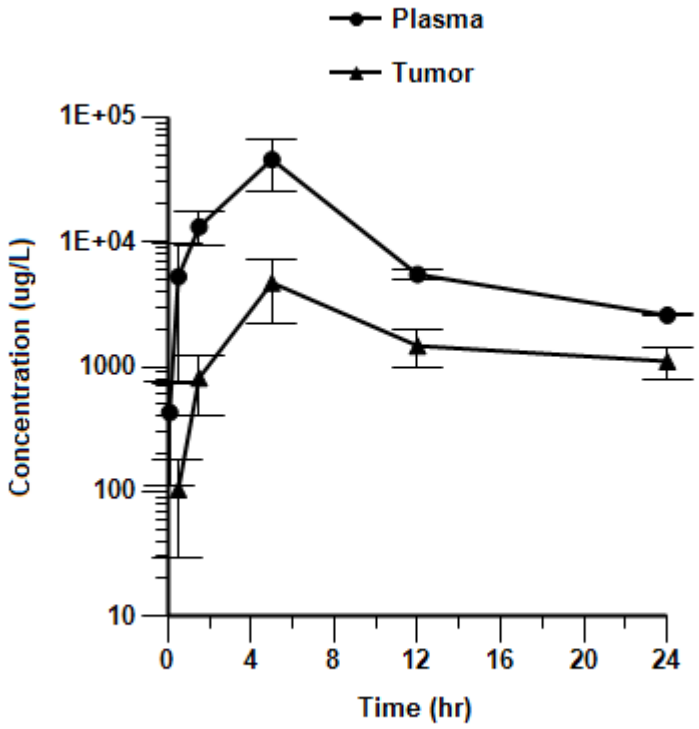

Table 4.1: NCA PK Parameter Estimates of Pazopanib by Group

		Analyte	
		Pazopanib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	45700	4700
Tmax	hr	5.00	5.00
AUClast	hr*ug/L	292000	44900
AUCinf	hr*ug/L	306000	58400
Kel	1/hr	0.142	0.0705
T1/2	hr	4.90	9.84
CL/F	L/hr/kg	0.326	1.71
Vz/F	L/kg	2.31	24.3
Clast	ug/L	2590	1110

		Analyte	
		Pazopanib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Tlast	hr	24.0	24.0
Kp,tumor	-	-	0.191

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

		Analyte	
		Pazopanib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.083	N	3	0
	Mean	430	
	SD	321	
	Min	63.1	
	Median	573	
	Max	656	
	CV%	74.5	
	Geometric Mean	287	
	CV% Geometric Mean	215	
0.500	N	3	3
	Mean	5260	104
	SD	4540	74.7
	Min	614	33.7
	Median	5480	95.4
	Max	9690	182
	CV%	86.3	71.9
	Geometric Mean	3190	83.7
	CV% Geometric Mean	271	103
1.500	N	3	3
	Mean	13200	813
	SD	4000	401
	Min	8590	411
	Median	15300	813
	Max	15700	1210
	CV%	30.3	49.4

Pazopanib Screening Plasma Tumor PK (SPTPK)

		Analyte	
		Pazopanib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	Geometric Mean	12700	740
	CV% Geometric Mean	35.1	59.1
5.000	N	3	3
	Mean	45700	4700
	SD	20500	2470
	Min	25300	2160
	Median	45500	4850
	Max	66200	7090
	CV%	44.8	52.4
	Geometric Mean	42400	4210
	CV% Geometric Mean	51.5	66.6
12.000	N	3	3
	Mean	5480	1470
	SD	564	500
	Min	4840	906
	Median	5720	1650
	Max	5900	1860
	CV%	10.3	34.0
	Geometric Mean	5460	1400
	CV% Geometric Mean	10.6	39.9
24.000	N	2	2
	Mean	2590	1110
	SD	28.3	329
	Min	2570	874
	Median	2590	1110
	Max	2610	1340
	CV%	1.09	29.7
	Geometric Mean	2580	1080
	CV% Geometric Mean	1.09	30.9

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
1.00	Pazopanib	Plasma	1.50	8585.00

Pazopanib Screening Plasma Tumor PK (SPTPK)

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
1.00	Pazopanib	Tumor	1.50	1213.80
2.00	Pazopanib	Plasma	1.50	15725.00
2.00	Pazopanib	Tumor	1.50	411.20
3.00	Pazopanib	Plasma	1.50	15265.00
3.00	Pazopanib	Tumor	1.50	812.90
4.00	Pazopanib	Plasma	0.50	614.20
4.00	Pazopanib	Tumor	0.50	33.70
5.00	Pazopanib	Plasma	0.50	9685.00
5.00	Pazopanib	Tumor	0.50	182.30
6.00	Pazopanib	Plasma	0.50	5480.00
6.00	Pazopanib	Tumor	0.50	95.40
7.00	Pazopanib	Plasma	0.08	63.10
7.00	Pazopanib	Tumor	0.08	
8.00	Pazopanib	Plasma	0.08	572.60
8.00	Pazopanib	Tumor	0.08	
9.00	Pazopanib	Plasma	0.08	655.50
9.00	Pazopanib	Tumor	0.08	
10.00	Pazopanib	Plasma	12.00	5895.00
10.00	Pazopanib	Tumor	12.00	1645.70
11.00	Pazopanib	Plasma	12.00	5715.00
11.00	Pazopanib	Tumor	12.00	1858.00
12.00	Pazopanib	Plasma	12.00	4840.00
12.00	Pazopanib	Tumor	12.00	905.80
13.00	Pazopanib	Plasma	5.00	66220.00
13.00	Pazopanib	Tumor	5.00	4852.70
14.00	Pazopanib	Plasma	5.00	45450.00
14.00	Pazopanib	Tumor	5.00	7088.30
15.00	Pazopanib	Plasma	5.00	25295.00
15.00	Pazopanib	Tumor	5.00	2163.50
16.00	Pazopanib	Plasma	24.00	2565.00
16.00	Pazopanib	Tumor	24.00	874.00
17.00	Pazopanib	Plasma	24.00	2605.00
17.00	Pazopanib	Tumor	24.00	1338.90

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Pazopanib	Plasma	0.08	430.40	320.78	3.00
Concentration	ug/L	Pazopanib	Plasma	0.50	5259.73	4539.41	3.00
Concentration	ug/L	Pazopanib	Plasma	1.50	13191.67	3996.11	3.00
Concentration	ug/L	Pazopanib	Plasma	5.00	45655.00	20463.27	3.00
Concentration	ug/L	Pazopanib	Plasma	12.00	5483.33	564.37	3.00

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Pazopanib	Plasma	24.00	2585.00	28.28	2.00
Concentration	ug/L	Pazopanib	Tumor	0.08			0.00
Concentration	ug/L	Pazopanib	Tumor	0.50	103.80	74.66	3.00
Concentration	ug/L	Pazopanib	Tumor	1.50	812.63	401.30	3.00
Concentration	ug/L	Pazopanib	Tumor	5.00	4701.50	2465.88	3.00
Concentration	ug/L	Pazopanib	Tumor	12.00	1469.83	499.87	3.00
Concentration	ug/L	Pazopanib	Tumor	24.00	1106.45	328.73	2.00

5.0 ATTACHED FILES

Attached File 5.1 Pazopanib Screening Plasma Tumor PK TLFs - *Report TLFs as a Word document for manipulation, plotting, and further presentation*