PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 18156-82375

STUDY TITLE:

SCREENING PLASMA AND VITREOUS HUMOR PHARMACOKINETICS OF FOSTAMATINIB IN FEMALE C57BL/6 NON-TUMOR BEARING MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Oral Fostamatinib Screening Plasma Vitreous PK**
TEST ARTICLE:	Fostamatinib (R788), phosphate prodrug of tamatinib (R406)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	18156-82375 Preclinical Pharmacokinetic Shared Resource
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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 vitreous humor pharmacokinetic (PK) profile of the Syk kinase inhibitor prodrug fostamatinib was evaluated in female non-tumor bearing C57BL/6 mice (The Jackson Laboratories), approximately 12 weeks in age. Fostamatinib free base (Selleck Chemicals, Lot # S262501, Purity 99.0%) was dissolved in 0.01 M sodium citrate buffer, pH 6, in ultrapure water, at a concentration of 2.5 mg/mL as a 10 mL/kg oral gavage, for a 25 mg/kg oral dose. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 8 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. Vitreous humor samples 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

Plasma and vitreous humor samples were analyzed for active tamatinib (Selleck Chemicals, lot # S219401, purity 99.1) by the Chemical Biology and Therapeutics Department, St. Jude Children's Research Hospital, using a sensitive and specific liquid chromatography – tandem mass spectrometry (LC-MS) assay. Matrix calibrators were spiked with solutions, corrected for salt content, prepared in DMSO. Plasma samples, 25 µL each, were protein precipitated with 100 µL of 4 µg/mL warfarin in acetonitrile as an internal standard. Vitreous humor samples, 5 µL each, were protein precipitated with 45 µL of 4 µg/mL warfarin in acetonitrile as an internal standard. A 10 µL aliquot of the extracted supernatant was injected onto a Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters Corporation, Milford, MA).

The LC separation was performed using an Acquity UPLC BEH C18 (1.7 µm, 2.1 mm x 50 mm) column (Waters Corporation, Milford, MA) maintained at 55 °C with gradient elution at a flow rate of 1.0 mL/min. The binary mobile phase consisted of 0.1% formic acid in MilliQ water in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 10% B and was followed by a linear increase to 30% B in 0.2 min. A second linear increase to 95% B in 1.4 min. was then followed by a 0.35 min. hold at 95% B. The column was then returned to the initial conditions over 0.05 min for a total run time of 2.0 min. Under these conditions, the analyte eluted at 0.68 min. Analyte and IS were detected with an Acquity UPLC/MS System (Waters Corporation, Milford, MA) equipped with a single-quadrapole mass spectrometer in the positive ESI mode. Single ion recording mass spectrometry was used to quantify relative amounts of the samples with monitoring of the following molecular ions: [M+H]+ ion m/z 471.4 for tamatinib and [M+H]+ ion m/z 309.1 for warfarin.

A linear model (1/X weighting) fit the calibrators across the 15 to 3704 nmol 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 15 nmol.

1.3 Pharmacokinetic (PK) Analysis

Concentration-time (Ct) data for tamatinib 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). The apparent plasma-to-vitreous humor 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

The tamatinib plasma and vitreous Ct data demonstrated high variability between and within mice, with coefficients of variation ranging from 17.1% to 73.7%. The absorption rate of fostamatinib and appearance of tamatinib in plasma was rapid, with the Tmax occurring at 0.5 hours post-dose. After Cmax, plasma concentrations diminished in a bi-exponential manner. Notable numbers of BLOQ data were observed starting at 4 hours post-dose, particularly for the vitreous. The apparent plasma terminal half-life of tamatinib was 0.791 hours. The apparent plasma clearance (CL/F) of tamatinib was very high at 645 mL/min/kg, far exceeding murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for tamatinib was also high at 44.2 L/kg, in excess of total body water. The oral bioavailability of fostamatinib and tamatinib is unknown in the current study, but has been reported to be 42% to 54% in rats [1].

Penetration of tamatinib from plasma to vitreous after a single oral fostamatinib dose was poor in mice, with concentrations being less than 10% of that observed in plasma. The vitreous Ct profile proportionally mirrored the plasma for 2 hours after dosing, with the subsequent observations being BLOQ.

After 9 days of fostamatinib 100 mg PO BID, the common clinical dosage, patients exhibited a total plasma tamatinib AUCtau of 4980 hr-ng/mL [2]. Plasma protein binding of tamatinib is 98% for humans, but is unreported for mice [1]. Assuming linear, dose-proportional, and time-invariant PK across species, and similar plasma protein binding, an AUC-based clinically relevant dose (CRD) of fostamatinib would be ~200 mg/kg PO BID for mice. However, regulatory review data suggests that tamatinib plasma PK is likely supra-proportional and time-dependent in mice, with female mice having significantly lower exposures than males [1]. Due to uncertainties in the PK in mice vs. humans, a firm PK-based CRD for fostamatinib cannot be recommended. Doses common in mouse models range from 60 to 100 mg/kg/day orally, and are suggested.

An alternate version of these PK results, using different PK analysis methods, was published in Pritchard et al. 2014 [3].

3.0 REFERENCES

1. FDA. Drug Approval Package: Tavalisse (fostamatinib disodium hexahydrate) NDA # 209299 Multi-Discipline Review/Summary, Clinical, Non-Clinical [Internet]. Drugs@FDA. 2018 [cited 2020 Apr 28]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/209299Orig1s000MultidisciplineR.pdf

2. Baluom M, Samara E, Grossbard EB, Lau DT-W. Fostamatinib, a Syk-Kinase Inhibitor, Does Not Affect Methotrexate Pharmacokinetics in Patients With Rheumatoid Arthritis. J Clin Pharmacol. 2011;51(9):1310–8.

3. Pritchard EM, Stewart E, Zhu F, Bradley C, Griffiths L, Yang L, Suryadevara PK, Zhang J, Iii BBF, Guy RK, Dyer MA. Pharmacokinetics and Efficacy of the Spleen Tyrosine Kinase Inhibitor R406 after Ocular Delivery for Retinoblastoma. Pharm Res. 2014 Jun 7;31(11):3060–72.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

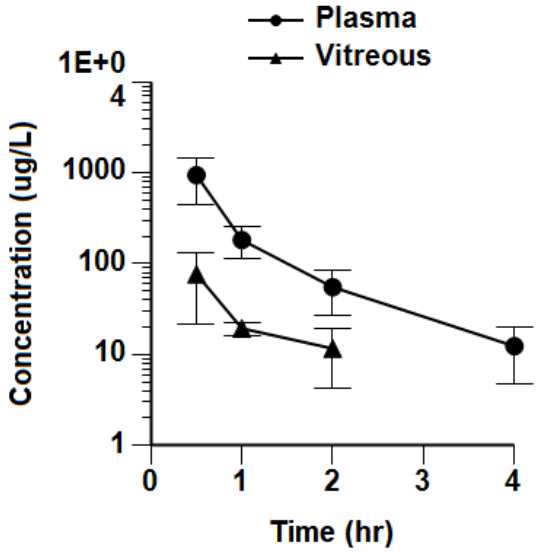

Table 4.1: NCA PK Parameter Estimates of Tamatinib by Group

		Analyte	
		Tamatinib	
		Group	
		Plasma	Vitreous
Parameter	Units	Estimate	
Cmax	ug/L	946	77.2
Tmax	hr	0.500	0.500
AUClast	hr*ug/L	633	55.5
AUCinf	hr*ug/L	646	64.1
Kel	1/hr	0.876	1.15
T1/2	hr	0.791	0.602
CL/F	L/hr/kg	38.7	390
Vz/F	L/kg	44.2	339
Clast	ug/L	12.4	11.7
Tlast	hr	4.00	2.00
Kp,inf	-	-	0.0992
Kp,last	-	-	0.0877

Table 4.2: Tamatinib Ct Data Listings by Subject, Analyte, Group, and Time

		Analyte	
		Tamatinib	
		Group	
		Plasma	Vitreous
Time (hr)		Concentration (ng/mL)	
0.500	N	3	3
	Mean	946	77.2
	SD	496	55.4
	Min	453	44.2
	Median	939	46.1
	Max	1440	141
	CV%	52.4	71.8
	Geometric Mean	850	66.0
	CV% Geometric Mean	64.0	73.7
1.000	N	3	3
	Mean	183	19.4
	SD	70.3	3.34
	Min	140	16.5
	Median	144	18.8
	Max	264	23.1
	CV%	38.5	17.2
	Geometric Mean	175	19.3
	CV% Geometric Mean	36.9	17.1
2.000	N	3	3
	Mean	55.4	11.7
	SD	28.7	7.37
	Min	22.3	3.53
	Median	69.9	13.6
	Max	73.9	17.9
	CV%	51.8	63.1
	Geometric Mean	48.7	9.51
	CV% Geometric Mean	75.9	106
4.000	N	3	
	Mean	12.4	
	SD	7.70	
	Min	3.53	
	Median	16.2	

		Analyte	
		Tamatinib	
		Group	
		Plasma	Vitreous
Time (hr)		Concentration (ng/mL)	
	Max	17.4	
	CV%	62.1	
	Geometric Mean	9.99	
	CV% Geometric Mean	112	

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

Subject	Analyte	Group	Time (hr)	Concentration (ng/mL)
M1	Tamatinib	Plasma	0.50	939.12
M1	Tamatinib	Vitreous	0.50	46.11
M2	Tamatinib	Plasma	0.50	1444.44
M2	Tamatinib	Vitreous	0.50	141.15
M3	Tamatinib	Plasma	0.50	453.09
M3	Tamatinib	Vitreous	0.50	44.23
M4	Tamatinib	Plasma	1.00	139.97
M4	Tamatinib	Vitreous	1.00	18.82
M5	Tamatinib	Plasma	1.00	144.44
M5	Tamatinib	Vitreous	1.00	16.47
M6	Tamatinib	Plasma	1.00	263.95
M6	Tamatinib	Vitreous	1.00	23.05
M7	Tamatinib	Plasma	2.00	69.87
M7	Tamatinib	Vitreous	2.00	13.64
M8	Tamatinib	Plasma	2.00	22.35
M8	Tamatinib	Vitreous	2.00	3.53
M9	Tamatinib	Plasma	2.00	73.87
M9	Tamatinib	Vitreous	2.00	17.88
M10	Tamatinib	Plasma	4.00	16.23
M11	Tamatinib	Plasma	4.00	3.53
M12	Tamatinib	Plasma	4.00	17.41

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ng/mL)	SD (ng/mL)	N
Concentration	ng/mL	Tamatinib	Plasma	0.50	945.55	495.70	3.00
Concentration	ng/mL	Tamatinib	Plasma	1.00	182.79	70.32	3.00
Concentration	ng/mL	Tamatinib	Plasma	2.00	55.36	28.66	3.00
Concentration	ng/mL	Tamatinib	Plasma	4.00	12.39	7.70	3.00

Variable	Units	Analyte	Group	Time (hr)	Mean (ng/mL)	SD (ng/mL)	N
Concentration	ng/mL	Tamatinib	Vitreous	0.50	77.16	55.42	3.00
Concentration	ng/mL	Tamatinib	Vitreous	1.00	19.45	3.34	3.00
Concentration	ng/mL	Tamatinib	Vitreous	2.00	11.68	7.37	3.00

5.0 ATTACHED FILES

Attached File 5.1 R788 PK Study.docx – *Final in vivo study plan as executed*
Attached File 5.2 R788 PO Study Worksheet v1.xlsx – *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 R778_worksheet.xlsx – *Submitted in vivo study worksheet #1*
Attached File 5.4 Fostamatinib Screening Plasma Vitreous PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*