PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 821575 - 3094399

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF ROBLITINIB IN FEMALE ATHYMIC NUDE MICE BEARING RHABDOMYOSARCOMA MAST39 XENOGRAFT TUMORS AFTER A SINGLE ORAL DOSE

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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 Roblitinib was evaluated in normal female athymic nude mice (Charles River) bearing rhabdomyosarcoma MAST39 xenograft tumors, approximately 12 to 16 weeks in age. Roblitinib (SJ001010844-7, Med Chem Express, CAT# HY-101568, Lot# 309056) was suspended in 0.5% methylcellulose (type 400 cPs) / 0.5% Tween 80 in UP water at 6 mg/mL for a 5 mL/kg oral gavage, yielding a 30 mg/kg dose. One blood sample was collected from each mouse after dosing using an IACUC-approved terminal cardiac puncture, with KEDTA as the anticoagulant and immediately processed to plasma, following which tumor tissue was extracted after perfusion with PBS. Samples were obtained at various times up to 24 hours post-dose and stored at -80 °C until analysis.

1.2 Bioanalysis

Tumor samples were weighed in 4.5 mL (tumor weight below 0.5 gram) or 15 mL (tumor weight above 0.5 gram) 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). Tumor samples were subjected to 3 - 10 cycles of 1 min vibration at 6.5 M/S speed, with 5 min ice baths between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor homogenate samples were analyzed for Roblitinib (SJ001010844-7, Med Chem Express, CAT# HY-101568, LOT# 309056) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in methanol. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of 20 mM ammonium acetate (pH 6) in water-methanol (10:90 v/v) and 25 µL of 200 ng/mL Erdafitinib (SJ000981035-1, Med Chem Express, CAT# HY-18708, LOT# 17782) in methanol as an internal standard (IS). A 2 µL aliquot of the extracted supernatant was injected onto a SCIEX ExionLC high performance liquid chromatography system via a SCIEX ExionLC autosampler. The LC separation was performed using a Phenomenex Kinetex Polar 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 20 mM ammonium acetate (pH 6) in water-methanol (90:10 v/v) in reservoir A and 20 mM ammonium acetate (pH 6) in water-methanol (10:90 v/v) in reservoir B. The initial mobile phase consisted of 0% B for 0.5 min with a linear increase to 100% B in 0.5 min. The column was then rinsed for 2.5 min at 100% B and then equilibrated at the initial conditions for 2.5 min for a total run time of 6 min. Under these conditions, the analyte and IS eluted at 3.02 and 3.04 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX Triple Quad 3500 in the positive ESI mode and the following mass transitions were monitored: Roblitinib 507.2 -> 175.1, Erdafitinib 447.2 -> 388.3 The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fits the calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9981. 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 in plasma and 6 ng/mL in tumor. Sample dilution integrity was confirmed. No significant matrix effects, ion enhancement or suppression, were detected in blank EDTA plasma and tumor homogenate harvested from untreated female athymic nude mice. The intra-run precision and accuracy was ≤ 4.61% CV and 92% to 106%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for Roblitinib were grouped by matrix and nominal time point. 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. Summary statistics were calculated and the arithmetic 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). Plasma-to-tumor partition coefficients (Kp,inf) were estimated (when possible) as ratios of AUCinf in tissue to AUCinf plasma, whereas Kp,last was similarly estimated using AUClast estimates

Roblitinib fractions unbound in mouse plasma (Fu,p,m) and human plasma (Fu,p,h) were determined using equilibrium dialysis device HTD-96B (12-14K MWCO, HTDialysis LLC, Gales Ferry, CT). Briefly, blank mouse and human plasma were spiked with Roblitinib DMSO stock, achieving back calculated final concentrations 5 µM. The spiked plasma was placed in one side of the membrane on the HTD-96B plate in sextuplicate and dialyzed against equal volume of the 100 mM PBS (pH 7.4), and permitted to equilibrate for 6 hours at 37 °C with 5% CO2. Compounds concentrations were assayed on both side of the dialysis membrane using the qualified LC-MS/MS assay, with the fraction unbound calculated as the ratio of concentration in receiver to donor [1].

A clinically relevant dose (CRD) for mice was estimated from unbound plasma PK and exposure. The CRD was defined as the mouse dose achieving a predicted mean steady state unbound plasma AUC (AUCu) similar to humans at the single agent maximum tolerated dose (MTD), recommended Phase II dose (RP2D), or FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed. Human and mouse plasma protein binding were assumed similar when data were not available. This is similar to the clinical relevance approach proposed by Spilker [2] which uses unbound plasma average steady state concentrations. Some latitude in dose rounding was permitted in the CRD recommendation, and an unbound exposure within 2-fold of the clinical target was considered acceptable. Additional considerations influenced the final recommended mouse dose, including mouse dosing regimens prevalent in the literature and the tolerability of the compound in mice.

2.0 RESULTS

The Roblitinib Ct data demonstrated moderate variability between and within mice, with coefficients of variation ranging from 15.1% to 70.6% for plasma, and 22.3% to 50.4% for tumor, except 4 hours, where most of the variability was seen for both plasma and tumor, 110% and 85.9%, respectively. The absorption rate of Roblitinib was moderate, with the Tmax occurring at 1 hour post-dose for both plasma and tumor. After Cmax, both plasma and tumor concentrations diminished in a bi-exponential manner, with BLOQ observations at 24 hours post-dose time points for all mice. The apparent plasma and tumor terminal half-life of Roblitinib was 1.92 and 3.07 hours, respectively. The apparent plasma clearance (CL/F) of Roblitinib was high at 129 mL/min/kg, or approximately 1.43-fold higher than the murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for Roblitinib was high at 21.5 L/kg, in excess of the total body water. The plasma to tumor partitioning of Roblitinib was moderate, with a Kp,inf of 0.361, and Kp,last of 0.356. The oral bioavailability of Roblitinib was unknown in the current study, but has been previously reported to be 21% in mice [3].

The plasma protein binding results of Roblitinib for mice and humans herein were as follows: Fu,p,m = 0.008968 ± 0.001995; Fu,p,h = 0.07211 ± 0.004268. Plasma protein binding was evaluated twice using both diluted and undiluted plasma, confirming the fact that Roblitinib in mouse plasma is much more highly bound compared to humans. Both the mouse and human plasma protein binding results in current study are appreciably different from the previous findings by Fairhurst et al. and Novartis, i.e., unbound fractions of 0.26 and 0.12 in mouse and human plasma respectively, using rapid equilibrium dialysis (RED) device [4]. In the Fairhurst paper, however, most of their synthesized FGFR4 inhibitor analogs

showed high plasma binding of >99% in mice. Given all of this, and the fact that Roblitinib exhibits physio-chemical properties consistent with high plasma binding, the low values reported by Fairhurst are suspect, for mice in particular. Interestingly, two other FGFR inhibitors, erdafitinib and futibatinib, exhibit unusual plasma protein binding specifically to the variably expressed, acute phase reactant alpha acid glycoprotein (AAG) [5,6]. It is speculated that some FGFR inhibitors may share a pharmacophore with a cross-specificity between AAG and FGF receptors, and that this makes plasma protein binding difficult to assess for this class of agent.

The PK profile of Roblitinib in the current study differs modestly from previous findings in mice. Weiss et al. reported a plasma Cmax of 2770 ng/mL (5.47µM) and AUC 0-24h of 6595 hr-ng/mL (13.02 µM-hr) for Roblitnib after 30 mg/kg PO in RH30 tumor-bearing female Athymic nude mice [3]. The plasma Cmax in the current study at the same dose in MAST39 tumor-bearing female Athymic nude mice was similar at 2280 ng/mL; however, plasma AUCinf in the current study was 1.7-fold lower at 3880 hr-ng/mL. This suggests higher clearance and volume of distribution, or a differing rate-extent of bioavailability, resulting in a lower exposure of Roblitinib in our mouse PK study.

In clinical studies, Chan et al. reported total AUCtau at steady state as 7590 hr-ng/mL for fasted and 7980 hr-ng/mL for fed in adult patients with progressive hepatocellular carcinoma at 120 mg PO QD on cycle 1 day 8 [7]. In another clinical study, Xu et al. reported total AUCtau at steady state as 4284 hr-ng/mL in adult patients with advanced solid tumors at 80 mg PO BID on cycle 1, day 8 [8]. Using our plasma protein binding results, a precise CRD, calculated by unbound AUCs between humans and our mice is Roblitinib 300 mg/kg PO BID. The highest dose of Roblitinib reportedly used in mice is 100 mg/kg [3], and a 300 mg/kg PO BID is not likely to be feasible.

Presently, there are two factors preventing the high-confidence identification of a murine Roblitinib CRD – the major one being the discrepant mouse plasma protein binding values, and secondly our 1.7-fold lower exposure vs. literature. Ignoring the plasma protein binding, and assuming that binding is similar between mice and humans, gives a more reasonable calculated CRD of 33 mg/kg. This value is more in line with the common preclinical Roblitinib dosage of 30 mg/kg PO BID.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profiles by Analyte and Group

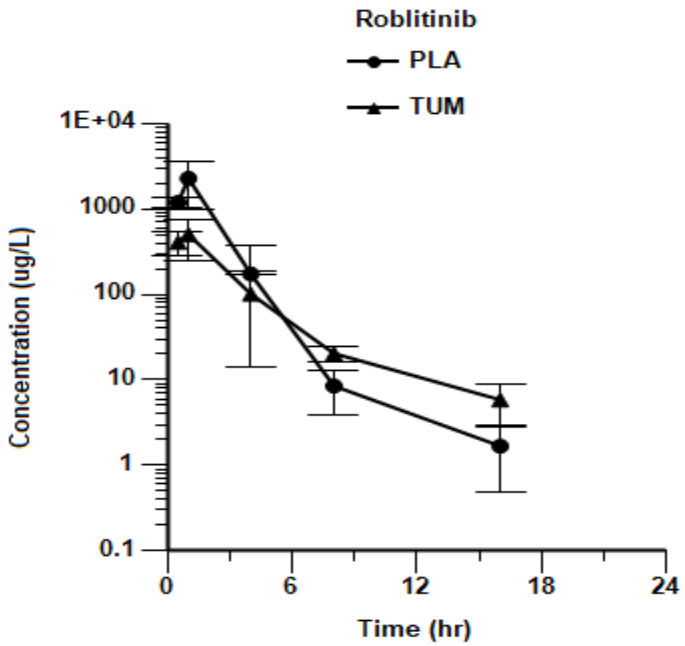

Table 4.1: NCA Parameter Estimates by Analyte and Group

		Analyte	
		Roblitinib	
		Group	
		PLA	TUM
Parameter	**Unit**	**Value**	
Cmax	ug/L	2280	504
Tmax	hr	1.00	1.00
AUClast	hr*ug/L	3870	1380
AUCinf	hr*ug/L	3880	1400
Kel	1/hr	0.360	0.226
T1/2	hr	1.92	3.07
CL/F	L/hr/kg	7.74	21.4
Vz/F	L/kg	21.5	94.7
Clast	ug/L	1.68	5.86
Tlast	hr	16.0	16.0
Kp_last			0.356
Kp_inf			0.361

Table 4.2: Full Summary Statistics of Ct Data by Analyte and Group

		Analyte	
		Roblitinib	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	1190	409
	SD	180	132
	Min	986	257
	Median	1280	479
	Max	1310	492
	CV%	15.1	32.2
	Geometric Mean	1180	393
	CV% Geometric Mean	15.9	37.9
1.00	N	3	3
	Mean	2280	504
	SD	1290	254
	Min	1430	356
	Median	1650	359
	Max	3760	797
	CV%	56.5	50.4
	Geometric Mean	2070	467
	CV% Geometric Mean	56.0	48.9
4.00	N	3	3
	Mean	174	101
	SD	191	87.0
	Min	35.6	39.9
	Median	93.1	63.1
	Max	392	201
	CV%	110	85.9
	Geometric Mean	109	79.7
	CV% Geometric Mean	182	100
8.00	N	3	3
	Mean	8.50	20.4
	SD	4.59	4.55
	Min	5.06	15.8
	Median	6.72	20.5
	Max	13.7	24.9
	CV%	54.0	22.3
	Geometric Mean	7.76	20.1
	CV% Geometric Mean	54.9	23.1
16.0	N	3	3
	Mean	1.68	5.86

		Analyte	
		Roblitinib	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
	SD	1.19	2.94
	Min	0.923	3.00
	Median	1.07	5.69
	Max	3.05	8.88
	CV%	70.6	50.2
	Geometric Mean	1.45	5.33
	CV% Geometric Mean	72.6	58.9
24.0	N		
	Mean		
	SD		
	Min		
	Median		
	Max		
	CV%		
	Geometric Mean		
	CV% Geometric Mean		

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Roblitinib	PLA	0.500	1309.70
M1	Roblitinib	TUM	0.500	478.52
M2	Roblitinib	PLA	0.500	1282.30
M2	Roblitinib	TUM	0.500	257.35
M3	Roblitinib	PLA	0.500	985.98
M3	Roblitinib	TUM	0.500	492.13
M4	Roblitinib	PLA	1.00	1429.80
M4	Roblitinib	TUM	1.00	358.64
M5	Roblitinib	PLA	1.00	3763.70
M5	Roblitinib	TUM	1.00	797.38
M6	Roblitinib	PLA	1.00	1649.40
M6	Roblitinib	TUM	1.00	356.44
M7	Roblitinib	PLA	4.00	93.13
M7	Roblitinib	TUM	4.00	63.13
M8	Roblitinib	PLA	4.00	35.62
M8	Roblitinib	TUM	4.00	39.95
M9	Roblitinib	PLA	4.00	392.15
M9	Roblitinib	TUM	4.00	200.90

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M10	Roblitinib	PLA	8.00	13.71
M10	Roblitinib	TUM	8.00	24.92
M11	Roblitinib	PLA	8.00	5.06
M11	Roblitinib	TUM	8.00	15.81
M12	Roblitinib	PLA	8.00	6.72
M12	Roblitinib	TUM	8.00	20.47
M13	Roblitinib	PLA	16.0	3.05
M13	Roblitinib	TUM	16.0	8.88
M14	Roblitinib	PLA	16.0	0.92
M14	Roblitinib	TUM	16.0	5.69
M15	Roblitinib	PLA	16.0	1.07
M15	Roblitinib	TUM	16.0	3.00*
M16	Roblitinib	PLA	24.0	BLOQ (< 1 ng/mL) *
M16	Roblitinib	TUM	24.0	BLOQ (< 6 ng/mL) *
M17	Roblitinib	PLA	24.0	BLOQ (< 1 ng/mL) *
M17	Roblitinib	TUM	24.0	BLOQ (< 6 ng/mL) *
M18	Roblitinib	PLA	24.0	BLOQ (< 1 ng/mL) *
M18	Roblitinib	TUM	24.0	BLOQ (< 6 ng/mL) *

*: Manual imputation of data below the lower limit of quantitation (BLOQ): IF ≥ 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.

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

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Roblitinib	PLA	0.500	1190	180	3
Roblitinib	PLA	1.00	2280	1290	3
Roblitinib	PLA	4.00	174	191	3
Roblitinib	PLA	8.00	8.50	4.59	3
Roblitinib	PLA	16.0	1.68	1.19	3
Roblitinib	PLA	24.0			0
Roblitinib	TUM	0.500	409	132	3
Roblitinib	TUM	1.00	504	254	3
Roblitinib	TUM	4.00	101	87.0	3
Roblitinib	TUM	8.00	20.4	4.55	3
Roblitinib	TUM	16.0	5.86	2.94	3
Roblitinib	TUM	24.0			0

5.0 ATTACHED FILES

Attached File 5.1 Roblitinib Screening Plasma and Tumor PK V1.0.docx – *Final in vivo study plan as executed*

Attached File 5.2 Roblitinib Screening Plasma and Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

Attached File 5.3 Roblitinib PK Sheet.pdf – *submitted in vivo study form from in vivo scientist*