P-PKSR
Preclinical
Pharmacokinetics

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 833884 - 3183341

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF VS-4718 IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	VS-4718 Screening Plasma and Tumor PK (SPTPK)
TEST ARTICLE:	VS-4718 (as hydrochloride salt) (PND-1186; SR-2516)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH O/R: SRM2	833884 - 3183341 Preclinical Pharmacokinetic s Shared Resource
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REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2025-01-16

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 tumor pharmacokinetic (PK) profiles of the FAK inhibitor VS-4718 were evaluated in female Athymic nude mice (Charles River), approximately 12 weeks in age. VS-4718 hydrochloride (SJ000982641-7, MedChemExpress, Cat# HY-13917A, Lot# 89929, purity 99.38%) was suspended in 0.5% sodium carboxymethylcellulose (NaCMC) / 0.1% polysorbate 80 (PS80) in ultrapure water (UP H2O), 10 mg/mL for a 5 mL/kg oral gavage, yielding a 50 mg/kg dose. Three survival blood samples were obtained from each mouse via retro-orbital plexus using 70 µL glass microhematocrit capillary tubes (Fisherbrand, Cat 22362574), and a fourth final sample by cardiac puncture, all using KEDTA as the anticoagulant. Samples were obtained at various times up to 24 hours post-dose, immediately processed to plasma. The carcass was then perfused with PBS, the tumor extracted, rinsed, and placed in a microcentrifuge tube. All samples were immediately stored on dry ice and transferred to -80 °C until analysis.

1.2 Bioanalysis (BA)

Tumor samples were weighed in 15 mL 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) for five cycles of 1 min vibration at 6.5 M/S speed, with 5 min in ice bath 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 VS-4718 (SJ000982641-7, MedChem Express, Cat# HY-13917A, Lot# 89929, purity 99.38%) 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, 20 µL each, were protein precipitated with 130 µL of 5 ng/mL defactinib (Medkoo Biosciences, CAT # 205583, LOT # A7T12K13, Purity 98%) in acetonitrile-methanol (90:10, v/v) as an internal standard (IS). A 3 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 F5 (2.6 µm, 50 mm x 2.1 mm) column maintained at 45 °C with gradient elution at a flow rate of 0.4 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 5% B for 0.5 min with a linear increase to 95% B in 1.5 min. The column was then rinsed for 1.5 min at 95% B and returned to initial mobile phase conditions in 0.3 mins, then equilibrated the column for another 1.2 min for a total run time of 5.0 min. Under these conditions, the analyte and IS eluted at 2.37 and 2.44 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: VS- 4718 502.2 → 471.2, defactinib 511.1 → 312.0. 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 0.5 to 100 ng/mL range, with a correlation coefficient (R) of ≥ 0.9963. 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 0.5 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 harvested from untreated female athymic nude mice or in tumor homogenates. The intra-run precision and accuracy was ≤ 5.36% CV and 97.0% to 111.44%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for VS-4718 were grouped by 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 WinNonlin 8.4 (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).

2.0 RESULTS

The VS-4718 Ct data demonstrated moderate-to-high variability between mice for plasma and tumor, with coefficients of variation ranging from 16.2% to 138%. The absorption rate of VS-4718 was rapid with the Tmax occurring at 0.5 hours post-dose for both plasma and tumor. After Cmax, plasma diminished in a nearly mono-exponential manner, while the tumor declining likewise, and two BLOQ observation at the 24 hour post-dose time point (M16 and M17). Therefore, 24 hr tumor data was excluded from the NCA.

The apparent plasma and tumor terminal half-lives of VS-4718 were 2.31 and 2.42 hours, respectively. The apparent plasma clearance (CL/F) of VS-4718 was moderate at 28.66 mL/min/kg, or approximately 32% of murine hepatic blood flow. The terminal volume of distribution (Vz/F) for VS-4718 was high at 5.74 L/kg, in excess of total body water. The tumor penetration of VS-4718 was moderate, with a Kp,inf of 0.362, and Kp,last of 0.360. The plasma AUCinf (29100 hr-ug/L) in the current study was 1.7-fold lower in comparison with our previous plasma PK study at the same 50 mg/kg dose (RPT.833591-3181183).

At the time of this study, limited preclinical PK was available in the literature for VS-4718. The PK profile of VS-4718 in the current study differed appreciably from previous literature reports in mice, with our mice PK exhibiting a higher Cmax. Walsh et al. reported a VS-4718 total plasma AUCinf of 77400 hr-ug/L with a slower absorption rate, Tmax occurring at 4 hr after a150 mg/kg PO in female BALB/c mice, and a Cmax of 13.98 µM [1]. Our current VS-4718 PK results at 50 mg/kg PO in female athymic nude mice showed a 2.42-fold higher plasma Cmax (33.93 µM), a 2.6-fold lower AUCinf (29100 hr-ng/mL) and a 8-fold faster absorption (Tmax, 0.5 hr). Back-extrapolating from the 150 mg/kg to our 50 mg/kg dose, the AUC values are proportionally lower, however the Cmax was much higher at the lower dose. Most likely, there is a slower rate or lower extent of absorption at higher VS-4718 doses for

As defactinib (VS-6063) is the clinically applied FAKi, and VS-4718 has not been advanced to humans, defining a CRD for VS-4718 is challenging. However, we estimated a CRD by adjusting plasma concentrations for protein binding and potency differences between the two agents. Using equilibrium dialysis, we determined plasma free fractions for defactinib and VS-4718 of 0.0437 ± 0.00178 and ≤ 0.01 respectively. Defactinib is ~2.5-fold more potent than VS-4718 in acellular FAK inhibition assays (0.6 vs 1.5 nM) [2,3]. Therefore, a total plasma correction factor for VS-4718 would be ~11. calculated concentration

In the limited clinical PK data available for defactinib to date, a 200 mg oral dose (the current RP2D in combination with avutometinib) yielded a mean steady state total plasma area under the concentration-time curve (AUC) of 6240 hr-ng/mL, with extraordinarily high variability (90-322 %CV) [4]. In our mouse PK study of VS-4718, we achieved an AUC of 29100 hr-ng/mL with a 50 mg/kg oral dose. Assuming dose-proportional PK, and with the 11-fold correction factor applied, a VS-4718 mouse dose of ~130 mg/kg would equate to defactinib 200 mg in humans by corrected plasma AUC.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

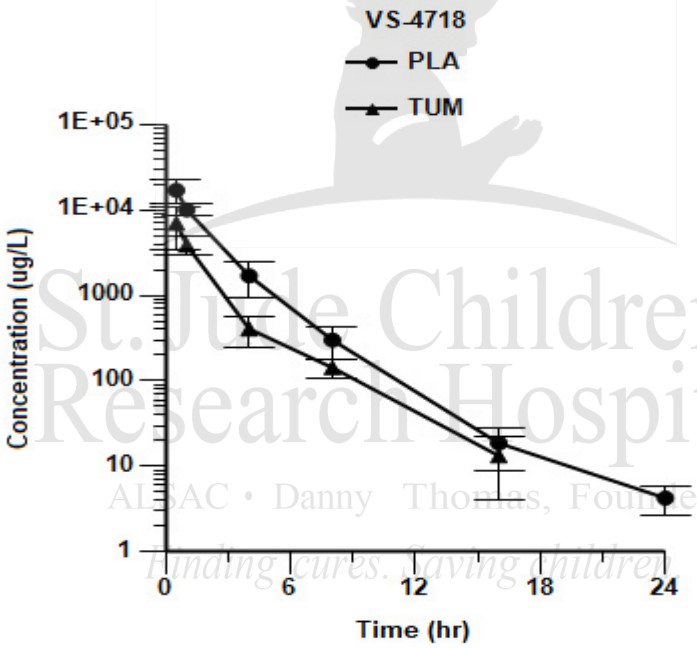

Table 4.1: NCA Parameter Estimates by Analyte and Group

		Analyte	
		VS-4718	
		Group	
		PLA	TUM
Parameter	Unit	Value	
Cmax	ug/L	17000	7010
Tmax	hr	0.500	0.500
AUClast	hr*ug/L	29100	10500
AUCinf	hr*ug/L	29100	10500
Kel	1/hr	0.300	0.287
T1/2	hr	2.31	2.42
CL/F	L/hr/kg	1.72	4.75
Vz/F	L/kg	5.74	16.6
Clast	ug/L	4.26	13.3
Tlast	hr	24.0	16.0
Kp,last			0.360
Kp,inf			0.362

VS-4718 Screening Plasma Tumor PK (SPTPK)

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

		Analyte	
		VS-4718	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	17000	7010
	SD	5100	3670
	Min	11800	3650
	Median	17300	6470
	Max	22000	10900
	CV%	30.0	52.3
	Geometric Mean	16500	6370
	Geometric CV% Mean	32.2	59.3
1.00	N	3	3
	Mean	10000	3880
	SD	1630	948
	Min	8300	2810
	Median	10300	4220
	Max	11500	4620
	CV%	16.2	24.4
	Geometric Mean	9940	3800
	Geometric CV% Mean	16.8	26.9
4.00	N	3	3
	Mean	1700	407
	SD	754	161
	Min	840	250
	Median	2000	399
	Max	2260	572
	CV%	44.4	39.6
	Geometric Mean	1560	385
	Geometric CV% Mean	58.0	43.3
8.00	N	3	3
	Mean	301	143
	SD	127	37.8
	Min	193	99.5
	Median	268	161
	Max	441	168
	CV%	42.3	26.4
	Geometric Mean	284	139
	Geometric CV% Mean	43.5	29.8
16.0	N	3	3
	Mean	18.7	13.3

		Analyte	
		VS-4718	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
	SD	9.70	9.20
	Min	7.98	2.66
	Median	21.3	18.5
	Max	26.8	18.6
	CV%	51.8	69.3
	Geometric Mean	16.6	9.72
	CV% Mean	71.7	159
24.0	N	3	
	Geometric Mean	4.26	
	SD	1.63	
	Min	2.45	
	Median	4.70	
	Max	5.62	
	CV%	38.3	
	Geometric Mean	4.02	
	CV% Mean	45.8	

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	VS-4718	PLA	0.500	21980.00
M1	VS-4718	TUM	0.500	6468.80
M2	VS-4718	PLA	0.500	17319.00
M2	VS-4718	TUM	0.500	10927.00
M3	VS-4718	PLA	0.500	11786.00
M3	VS-4718	TUM	0.500	3648.40
M4	VS-4718	PLA	1.00	10281.00
M4	VS-4718	TUM	1.00	4617.40
M5	VS-4718	PLA	1.00	11528.00
M5	VS-4718	TUM	1.00	4221.60
M6	VS-4718	PLA	1.00	8296.50
M6	VS-4718	TUM	1.00	2813.90
M7	VS-4718	PLA	4.00	840.13
M7	VS-4718	TUM	4.00	398.86
M8	VS-4718	PLA	4.00	2257.60
M8	VS-4718	TUM	4.00	572.40
M9	VS-4718	PLA	4.00	1995.80
M9	VS-4718	TUM	4.00	250.40

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M10	VS-4718	PLA	8.00	268.34
M10	VS-4718	TUM	8.00	168.28
M11	VS-4718	PLA	8.00	193.14
M11	VS-4718	TUM	8.00	161.10
M12	VS-4718	PLA	8.00	441.17
M12	VS-4718	TUM	8.00	99.50
M13	VS-4718	PLA	16.0	21.29
M13	VS-4718	TUM	16.0	18.64
M14	VS-4718	PLA	16.0	7.98
M14	VS-4718	TUM	16.0	2.66
M15	VS-4718	PLA	16.0	26.84
M15	VS-4718	TUM	16.0	18.55
M16	VS-4718	PLA	24.0	2.45
M16	VS-4718	TUM	24.0	BLOQ
M17	VS-4718	PLA	24.0	4.70
M17	VS-4718	TUM	24.0	BLOQ
M18	VS-4718	PLA	24.0	5.62
M18	VS-4718	TUM	24.0	34.814

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

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
VS-4718	PLA	0.500	17000	5100	3
VS-4718	PLA	1.00	10000	1630	3
VS-4718	PLA	4.00	1700	754	3
VS-4718	PLA	8.00	301	127	3
VS-4718	PLA	16.0	18.7	9.70	3
VS-4718	PLA	24.0	4.26	1.63	3
VS-4718	TUM	0.500	7010	3670	3
VS-4718	TUM	1.00	3880	948	3
VS-4718	TUM	4.00	407	161	3
VS-4718	TUM	8.00	143	37.8	3
VS-4718	TUM	16.0	13.3	9.20	3
VS-4718	TUM	24.0			0

5.0 ATTACHED FILES

Attached File 5.1 VS-4718 Screening Plasma PK V1.0.docx – *Final in vivo study plan as executed*

Attached File 5.2 VS-4718 Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

Attached File 5.3 Copy of eDCF_833884__SPTPK_2024-10-18.xlsx – *Digital data collection form for VS-4718 in vivo from client*

Attached File 5.4 VS-4718 Tumor Bearing PK .pdf – *Digital data collection form for VS-4718 in vivo from client*