PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 134364-1407132

STUDY TITLE:

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

SHORT TITLE:	**Ensartinib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Ensartinib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	134364-1407132 Preclinical Pharmacokinetic Shared Resource 124312-1298568
REFERENCE STUDY NUMBERS:	NA NA
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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

Two separate PK studies of ensartinib were conducted in female Athymic nude mice (Charles River Laboratories, Frederick, MD) and are summarized below.

The first ensartinib PK study (SRM2 O/R 124312-1298568, SPPK) was a survival plasma PK evaluation using non-tumor bearing Athymic nude mice. Ensartinib was suspended in 1% hydroxyethylcellulose (MW 720,000), 0.25% Tween 80, and ~0.05% simethicone at 2.5 mg/mL and administered as a 10 mL/kg oral gavage for a 25 mg/kg dose. A batch sampling design was implemented where 3 samples were collected per mouse. Mice were divided into 3 groups for sample collection. Mice from group 1 were sampled at 0.125, 1, and 16 hr post-dose. Mice from group 2 were sampled at 0.25, 2, and 24 hr, and mice from group 3 were sampled at 0.5, 4, and 8 hr post-dose. Blood samples (~ 50 µL) were collected by retro-orbital eye bleed technique using Minivette POCT 50 µL capillary devices containing K3EDTA (Sarstedt AG, Germany). Terminal samples at the last time point were collected by cardiac puncture using a 1 mL syringe, and the blood placed in a Sarstedt Microvette K3EDTA 500 µL tube.

In the second ensartinib PK study (SRM2 O/R 134364-1407132, SPTPK), the plasma and tumor PK were evaluated after a single oral dose of the ensartinib 25 mg/kg suspension. Female Athymic nude mice bearing neuroblastoma (MAST 3) orthotopic xenografts in the adrenal capsule were sacrificed using an IACUC-approved method at 0.125, 1, 4, 8, 16 hr post-dose (3 mice per timepoint). Blood was collected by cardiac puncture, after which the carcass was perfused with PBS, the tumor extracted, rinsed, and placed in a microcentrifuge tube.

In all instances, blood samples were immediately centrifuged to plasma. Plasma and tumor samples were temporarily placed on dry ice until transfer to a deep freezer, and samples were stored at -80 °C until analysis.

Additionally, ensartinib fraction unbound in mouse and human plasma (Fu,p,m and Fu,p,h), and patient derived rhabdomyosarcoma tumor homogenate (Fu,t) was determined using rapid equilibrium dialysis (RED, Pierce Biotechnology, ThermoFisher Scientific, Waltham, MA). Briefly, blank mouse plasma and tumor homogenates, diluted with PBS, were spiked with compounds in triplicate achieving final concentrations of 10 µM, placed in donor wells of RED apparatus, and permitted to equilibrate for 4-6 hours at 37 °C. Compounds were assayed in donor and receiver well samples using LC-MS, with the fraction unbound calculated as the ratio of concentration in receiver to donor adjusted for any dilution [1]. These experiments were conducted fully by SJCRH Chemical Biology and Therapeutics (CBT) Analytical Technologies Center (ATC) personnel under the direction of Lei Yang.

1.2 Bioanalysis

Frozen tumor samples were weighed in tared 15 mL Lysing Matrix D tubes (MP Biomedical, Santa Ana, CA) and diluted with a 5:1 volume of ultra-pure water. The tumor samples were then homogenized with a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). The homogenization consisted of four 6.0 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.

Plasma and tumor samples were analyzed for ensartinib (Selleckchem, Lot # S823001, purity 99.4%) with a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in acetonitrile. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of 25 ng/mL tazemetostat (ADOOQ, Lot # L12712B002, purity 99.7%) in acetonitrile as an internal standard. A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a LEAP CTC PAL autosampler.

The LC separation was performed using a Phenomenex Kinetex EVO (2.6 µm C18 100Å, 50 x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.5 mL/min. The binary mobile phase consisted of water-acetonitrile-200 mM ammonium acetate pH 6.0 (90:10:10 v/v/v) in reservoir A and acetonitrile-water-200 mM ammonium acetate pH 6.0 (90:10:10 v/v/v) in reservoir B. The initial mobile phase consisted of 10% B with a linear increase to 100% B in 4 min. The column was then rinsed for 1 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 7 min. Under these conditions, the analyte and IS eluted at 2.01 and 2.28 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode with the following mass transitions were monitored: ensartinib 561.16 -> 257.10, and tazemetostat 573.30 -> 486.30.

The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of 0.9995. 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. Sample dilution integrity was confirmed. For the plasma matrix, the intra-run precision and accuracy was ≤ 4.51% CV and 94.0% to 99.9%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The resultant ensartinib concentration-time (Ct) data were grouped by study, 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 were generated, and the ensartinib arithmetic mean Ct data for 1) each study and matrix, and for 2) plasma as an aggregate across studies (Study = Aggregate), was subjected to noncompartmental pharmacokinetic analysis (NCA).

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 ensartinib 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 human unbound plasma PK value at the recommended phase 2 dose (RP2D) of ensartinib of 225 mg PO QD [2]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

The PK results for individual studies and as an aggregate for plasma across all the studies are presented in Section 4.0. The aggregate plasma results are being referenced for overall inferences, including the clinically relevant dose (CRD) derivations. However, the plasma PK results between the two PK studies were similar and within two-fold for most paramters.

Prior to the plasma Tmax of 2 hr, plasma ensartinib concentrations demonstrated appreciable variability. The mouse plasma PK was similar to that observed by Lovly, with the 2 hr total plasma concentrations being within 2-fold [3]. Ensartinib showed a slightly delayed but high distribution to the tumor, with a Cmax at 4 hours, and a Kp,tumor of 3.23. The estimated total plasma AUC at the RP2D of 225 mg PO QD in humans was 5530 hr-ug/L [2]. The observed Fu,p,h in the RED binding studies was 0.0349, which yields a human AUCu of 193 hr-ug/L. As the mouse AUCu was calculated as 45 hr-ug/L at 25 mg/kg (Fu,p,m = 0.0146), a CRD would be ensartinib 50 mg/kg BID. The twice daily dosing is suggested to achieve a lower peak to trough fluctuation and prolong pharmacodynamic coverage, secondary to the short mouse half-life versus humans.

3.0 REFERENCES

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3. Lovly CM, Heuckmann JM, Stanchina E de, Chen H, Thomas RK, Liang C, Pao W. Insights into ALK-Driven Cancers Revealed through Development of Novel ALK Tyrosine Kinase Inhibitors. Cancer Res. 2011 Jul 15;71(14):4920–31.

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of Ensartinib by Study and Group

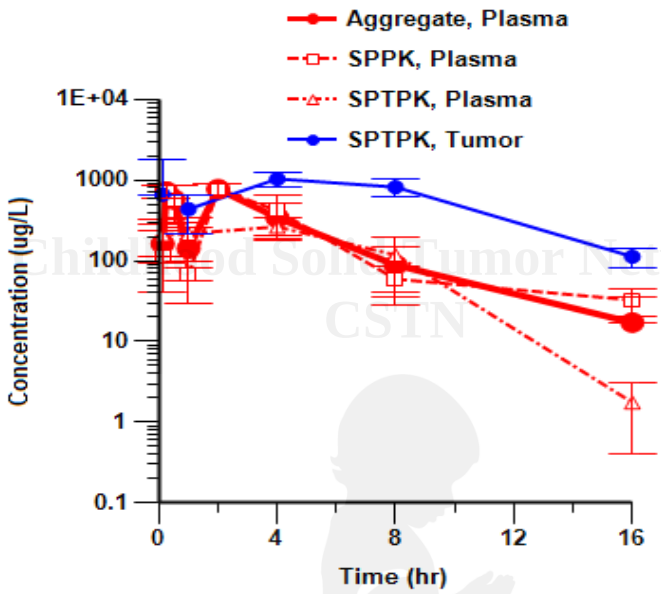

Table 4.1: NCA PK Parameter Estimates of Ensartinib by Study and Group

		Analyte			
		Ensartinib			
		Study			
		Aggregate	SPPK	SPTPK	
		Group	Group	Group	
		Plasma	Plasma	Plasma	Tumor
Parameter	Units	Estimate			
Cmax	ug/L	774	774	265	1040
Tmax	hr	2.00	2.00	4.00	4.00
AUClast	hr*ug/L	3000	3020	1870	9280
AUCinf	hr*ug/L	3070	3150	1880	9920
Kel	1/hr	0.243	0.193	0.433	0.194
T1/2	hr	2.85	3.59	1.60	3.58
CL/F	L/hr/kg	8.16	7.94	13.3	2.52
Vz/F	L/kg	33.6	41.1	30.8	13.0
Clast	ug/L	17.2	32.6	1.76	113
Tlast	hr	16.0	16.0	16.0	16.0
Kp,tumor*	-	*	-	-	3.23

* Kp,tumor calculated as AUCinf, tumor / AUCinf, plasma, Aggregate

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

		Analyte			
		Ensartinib			
		Study			
		Aggregate	SPPK	SPTPK	
		Group	Group	Group	
		Plasma	Plasma	Plasma	Tumor
Time (hr)		Concentration (ug/L)			
0.125	N	6	3	3	3
	Mean	163	114	211	660
	SD	121	126	117	1110
	Min	11.7	11.7	133	15.5
	Median	144	75.2	154	18.4
	Max	346	255	346	1940
	CV%	74.6	111	55.5	169
	Geometric Mean	108	60.7	192	82.2
	CV% Geometric Mean	183	320	54.9	4280
0.250	N	3	3		
	Mean	723	723		
	SD	130	130		
	Min	574	574		
	Median	789	789		
	Max	806	806		
	CV%	17.9	17.9		
	Geometric Mean	715	715		
	CV% Geometric Mean	19.2	19.2		
0.500	N	3	3		
	Mean	577	577		
	SD	279	279		
	Min	353	353		
	Median	488	488		
	Max	889	889		
	CV%	48.3	48.3		
	Geometric Mean	535	535		
	CV% Geometric Mean	49.5	49.5		
1.000	N	6	3	3	3
	Mean	142	67.9	217	437
	SD	112	12.4	121	225
	Min	57.9	57.9	77.4	177

Ensartinib Screening Plasma Tumor PK (SPTPK)

		Analyte			
		Ensartinib			
		Study			
		Aggregate	SPPK	SPTPK	
		Group	Group	Group	
		Plasma	Plasma	Plasma	Tumor
Time (hr)		Concentration (ug/L)			
	Median	79.6	63.9	280	551
	Max	294	81.8	294	583
	CV%	78.8	18.3	55.8	51.6
	Geometric Mean	112	67.1	185	385
	CV% Geometric Mean	85.7	18.0	87.9	75.3
2.000	N	3	3		
	Mean	774	774		
	SD	112	112		
	Min	661	661		
	Median	774	774		
	Max	886	886		
	CV%	14.5	14.5		
	Geometric Mean	768	768		
	CV% Geometric Mean	14.7	14.7		
4.000	N	6	3	3	2
	Mean	343	420	265	1040
	SD	168	216	76.2	202
	Min	173	173	187	894
	Median	303	521	267	1040
	Max	568	568	339	1180
	CV%	49.1	51.4	28.8	19.5
	Geometric Mean	309	371	257	1030
	CV% Geometric Mean	53.6	74.5	30.7	19.8
8.000	N	6	3	3	3
	Mean	88.8	59.5	118	820
	SD	60.8	24.7	77.8	191
	Min	35.6	35.6	44.4	680
	Median	71.4	57.9	111	743
	Max	199	84.9	199	1040
	CV%	68.5	41.6	65.8	23.2
	Geometric Mean	74.5	55.9	99.3	807
	CV% Geometric Mean	70.5	45.8	87.9	22.5

		Analyte			
		Ensartinib			
		Study			
		Aggregate	SPPK	SPTPK	
		Group	Group	Group	
		Plasma	Plasma	Plasma	Tumor
Time (hr)		Concentration (ug/L)			
16.000	N	6	3	3	3
	Mean	17.2	32.6	1.76	113
	SD	18.7	12.5	1.35	30.9
	Min	0.500	22.6	0.500	77.6
	Median	12.9	28.7	1.59	126
	Max	46.6	46.6	3.19	135
	CV%	109	38.2	76.8	27.3
	Geometric Mean	6.52	31.1	1.36	110
	CV% Geometric Mean	522	38.1	118	30.8
24.000	N	0	0		
	Mean				
	SD				
	Min				
	Median				
	Max				
	CV%				
	Geometric Mean				
	CV% Geometric Mean				

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

Study	Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
Aggregate	73.00	Ensartinib	Plasma	0.13	255.48
Aggregate	73.00	Ensartinib	Plasma	1.00	81.80
Aggregate	73.00	Ensartinib	Plasma	16.00	46.58
Aggregate	74.00	Ensartinib	Plasma	0.13	11.66
Aggregate	74.00	Ensartinib	Plasma	1.00	57.87
Aggregate	74.00	Ensartinib	Plasma	16.00	22.61
Aggregate	75.00	Ensartinib	Plasma	0.13	75.18
Aggregate	75.00	Ensartinib	Plasma	1.00	63.92
Aggregate	75.00	Ensartinib	Plasma	16.00	28.67
Aggregate	76.00	Ensartinib	Plasma	0.25	789.32
Aggregate	76.00	Ensartinib	Plasma	2.00	774.04

Ensartinib Screening Plasma Tumor PK (SPTPK)

Study	Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
Aggregate	76.00	Ensartinib	Plasma	24.00	
Aggregate	77.00	Ensartinib	Plasma	0.25	805.99
Aggregate	77.00	Ensartinib	Plasma	2.00	885.69
Aggregate	77.00	Ensartinib	Plasma	24.00	
Aggregate	78.00	Ensartinib	Plasma	0.25	573.76
Aggregate	78.00	Ensartinib	Plasma	2.00	661.38
Aggregate	78.00	Ensartinib	Plasma	24.00	
Aggregate	79.00	Ensartinib	Plasma	0.50	888.79
Aggregate	79.00	Ensartinib	Plasma	4.00	520.92
Aggregate	79.00	Ensartinib	Plasma	8.00	35.58
Aggregate	80.00	Ensartinib	Plasma	0.50	353.34
Aggregate	80.00	Ensartinib	Plasma	4.00	172.58
Aggregate	80.00	Ensartinib	Plasma	8.00	57.90
Aggregate	81.00	Ensartinib	Plasma	0.50	487.76
Aggregate	81.00	Ensartinib	Plasma	4.00	567.84
Aggregate	81.00	Ensartinib	Plasma	8.00	84.94
Aggregate	82.00	Ensartinib	Plasma	0.13	154.41
Aggregate	83.00	Ensartinib	Plasma	0.13	345.73
Aggregate	84.00	Ensartinib	Plasma	0.13	133.13
Aggregate	85.00	Ensartinib	Plasma	1.00	279.51
Aggregate	86.00	Ensartinib	Plasma	1.00	293.99
Aggregate	87.00	Ensartinib	Plasma	1.00	77.36
Aggregate	88.00	Ensartinib	Plasma	4.00	339.43
Aggregate	89.00	Ensartinib	Plasma	4.00	267.42
Aggregate	90.00	Ensartinib	Plasma	4.00	187.06
Aggregate	91.00	Ensartinib	Plasma	8.00	199.35
Aggregate	92.00	Ensartinib	Plasma	8.00	110.54
Aggregate	93.00	Ensartinib	Plasma	8.00	44.39
Aggregate	94.00	Ensartinib	Plasma	16.00	1.59
Aggregate	95.00	Ensartinib	Plasma	16.00	3.19
Aggregate	96.00	Ensartinib	Plasma	16.00	0.50
SPPK	73.00	Ensartinib	Plasma	0.13	255.48
SPPK	73.00	Ensartinib	Plasma	1.00	81.80
SPPK	73.00	Ensartinib	Plasma	16.00	46.58
SPPK	74.00	Ensartinib	Plasma	0.13	11.66
SPPK	74.00	Ensartinib	Plasma	1.00	57.87
SPPK	74.00	Ensartinib	Plasma	16.00	22.61
SPPK	75.00	Ensartinib	Plasma	0.13	75.18
SPPK	75.00	Ensartinib	Plasma	1.00	63.92
SPPK	75.00	Ensartinib	Plasma	16.00	28.67
SPPK	76.00	Ensartinib	Plasma	0.25	789.32
SPPK	76.00	Ensartinib	Plasma	2.00	774.04

Ensartinib Screening Plasma Tumor PK (SPTPK)

Study	Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
SPPK	76.00	Ensartinib	Plasma	24.00	
SPPK	77.00	Ensartinib	Plasma	0.25	805.99
SPPK	77.00	Ensartinib	Plasma	2.00	885.69
SPPK	77.00	Ensartinib	Plasma	24.00	
SPPK	78.00	Ensartinib	Plasma	0.25	573.76
SPPK	78.00	Ensartinib	Plasma	2.00	661.38
SPPK	78.00	Ensartinib	Plasma	24.00	
SPPK	79.00	Ensartinib	Plasma	0.50	888.79
SPPK	79.00	Ensartinib	Plasma	4.00	520.92
SPPK	79.00	Ensartinib	Plasma	8.00	35.58
SPPK	80.00	Ensartinib	Plasma	0.50	353.34
SPPK	80.00	Ensartinib	Plasma	4.00	172.58
SPPK	80.00	Ensartinib	Plasma	8.00	57.90
SPPK	81.00	Ensartinib	Plasma	0.50	487.76
SPPK	81.00	Ensartinib	Plasma	4.00	567.84
SPPK	81.00	Ensartinib	Plasma	8.00	84.94
SPTPK	82.00	Ensartinib	Plasma	0.13	154.41
SPTPK	82.00	Ensartinib	Tumor	0.13	1944.80
SPTPK	83.00	Ensartinib	Plasma	0.13	345.73
SPTPK	83.00	Ensartinib	Tumor	0.13	18.37
SPTPK	84.00	Ensartinib	Plasma	0.13	133.13
SPTPK	84.00	Ensartinib	Tumor	0.13	15.53
SPTPK	85.00	Ensartinib	Plasma	1.00	279.51
SPTPK	85.00	Ensartinib	Tumor	1.00	550.58
SPTPK	86.00	Ensartinib	Plasma	1.00	293.99
SPTPK	86.00	Ensartinib	Tumor	1.00	582.67
SPTPK	87.00	Ensartinib	Plasma	1.00	77.36
SPTPK	87.00	Ensartinib	Tumor	1.00	177.49
SPTPK	88.00	Ensartinib	Plasma	4.00	339.43
SPTPK	89.00	Ensartinib	Plasma	4.00	267.42
SPTPK	89.00	Ensartinib	Tumor	4.00	1180.00
SPTPK	90.00	Ensartinib	Plasma	4.00	187.06
SPTPK	90.00	Ensartinib	Tumor	4.00	893.79
SPTPK	91.00	Ensartinib	Plasma	8.00	199.35
SPTPK	91.00	Ensartinib	Tumor	8.00	743.47
SPTPK	92.00	Ensartinib	Plasma	8.00	110.54
SPTPK	92.00	Ensartinib	Tumor	8.00	680.43
SPTPK	93.00	Ensartinib	Plasma	8.00	44.39
SPTPK	93.00	Ensartinib	Tumor	8.00	1037.40
SPTPK	94.00	Ensartinib	Plasma	16.00	1.59
SPTPK	94.00	Ensartinib	Tumor	16.00	125.53
SPTPK	95.00	Ensartinib	Plasma	16.00	3.19

Study	Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
SPTPK	95.00	Ensartinib	Tumor	16.00	135.26
SPTPK	96.00	Ensartinib	Plasma	16.00	0.50
SPTPK	96.00	Ensartinib	Tumor	16.00	77.63

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

Variable	Units	Analyte	Study	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Ensartinib	Aggregate	Plasma	0.13	162.60	121.26	6.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	0.25	723.02	129.53	3.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	0.50	576.63	278.57	3.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	1.00	142.41	112.24	6.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	2.00	773.70	112.16	3.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	4.00	342.54	168.10	6.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	8.00	88.78	60.77	6.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	16.00	17.19	18.67	6.00
Concentration	ug/L	Ensartinib	Aggregate	Plasma	24.00			0.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	0.13	114.11	126.49	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	0.25	723.02	129.53	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	0.50	576.63	278.57	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	1.00	67.86	12.44	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	2.00	773.70	112.16	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	4.00	420.45	215.94	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	8.00	59.47	24.72	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	16.00	32.62	12.46	3.00
Concentration	ug/L	Ensartinib	SPPK	Plasma	24.00			0.00
Concentration	ug/L	Ensartinib	SPTPK	Plasma	0.13	211.09	117.09	3.00
Concentration	ug/L	Ensartinib	SPTPK	Plasma	1.00	216.95	121.11	3.00
Concentration	ug/L	Ensartinib	SPTPK	Plasma	4.00	264.64	76.22	3.00
Concentration	ug/L	Ensartinib	SPTPK	Plasma	8.00	118.09	77.76	3.00
Concentration	ug/L	Ensartinib	SPTPK	Plasma	16.00	1.76	1.35	3.00
Concentration	ug/L	Ensartinib	SPTPK	Tumor	0.13	659.57	1113.05	3.00
Concentration	ug/L	Ensartinib	SPTPK	Tumor	1.00	436.91	225.24	3.00
Concentration	ug/L	Ensartinib	SPTPK	Tumor	4.00	1036.90	202.38	2.00
Concentration	ug/L	Ensartinib	SPTPK	Tumor	8.00	820.43	190.52	3.00
Concentration	ug/L	Ensartinib	SPTPK	Tumor	16.00	112.81	30.85	3.00

5.0 ATTACHED FILES

Attached File 5.1 Ensartinib Screening Plasma PK.docx – *Final in vivo study plan as executed (SRM2 O/R 124312-1298568, SPPK)*

Attached File 5.2 Ensartinib Screening Plasma and Tumor PK.docx – *Final in vivo study plan as executed (SRM2 O/R 134364-1407132, SPTPK)*

Attached File 5.3 Ensartinib PK_non tumor.docx – *Digital data collection form from SPPK in vivo study*

Attached File 5.4 Ensartinib PK tumor bearing study sheet .docx – *Digital data collection form from in vivo SPTPK study*

Attached File 5.5 Ensartinib Screening Plasma and Tumor PK TLFs.docx – *Tables, listings, and figures from SPTPK report in Word document for reformatting or manipulations*