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 and St. Jude Children's Research Hospital, 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, 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.

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 244899-2289956

STUDY TITLE:

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

SHORT TITLE:	Elimusertib Screening Plasma, Brain and Tumor PK (SPTPK)
TEST ARTICLE:	Elimusertib (as the HCl salt)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	244899-2289956 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	244899 P-PKSR Study ID
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FOR P-PKSR APPROVED USE AND DISTRIBUTION

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1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma, brain and MAST39 rhabdomyosarcoma orthotopic tumor pharmacokinetic (PK) profile of elimusertib was evaluated in female Athymic Nude mice (Charles River) approximately 8-12 weeks in age. Elimusertib hydrochloride (SJ001007686-04, MedChemExpress, Cat # HY-101566A, Lot # 46170, 97.30% purity) was formulated in 1% hydroxyethylcellulose (HEC, MW 90000) / 0.25% Tween 80 at 5 mg/mL for a 50 mg/kg free base equivalent dose, and a10 mL/kg oral gavage (formulation performed by CBT ATC staff).Terminal samples were collected over a 24 hour post-dose period by cardiac puncture using a 1 mL syringe, and the blood placed in a Sarstedt Microvette K3EDTA 500 µL tube, and immediately processed to plasma.
The carcasses were perfused with PBS, brains and tumors extracted, rinsed, and placed in microcentrifuge tubes. All samples were immediately stored on dry ice and transferred to -80 °C until analysis. Remaining formulation, stored at 4°C protected from light, was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Brain and tumor samples were weighed in 4.5 mL and 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 for 24 hours until analysis.

Plasma, brain and tumor homogenate samples were analyzed for elimusertib (SJ001007686-04, MedChemExpress, Cat # HY-101566A, Lot # 46170, 97.30% purity) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls (QCs) were spiked with solutions, corrected for salt content and purity as necessary, prepared in methanol. Plasma, brain and tumor homogenate samples, 25 µL each, were protein precipitated with 120 µL of 8 ng/mL AZD-6738 (SJ000861428-02, LC-LABS, Cat/Lot # L-15794B001, purity 98%) in acetonitrile-methanol (90%:10%, v/v) as an internal standard (IS). A 5 µL aliquot of the extracted supernatant was injected onto a AB Sciex ExionLC high performance liquid chromatography system via a AB 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 50 °C with gradient elution at a flow rate of 0.40 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 15% B for 0.5 min with a linear increase to 98% B in 1.0 min. The column was then rinsed for 1.3 min at 98% B and returned to initial mobile phase conditions in 0.4 mins, then equilibrated the column for another 1.4 min for a total run time of 4.6 min. Under these conditions, the analyte and IS eluted at 1.87 and 1.84 min, respectively for both plasma and brain homogenate samples.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX Triple Quad™ 3500 LC-MS/MS system in the positive ESI mode and the following mass transitions were monitored: elimusertib 376.1 -> 318.0, AZD-6738 413.1 -> 334.1. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the plasma calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9991. Sample dilution integrity was confirmed. 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 brain. The intra-run precision and accuracy was ≤ 4.5% CV and 98% to 104%, respectively. No matrix effects, ion enhancement or suppression, were detected in blank EDTA plasma harvested from untreated female Athymic Nude mice, brain homogenates, or pooled human and CD-1 mouse plasma mixed 1:1 with PBS. Elimusertib was stable for 3 weeks at -80 °C in CD-1 mouse plasma and tumor homogenate QCs at two levels, showing accuracies of 90% to 109%. However, Athymic Nude mouse brain homogenate QCs failed to meet stability requirements under these conditions, showing an accuracy of 120% to 127%. This

finding has minimal impact, as brain samples from our PK studies of elimusertib were frozen whole at -80 °C, and processed for analysis within 1 week of storage.

1.3 Pharmacokinetic (PK) Analysis

Plasma, brain and tumor concentration-time (Ct) data for elimusertib 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, or 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-tissue partition coefficients (Kp,inf) were estimated as the ratio of the AUCinf in tissue to AUCinf plasma, whereas Kp,last was similarly estimated using AUClast values.

Elimusertib fractions unbound in mouse and human plasma (Fu,p,m and Fu,p,h), and in mouse brain homogenate (Fu,b) were determined using rapid equilibrium dialysis (RED, 8K MWCO, Pierce Biotechnology, ThermoFisher Scientific, Waltham, MA). Briefly, blank plasma and brain homogenates were spiked with compounds and diluted with 100 mM PBS (6 replicates per group), achieving back-calculated final concentrations in blank plasma and brain tissue of 5 µM. The diluted samples were placed in donor wells of RED apparatus, and permitted to equilibrate for 6 hours (plasma) and 4 hours (brain) at 37 °C with 5% CO2. Compounds concentrations were assayed in donor and receiver well samples using the qualified LC-MS/MS assay, with the fraction unbound calculated as the ratio of concentration in receiver to donor, adjusted for any dilution [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 elimusertib plasma, brain and tumor Ct data demonstrated moderate variability between and within mice for plasma, with coefficients of variation ranging from 18.5% to 81.1%; 25.8% to 80.8% for brain and 16.2% to 73.6% for tumor. The absorption rate of elimusertib was rapid, with the Tmax occurring at 0.5 hours post-dose for plasma, brain, and tumor, suggesting a rapid equilibrium with tissues. After Cmax, plasma, brain and tumor concentrations diminished in a bi-exponential manner with BLOQ observations at 16 and 24 hour post-dose time points for all mice. The apparent plasma, brain and tumor terminal half-life of elimusertib were 1.58, 2.26 and 1.73 hours respectively. The apparent plasma clearance (CL/F) of elimusertib was high at 143 mL/min/kg, or approximately 1.6-fold higher than the murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for elimusertib was also high at 19.6 L/kg, in excess of total body water. The plasma to tumor partitioning of elimusertib was high, with a Kp,inf

of 0.869, and Kp,last of 0.859, whereas the brain penetration was low-to-moderate with a Kp,inf of 0.305, and Kp,last of 0.293. The fractions unbound in mouse brain and plasma were 0.0293 and 0.0567 respectively, resulting in an unbound Kp,inf (Kpuu,inf) estimate of 0.158 for elimusertib in this study. The formulation was found to be in specification, with an elimusertib concentration of 4.81 ± 0.418 mg/mL (CV = 8.45%) after storing at 4°C protected from light for 28 days.

Compared with our previous elimusertib plasma and brain PK study (RPT.244898-2289955), the current mice had a 1.54-fold higher CL/F, resulting in a similar fold decrease in AUCinf (8960 to 5820 hr-ug/L). There was also a 1.4-fold increase in Vz/F, contributing to the lower exposure and Cmax in the current study. While the T1/2 in the current study was slightly shorter, it was similar to that observed in the previous study (1.58 vs. 1.73 hr).

Elimusertib brain exposures in the current study, calculated using brain and plasma AUC values, appeared about 30% lower than our previous assessment. This is a confounded comparison however, as the previous study's assessment was based on a brain:plasma concentration ratio at only 8 hours due to BLOQ data.

Wengner et al. presented plasma Ct profile figures from mice dosed with elimusertib from 3 to 50 mg/kg PO BID for 3 days [3]. Digital extraction of this Ct data suggested a super-proportionality in elimusertib exposure with either dose or time. The AUC estimated with NCA showed a 59-fold increase with only a ~17-fold increase in dosage from 3 to 50 mg/kg. It is difficult to compare our PK findings with Wengner's, given the differences between studies, such as single dose vs. multiple doses and the different formulations used. However, our current elimusertib PK results show a similar or higher Cmax, a substantially higher CL/F, and shorter T1/2 compared with Wengner's.

The single agent MTD and optimal dosing schedule of elimusertib was reported as 50 mg/kg PO BID for 3 days on / 4 days off in preclinical models for antitumor efficacy [3]. In the first-in-human clinical study, the MTD of elimusertib was estimated at 40 mg PO BID for 3 days on / 4 days off in adults, with the total plasma AUCtau values at steady state being 11432-15642 hr-ng/mL [4].The fraction unbound in plasma (Fu,p) for elimusertib has been estimated as 0.0567 and 0.0294 in mice and humans respectively from our protein binding studies. Using the PK findings from our current study, precise elimusertib CRDs calculated by unbound AUCs between humans and mice range from 50 - 70 mg/kg PO BID. However, a dosage of 50 mg/kg PO BID 3 days on / 4 days off every 21 days is comparable with the clinically relevant MTD range (i.e. within 2-fold), is supported by the preclinical literature, and is therefore recommended.

3.0 REFERENCES

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Ataxia Telangiectasia and RAD3-Related (ATR) Inhibitor BAY 1895344 in Patients with Advanced Solid Tumors. Cancer Discov. 2021 Jan 1;11(1):80–91.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

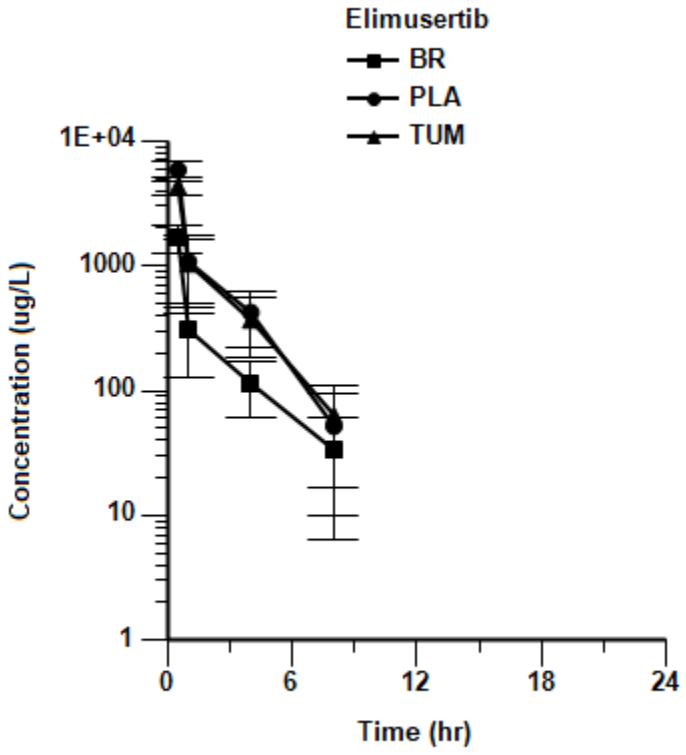

Table 4.1: NCA Parameter Estimates by Analyte and Group

		Analyte		
		Elimusertib		
		Group		
		BR	PLA	TUM
Parameter	Unit	Value		
Cmax	ug/L	1660	5870	4360
Tmax	hr	0.500	0.500	0.500
AUClast	hr*ug/L	1670	5690	4890
AUCinf	hr*ug/L	1780	5820	5060
Kel	1/hr	0.307	0.439	0.401
T1/2	hr	2.26	1.58	1.73
CL/F	L/hr/kg	28.1	8.58	9.88
Vz/F	L/kg	91.8	19.6	24.7
Clast	ug/L	33.6	51.9	63.9

		Analyte		
		Elimusertib		
		Group		
		BR	PLA	TUM
Parameter	Unit	Value		
Tlast	hr	8.00	8.00	8.00
Kp,last		0.305		0.859
Kp,inf		0.293		0.869

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

		Analyte		
		Elimusertib		
		Group		
		BR	PLA	TUM
Time (hr)		Concentration (ug/L)		
0.500	N	3	3	3
	Mean	1660	5870	4360
	SD	429	1090	706
	Min	1360	5000	3590
	Median	1470	5520	4520
	Max	2150	7090	4980
	CV%	25.8	18.5	16.2
	Geometric Mean	1630	5810	4320
	CV% Geometric Mean	24.9	18.1	16.9
1.00	N	3	3	3
	Mean	308	1080	1040
	SD	183	660	584
	Min	177	654	521
	Median	231	746	928
	Max	517	1840	1670
	CV%	59.3	61.1	56.1
	Geometric Mean	276	965	932
	CV% Geometric Mean	60.5	61.1	63.6
4.00	N	3	3	3
	Mean	114	423	370
	SD	53.2	203	183
	Min	67.2	247	249
	Median	104	377	280
	Max	172	645	581
	CV%	46.5	47.9	49.5
	Geometric Mean	106	392	343
	CV% Geometric Mean	49.8	51.0	48.4
8.00	N	3	3	3
	Mean	33.6	51.9	63.9

		Analyte		
		Elimusertib		
		Group		
		BR	PLA	TUM
Time (hr)		Concentration (ug/L)		
	SD	27.1	42.1	47.0
	Min	6.31	8.91	20.1
	Median	33.9	53.8	57.9
	Max	60.6	93.0	114
	CV%	80.8	81.1	73.6
	Geometric Mean	23.5	35.5	51.0
	CV% Geometric Mean	172	187	107
16.0	N	0	0	0
	Mean			
	SD			
	Min			
	Median			
	Max			
	CV%			
	Geometric Mean			
	CV% Geometric Mean			
24.0	N	0	0	0
	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	Elimusertib	BR	0.500	2152.00
M1	Elimusertib	PLA	0.500	5523.00
M1	Elimusertib	TUM	0.500	4521.60
M2	Elimusertib	BR	0.500	1360.50
M2	Elimusertib	PLA	0.500	4998.50
M2	Elimusertib	TUM	0.500	3591.10
M3	Elimusertib	BR	0.500	1469.20
M3	Elimusertib	PLA	0.500	7086.10
M3	Elimusertib	TUM	0.500	4976.80
M4	Elimusertib	BR	1.00	176.64

Elimusertib Screening Plasma, Brain And Tumor PK (SPTPK)

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M4	Elimusertib	PLA	1.00	654.31
M4	Elimusertib	TUM	1.00	521.21
M5	Elimusertib	BR	1.00	231.27
M5	Elimusertib	PLA	1.00	746.25
M5	Elimusertib	TUM	1.00	1672.80
M6	Elimusertib	BR	1.00	516.71
M6	Elimusertib	PLA	1.00	1840.10
M6	Elimusertib	TUM	1.00	928.10
M7	Elimusertib	BR	4.00	104.18
M7	Elimusertib	PLA	4.00	376.85
M7	Elimusertib	TUM	4.00	248.69
M8	Elimusertib	BR	4.00	67.18
M8	Elimusertib	PLA	4.00	247.44
M8	Elimusertib	TUM	4.00	280.20
M9	Elimusertib	BR	4.00	172.13
M9	Elimusertib	PLA	4.00	645.23
M9	Elimusertib	TUM	4.00	580.52
M10	Elimusertib	BR	8.00	33.86
M10	Elimusertib	PLA	8.00	53.78
M10	Elimusertib	TUM	8.00	57.89
M11	Elimusertib	BR	8.00	60.58
M11	Elimusertib	PLA	8.00	93.01
M11	Elimusertib	TUM	8.00	113.64
M12	Elimusertib	BR	8.00	6.31
M12	Elimusertib	PLA	8.00	8.91
M12	Elimusertib	TUM	8.00	20.12
M13	Elimusertib	BR	16.0	BLOQ
M13	Elimusertib	PLA	16.0	BLOQ
M13	Elimusertib	TUM	16.0	BLOQ
M14	Elimusertib	BR	16.0	BLOQ
M14	Elimusertib	PLA	16.0	BLOQ
M14	Elimusertib	TUM	16.0	4.95
M15	Elimusertib	BR	16.0	BLOQ
M15	Elimusertib	PLA	16.0	BLOQ
M15	Elimusertib	TUM	16.0	BLOQ
M16	Elimusertib	BR	24.0	BLOQ
M16	Elimusertib	PLA	24.0	BLOQ
M16	Elimusertib	TUM	24.0	BLOQ
M17	Elimusertib	BR	24.0	BLOQ
M17	Elimusertib	PLA	24.0	BLOQ
M17	Elimusertib	TUM	24.0	BLOQ
M18	Elimusertib	BR	24.0	BLOQ
M18	Elimusertib	PLA	24.0	BLOQ

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M18	Elimusertib	TUM	24.0	BLOQ

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

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Elimusertib	BR	0.500	1660	429	3
Elimusertib	BR	1.00	308	183	3
Elimusertib	BR	4.00	114	53.2	3
Elimusertib	BR	8.00	33.6	27.1	3
Elimusertib	BR	16.0			0
Elimusertib	BR	24.0			0
Elimusertib	PLA	0.500	5870	1090	3
Elimusertib	PLA	1.00	1080	660	3
Elimusertib	PLA	4.00	423	203	3
Elimusertib	PLA	8.00	51.9	42.1	3
Elimusertib	PLA	16.0			0
Elimusertib	PLA	24.0			0
Elimusertib	TUM	0.500	4360	706	3
Elimusertib	TUM	1.00	1040	584	3
Elimusertib	TUM	4.00	370	183	3
Elimusertib	TUM	8.00	63.9	47.0	3
Elimusertib	TUM	16.0			0
Elimusertib	TUM	24.0			0

5.0 ATTACHED FILES

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

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

Attached File 5.3 CBT-ATC_ElimusertibQCcertificate_Passed.pdf

Attached File 5.4 Elimusertib TB PK Study Sheet.xlsx –*Dosing and collection and weight of tumors form for elimusertib in vivo from client*

Attached File 5.5 Copy of 244899-2289956_ELM_SPTPK_2021-12-08.xlsx – *Digital data collection form for elimusertib in vivo from client*