PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 54548-487607

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF SELINEXOR IN FEMALE CD1 NU MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Selinexor Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Selinexor
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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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 total plasma and tumor PK of selinexor in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single oral dose. Selinexor (ABMOLE, M3152, Purity > 98%) was suspended in 0.5% Pluronic F68 at 2.5 mg/mL for a 10 mL/kg gavage. Mice were sacrificed using an IACUC-approved method at 10 min, 1, 4, 8, and 16 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for remainder of study. Mice were then perfused with PBS via the aorta, the orthotopic xenografts excised, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

1.2 Bioanalysis

Frozen tumor and brain samples were weighed in tared 15 mL Lysing Matrix D (MP Biomedical, Santa Ana, CA) tubes and diluted with a 5:1 volume of ultra-pure water. The tissue 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, brain and tumor samples were analyzed for selinexor (ABMOLE, M3152, Purity > 98%) with a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Matrix calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in acetonitrile. Plasma and tissue samples, 25 µL each, were protein precipitated with 100 µL of 1 µg/mL diclofenac sodium (Alfa Aesar, Lot # P03C024, purity 98.5%) in acetonitrile as an internal standard. A 3 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu SIL-20AC XR autosampler.

The LC separation was performed using a Phenomenex Luna C18 (3 µm, 50 mm x 2 mm) column maintained at ambient temperature with gradient elution at a flow rate of 0.25 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 a 0.5 min hold at 20% B and a linear increase to 100% B in 3 min. The column was then rinsed for 2 min at 100% B and then equilibrated at the initial conditions for 1.5 min for a total run time of 6.5 min. Under these conditions, the analyte and IS eluted at 3.76 and 4.04 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode with monitoring of the following mass transitions: selinexor 444.30 -> 334.20, and diclofenac sodium 296.20 -> 215.20

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 2.5 to 100 ng/mL range, with a correlation coefficient (R) of 0.996. 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 2.5 ng/mL. Sample dilution integrity was confirmed. For the plasma matrix, the intra-run precision and accuracy was ≤ 13.8% CV and 93.4% to 109%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The resultant selinexor concentration-time (Ct) data were grouped by 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 (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the selinexor arithmetic mean Ct data for each matrix

was subjected to noncompartmental pharmacokinetic analysis (NCA). The extravascular model (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 clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The apparent partition coefficient of selinexor 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 mouse dosage, the resultant mouse plasma AUCinf was compared with the reported human plasma PK values at the FDA approved dose of 80 mg PO. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Selinexor concentrations showed moderate variability in the plasma, demonstrating coefficients of variation of 35.2% to 63.4% across the sampling time points. Most tumor concentrations were below the LLOQ, except for observations at 1 and 4 hours. Caution should be used when interpreting the tumor PK parameters, given that only two points were used for terminal phase extrapolation.

The absorption rate of selinexor was moderate, with the Cmax occurring at 1 hour post-dose. The plasma Ct profile for selinexor appeared monoexponential, with an apparent terminal half-life of 1.65 hr. The apparent oral clearance was low at 25.5 mL/min/kg, or 28% of hepatic blood flow. The terminal volume of distribution was high at 3.64 L/kg. The bioavailability of selinexor was not evaluated in this study but has been reported to be ~65%. Selinexor tumor penetration was low, with a Kp,tumor of 0.0117 based on AUCinf.

A pharmacokinetically-guided clinically relevant dose (CRD) for selinexor was determined as the mouse dose providing the same unbound AUC at steady state at the MTD, RP2D, or FDA-approved dose, assuming linear and dose-proportional PK in both species. A CRD equivalent to selinexor 80 mg PO on Days 1 and 3 of each week (plasma AUC 4470 hr-ug/L)[1] would be approximately 15 mg/kg, assuming similar protein binding across species.

3.0 REFERENCES

1. XPOVIO (selinexor) package insert [Internet]. Karyopharm Therapeutics; 2019 [cited 2019 Oct 7]. Available from: https://www.karyopharm.com/wp-content/uploads/2019/07/NDA-212306-SN-0071-Prescribing-Information-01July2019.pdf

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

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

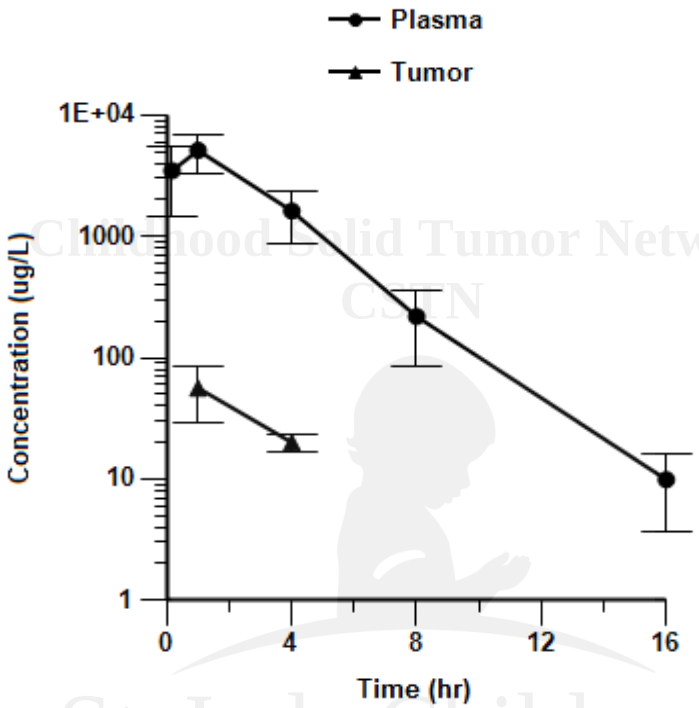

Table 4.1: NCA PK Parameter Estimates of Selinexor by Group

		Analyte	
		Selinexor	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	5120	56.3
Tmax	hr	1.00	1.00
AUClast	hr*ug/L	16300	133
AUCinf	hr*ug/L	16400	192
Kel	1/hr	0.420	0.344
T1/2	hr	1.65	2.01
CL/F	L/hr/kg	1.53	130
Vz/F	L/kg	3.64	379
Clast	ug/L	9.89	20.0
Tlast	hr	16.0	4.00

		Analyte	
		Selinexor	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Kp,tumor	-	-	0.0117

Table 4.2: Full Summary Statistics of Selinexor Ct Data by Group

		Analyte	
		Selinexor	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.167	N	3	0
	Mean	3480	
	SD	2050	
	Min	2130	
	Median	2470	
	Max	5830	
	CV%	58.8	
	Geometric Mean	3130	
	CV% Geometric Mean	58.6	
1.000	N	3	3
	Mean	5120	56.3
	SD	1800	27.2
	Min	3050	27.5
	Median	5900	60.0
	Max	6390	81.5
	CV%	35.2	48.3
	Geometric Mean	4870	51.2
	CV% Geometric Mean	42.3	60.8
4.000	N	3	3
	Mean	1620	20.0
	SD	748	3.15
	Min	775	16.6
	Median	1890	20.6
	Max	2190	22.9
	CV%	46.2	15.7
	Geometric Mean	1480	19.9

		Analyte	
		Selinexor	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	CV% Geometric Mean	61.1	16.3
8.000	N	3	0
	Mean	219	
	SD	133	
	Min	79.6	
	Median	232	
	Max	345	
	CV%	60.9	
	Geometric Mean	185	
	CV% Geometric Mean	88.2	
16.000	N	3	0
	Mean	9.89	
	SD	6.27	
	Min	5.93	
	Median	6.62	
	Max	17.1	
	CV%	63.4	
	Geometric Mean	8.76	
	CV% Geometric Mean	63.6	

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Selinexor	Plasma	0.17	2471.62
M1	Selinexor	Tumor	0.17	
M2	Selinexor	Plasma	0.17	2131.28
M2	Selinexor	Tumor	0.17	
M3	Selinexor	Plasma	0.17	5833.92
M3	Selinexor	Tumor	0.17	
M4	Selinexor	Plasma	1.00	5898.66
M4	Selinexor	Tumor	1.00	81.48
M5	Selinexor	Plasma	1.00	6394.02
M5	Selinexor	Tumor	1.00	27.47

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M6	Selinexor	Plasma	1.00	3053.89
M6	Selinexor	Tumor	1.00	59.96
M7	Selinexor	Plasma	4.00	2194.94
M7	Selinexor	Tumor	4.00	16.65
M8	Selinexor	Plasma	4.00	774.69
M8	Selinexor	Tumor	4.00	22.88
M9	Selinexor	Plasma	4.00	1889.97
M9	Selinexor	Tumor	4.00	20.59
M10	Selinexor	Plasma	8.00	231.77
M10	Selinexor	Tumor	8.00	
M11	Selinexor	Plasma	8.00	79.59
M11	Selinexor	Tumor	8.00	
M12	Selinexor	Plasma	8.00	344.99
M13	Selinexor	Plasma	16.00	17.11
M13	Selinexor	Tumor	16.00	
M14	Selinexor	Plasma	16.00	5.93
M14	Selinexor	Tumor	16.00	
M15	Selinexor	Plasma	16.00	6.62
M15	Selinexor	Tumor	16.00	

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Selinexor	Plasma	0.17	3478.94	2046.56	3.00
Concentration	ug/L	Selinexor	Plasma	1.00	5115.52	1802.53	3.00
Concentration	ug/L	Selinexor	Plasma	4.00	1619.87	747.66	3.00
Concentration	ug/L	Selinexor	Plasma	8.00	218.78	133.18	3.00
Concentration	ug/L	Selinexor	Plasma	16.00	9.89	6.27	3.00
Concentration	ug/L	Selinexor	Tumor	0.17			0.00
Concentration	ug/L	Selinexor	Tumor	1.00	56.30	27.19	3.00
Concentration	ug/L	Selinexor	Tumor	4.00	20.04	3.15	3.00
Concentration	ug/L	Selinexor	Tumor	8.00			0.00
Concentration	ug/L	Selinexor	Tumor	16.00			0.00

5.0 ATTACHED FILES

Attached File 5.1 Selinexor Prelim PK.docx – *Final in vivo study plan as executed*
Attached File 5.2 Selinexor_RMS_PK_Study Results.xlsx– *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 Selinexor Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*