PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 47869-406696

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF CARFILZOMIB IN FEMALE CD1 NUDE MICE AFTER A SINGLE INTRAPERITONEAL INJECTION

SHORT TITLE:	**Carfilzomib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Carfilzomib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Dyer, Michael A <Michael.Dyer@STJUDE.ORG>
SJCRH SRM2 O/R:	47869-406696 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA NA
IN VIVO SCIENTIST(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>; Bradley, Cori; Goshorn, Ross; Underwood, John
BIOANALYTICAL SCIENTIST:	Vuppala, Pradeep K
REPORT AUTHOR(S):	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>; Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2020-04-10

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 tumor pharmacokinetic (PK) profile of carfilzomib was evaluate in female CD-1 nude mice (Jax Mice Service), approximately 12 weeks in age, bearing MAST39 rhabdomyosarcoma orthotopic xenografts. Carfilzomib (SJ000782378, CFZ-101, LC Labs) was dissolved in 5% hydroxypropyl beta cyclodextrin (HPBCD) in 50 mM sodium citrate (pH 3.35), for a 3.6 mg/kg dose as a 5 mL/kg intraperitoneal injection. The nominal dosage was to be 5 mg/kg, but the formulation was found to be below specification upon post-hoc evaluation at 70% of the nominal concentration. Therefore, the estimated administered dose was adjusted downward.

Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 24 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. Tumors 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

Frozen tumor 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 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 homogenate samples were analyzed for carfilzomib with a sensitive and specific 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 or tumor homogenate samples, 50 µL each, plus 10 µL of 100 ng/mL ONX0914 in acetonitrile as an internal standard, was extracted with 700 µL methyl tert-butyl ether. After 5 min vortex and 5 min centrifuge at 10000 rpm 4°C, a 650 µL of top layer was evaporated to dryness using CentriVap Vacuum Concentrator (LABCONO, Kansas City, MO) at 35 °C for 25 min. The dried samples were reconstituted with 100 µL of acetonitrile: Ultrapure water 75:25 (v/v). A 5 µL aliquot of the reconstitute 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 0.4% formic acid in water (pH 3.2, adjust with ammonium acetate) in reservoir A and acetonitrile in reservoir B. The initial mobile phase consisted of 0% B for 0.5 min with a linear increase to 90% B in 1.5 min. The column was then rinsed for 3 min at 90% B, then reduce back to 0%B in 2 min, and equilibrated at the initial conditions for 2 min for a total run time of 9 min. Under these conditions, the analyte and IS eluted at 3.60 and 3.14 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode and the following mass transitions were monitored: carfilzomib 720.5 -> 402.3, ONX0914 581.5 -> 199.1.

The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A quadratic model (1/X2 weighting) fit the calibrators across the 0.2 to 50 ng/mL range, with a correlation coefficient (R) of ≥ 0.9948. 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 0.2 ng/mL for both matrices, with a functional LLOQ of 1.2 ng/mL for tumor considering dilution. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 7.36% CV and 98.06% to 115%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Carfilzomib plasma and tumor Ct data were grouped by matrix and nominal time point, and the 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-tumor 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

Carfilzomib plasma concentrations demonstrated modest variability, with coefficients of variation ranging from 31.9% to 55.4%. Carfilzomib demonstrated a rapid absorption phase, followed by an apparent and slight distribution and elimination phase, and an apparent terminal half-life of ~8 minutes. As expected, carfilzomib was rapidly cleared from mouse plasma after IP injection, with an apparent clearance of 518 mL/min/kg – several fold higher than hepatic blood flow. The apparent terminal volume of distribution was also high at 6.21 L/kg. The bioavailability (F) of carfilzomib 3.6 mg/kg IP was approximately 30%, when compared with 5 mg/kg IV in mice [1].

Tumor penetration of the irreversible proteasome inhibitor carfilzomib was poor. Only two samples were above the LLOQ in tumor when corrected for a 6-fold dilution factor (M4 0.133 hr, M6 0.133 hr). Therefore, tumor PK parameters could not be estimated.

The plasma protein binding of carfilzomib has not been reported in mice, but has been found to be high (93-97%) in rats, monkeys, and humans [1]. Assuming similar pPB, linear, and time-invariant PK across species, a clinically relevant dose (CRD) of carfilzomib is approximately 10 mg/kg IP, equating to 27 mg/m^2 IV in humans by plasma AUC [2] .

3.0 REFERENCES

1. FDA. Drug Approval Package: Kyprolis (carfilzomib) NDA # 202714 Pharmacology Review [Internet]. Drugs@FDA. 2012 [cited 2020 Apr 10]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/202714Orig1s000TOC.cfm

2. Ou Y, Doshi S, Nguyen A, Jonsson F, Aggarwal S, Rajangam K, Dimopoulos MA, Stewart AK, Badros A, Papadopoulos KP, Siegel D, Jagannath S, Vij R, Niesvizky R, Graham R, Visich J. Population Pharmacokinetics and Exposure–Response Relationship of Carfilzomib in Patients With Multiple Myeloma. J Clin Pharmacol. 2017;57(5):663–77.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Carfilzomib Ct Summary (Mean, SD, N) by Group

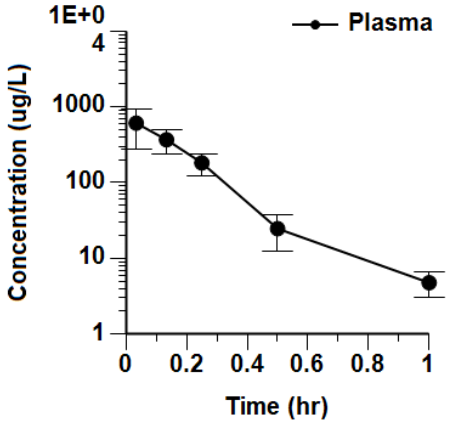

Table 4.1: NCA PK Parameter Estimates of Carfilzomib by Group

		Analyte	
		Carfilzomib	
		Group	
		Plasma	Tumor
Parameter	**Units**	**Estimate**	
Flag_N_Samples			Insufficient
Cmax	ug/L	612	
Tmax	hr	0.0333	
AUClast	hr*ug/L	115	
AUCinf	hr*ug/L	116	
Kel	1/hr	5.01	
T1/2	hr	0.138	
CL/F	L/hr/kg	31.1	
Vz/F	L/kg	6.21	
Clast	ug/L	4.78	
Tlast	hr	1.00	

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

		Analyte	
		Carfilzomib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.033	N	3	0
	Mean	612	
	SD	339	
	Min	268	
	Median	621	
	Max	946	
	CV%	55.4	
	Geometric Mean	540	
	CV% Geometric Mean	71.3	
0.133	N	3	0
	Mean	369	
	SD	129	
	Min	280	
	Median	311	
	Max	516	
	CV%	34.9	
	Geometric Mean	355	
	CV% Geometric Mean	33.7	
0.250	N	3	0
	Mean	183	
	SD	58.2	
	Min	130	
	Median	172	
	Max	245	
	CV%	31.9	
	Geometric Mean	177	
	CV% Geometric Mean	32.5	
0.500	N	3	0
	Mean	24.7	
	SD	12.4	
	Min	16.4	
	Median	18.8	
	Max	39.0	
	CV%	50.3	
	Geometric Mean	22.9	
	CV% Geometric Mean	49.3	
1.000	N	3	0
	Mean	4.78	

		Analyte	
		Carfilzomib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	SD	1.73	
	Min	2.94	
	Median	5.02	
	Max	6.38	
	CV%	36.2	
	Geometric Mean	4.55	
	CV% Geometric Mean	41.2	

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Carfilzomib	Plasma	0.03	621.24
M1	Carfilzomib	Tumor	0.03	
M2	Carfilzomib	Plasma	0.03	945.74
M2	Carfilzomib	Tumor	0.03	
M3	Carfilzomib	Plasma	0.03	268.33
M3	Carfilzomib	Tumor	0.03	
M4	Carfilzomib	Plasma	0.13	279.57
M4	Carfilzomib	Tumor	0.13	
M5	Carfilzomib	Plasma	0.13	310.76
M5	Carfilzomib	Tumor	0.13	
M6	Carfilzomib	Plasma	0.13	516.30
M6	Carfilzomib	Tumor	0.13	
M7	Carfilzomib	Plasma	0.25	245.30
M7	Carfilzomib	Tumor	0.25	
M8	Carfilzomib	Plasma	0.25	172.14
M8	Carfilzomib	Tumor	0.25	
M9	Carfilzomib	Plasma	0.25	130.23
M9	Carfilzomib	Tumor	0.25	
M10	Carfilzomib	Plasma	0.50	16.36
M10	Carfilzomib	Tumor	0.50	
M11	Carfilzomib	Plasma	0.50	18.78
M11	Carfilzomib	Tumor	0.50	
M12	Carfilzomib	Plasma	0.50	38.99
M12	Carfilzomib	Tumor	0.50	
M13	Carfilzomib	Plasma	1.00	2.94
M13	Carfilzomib	Tumor	1.00	
M14	Carfilzomib	Plasma	1.00	5.02

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M14	Carfilzomib	Tumor	1.00	
M15	Carfilzomib	Plasma	1.00	6.38
M15	Carfilzomib	Tumor	1.00	

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Carfilzomib	Plasma	0.03	611.77	338.80	3.00
Concentration	ug/L	Carfilzomib	Plasma	0.13	368.88	128.62	3.00
Concentration	ug/L	Carfilzomib	Plasma	0.25	182.56	58.24	3.00
Concentration	ug/L	Carfilzomib	Plasma	0.50	24.71	12.43	3.00
Concentration	ug/L	Carfilzomib	Plasma	1.00	4.78	1.73	3.00
Concentration	ug/L	Carfilzomib	Tumor	0.03			0.00
Concentration	ug/L	Carfilzomib	Tumor	0.13			0.00
Concentration	ug/L	Carfilzomib	Tumor	0.25			0.00
Concentration	ug/L	Carfilzomib	Tumor	0.50			0.00
Concentration	ug/L	Carfilzomib	Tumor	1.00			0.00

5.0 ATTACHED FILES

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