PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program (DBSTP)

P-PKSR Study 137797-1443223

STUDY TITLE:

MULTIPLE DOSE SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF TAZEMETOSTAT IN FEMALE ATHYMIC NUDE MICE BEARING MAST39 RHB ORTHOTOPIC XENOGRAFTS

SHORT TITLE:	**Tazemetostat MD SPTPK**
TEST ARTICLE:	Tazemetostat
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	137797-1443223 Preclinical Pharmacokinetic Shared Resource
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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 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, 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 STUDY SUMMARY

Tazemetostat (EPZ-6438) is a clinically advanced EZH2 inhibitor, which primarily functions to impair histone H3K27 methylation on chromatin proteins, thereby modulating expression of various genes. The plasma and tumor PK of tazemetostat was evaluated in a PK/PD study of female Athymic nude mice bearing orthotopically placed, patient-derived xenografts (o-PDX) of rhabdomyosarcoma (MAST39). The results were then compared to tazemetostat single dose mouse PK from previous DBSTP studies, as well as to that published in the literature for mice and humans. Given reports of increased apparent clearance of tazemetostat with time in the clinic after 15 days of dosing, we speculated that this may also occur in mice.

Tazemetostat free base was suspended in in 1% hydroxyethylcellulose (HEC) / 0.25% Tween 80 / 0.05% simethicone at 20 mg/mL for a 10 mL/kg oral gavage, 200 mg/kg dosage administered twice daily (BID) for 5 days. Plasma was sampled using an IACUC-approved, terminal cardiac puncture technique. Mice were perfused with PBS, tumors extracted, and placed at -80 °C until analysis.

Samples were analyzed with a qualified LC MS/MS assay demonstrating a LLOQ of 1 ng/mL for plasma and 6 ng/mL for tumor homogenates, and an intra-run precision and accuracy of 20% or less. If at any time point, ≥ 2/3rds of the results were below the assay LLOQ (BLOQ), then the entire time point was treated as missing. Otherwise, any data BLOQ were replaced with a value of ½ LLOQ, and the concentration statistics calculated.

Tazemetostat plasma exposure, as quantified by total plasma AUCtau, was similar to that expected from our previous single dose mouse studies. No increase in apparent clearance occurred over the 5-day dosing period in mice. Assuming a traditional mechanism of clearance induction (i.e. PXR), this data suggests that tazemetostat lacks such an effect. However, if non-traditional mechanisms of induction are at play, such as epigeneitic modulation of DMEs or transporters, mice were likely dosed for an insufficient period to attain such an effect.

Also within expectations was a slight accumulation index of tazemetostat in plasma with multiple doses, 8.1%. The tumor penetration ratio, calculated as the ratio of tumor to plasma AUCtau values, was 0.447. Free fraction of tazemetostat in mouse and human plasma (Fu,p) is estimated at 0.0361 and 0.0726, respectively (via in vitro rapid equilibrium dialysis, courtesy of Lei Yang and Zoran Rankovic, CBT ATC). The free fraction in MAST39 tumor homogenate was 0.0575.

In a Phase 1 study in pediatric patients, the mean total plasma AUC at the single agent RP2D (1200 mg/m2 BID) was reported to be ~4 fold greater than adults at 800 mg BID [1]. Thus, as the adult AUC on Day 15 was reported as 3340 hr-ng/mL [2], the pediatric plasma AUC at steady state is estimated at 13400 hr-ng/mL. When adjusted for protein binding between mice and humans, and based off of the data from this mouse study, a precise clinically relevant dose of tazemetostat is 160 mg/kg PO BID in mice. This is calculated by unbound steady state plasma AUCs, and is assuming dose proportional and time invariant PK in mice.

2.0 REFERENCES

1. Chi S, Fouladi M, Shukla N, Bourdeaut F, Margol A, Makin G, McCowage G, Wetmore C, Macy M, Laetsch T, Hargrave D, Pinto N, Yi J, Ebb D, Robinson G, Roche M, Suttle B, Clawson A, Ho P, Rodstrom J, Daigle S, Nysom K. Abstract A175: Phase 1 study of the EZH2 inhibitor, tazemetostat, in children with relapsed or refractory INI1-negative tumors including rhabdoid tumors, epithelioid sarcoma, chordoma, and synovial sarcoma. Mol Cancer Ther. 2018 Jan 1;17(1 Supplement):A175–A175.

2. Italiano A, Soria J-C, Toulmonde M, Michot J-M, Lucchesi C, Varga A, Coindre J-M, Blakemore SJ, Clawson A, Suttle B, McDonald AA, Woodruff M, Ribich S, Hedrick E, Keilhack H, Thomson B, Owa

T, Copeland RA, Ho PTC, Ribrag V. Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study. Lancet Oncol. 2018 Apr 9;

3.0 TABLES, LISTINGS, AND FIGURES

Figure 1: Mean (SD) Ct Profile of Test Article by Matrix

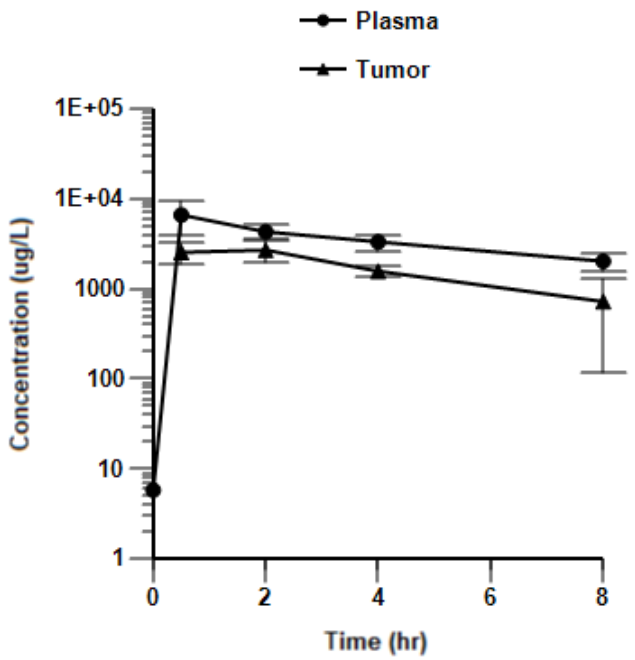

Table 1: NCA PK Parameter Estimates of Test Article by Matrix

		Matrix	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	6590	2690
Tmax	hr	0.500	2.00
AUClast	hr*ug/L	27700	13200
AUCtau	hr*ug/L	34000	15200
Kel	1/hr	0.124	0.216
T1/2	hr	5.60	3.21
Clast	ug/L	2020	721
Tlast	hr	8.00	8.00

Table 2: Summary Statistics of Test Article Ct Data by Matrix

		Matrix	
		Tumor	Plasma
Time (hr)		Concentration (ug/L)	
0.000	N	0	3
	Mean		5.83
	SD		6.16
	Min		1.36
	Median		3.28
	Max		12.9
	CV%		106
	Geometric Mean		3.85
	CV% Geometric Mean		162
0.500	N	2	3
	Mean	2530	6590
	SD	685	2770
	Min	2050	3470
	Median	2530	7570
	Max	3020	8730
	CV%	27.0	42.0
	Geometric Mean	2490	6120
	CV% Geometric Mean	27.9	53.0
2.000	N	3	3
	Mean	2690	4280
	SD	747	763
	Min	1950	3430
	Median	2670	4500
	Max	3450	4910
	CV%	27.8	17.8
	Geometric Mean	2620	4230
	CV% Geometric Mean	29.1	18.9
4.000	N	3	3
	Mean	1570	3310
	SD	228	679
	Min	1350	2620

		Matrix	
		Tumor	Plasma
Time (hr)		Concentration (ug/L)	
	Median	1550	3330
	Max	1800	3980
	CV%	14.5	20.5
	Geometric Mean	1550	3260
	CV% Geometric Mean	14.6	21.2
8.000	N	3	3
	Mean	721	2020
	SD	601	427
	Min	219	1750
	Median	557	1790
	Max	1390	2510
	CV%	83.4	21.2
	Geometric Mean	553	1990
	CV% Geometric Mean	116	20.4

Table 3: Test Article Ct Data Listings by Component, Matrix, Subject, and Time

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Tazemetostat	Plasma	M1	0.00	1.36
Tazemetostat	Plasma	M2	0.00	12.85
Tazemetostat	Plasma	M3	0.00	3.28
Tazemetostat	Plasma	M4	0.50	3466.00
Tazemetostat	Plasma	M5	0.50	8730.10
Tazemetostat	Plasma	M6	0.50	7572.90
Tazemetostat	Plasma	M7	2.00	4907.50
Tazemetostat	Plasma	M8	2.00	4503.70
Tazemetostat	Plasma	M9	2.00	3430.80
Tazemetostat	Plasma	M10	4.00	3333.30
Tazemetostat	Plasma	M11	4.00	2620.20
Tazemetostat	Plasma	M12	4.00	3978.30
Tazemetostat	Plasma	M13	8.00	2510.60
Tazemetostat	Plasma	M14	8.00	1792.50

Tazemetostat MD SPTPK

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Tazemetostat	Plasma	M15	8.00	1749.80
Tazemetostat	Tumor	M1	0.00	
Tazemetostat	Tumor	M2	0.00	
Tazemetostat	Tumor	M3	0.00	
Tazemetostat	Tumor	M4	0.50	No Tumor
Tazemetostat	Tumor	M5	0.50	3018.60
Tazemetostat	Tumor	M6	0.50	2049.80
Tazemetostat	Tumor	M7	2.00	3445.60
Tazemetostat	Tumor	M8	2.00	2665.30
Tazemetostat	Tumor	M9	2.00	1951.70
Tazemetostat	Tumor	M10	4.00	1345.60
Tazemetostat	Tumor	M11	4.00	1551.60
Tazemetostat	Tumor	M12	4.00	1800.40
Tazemetostat	Tumor	M13	8.00	1387.40
Tazemetostat	Tumor	M14	8.00	556.68
Tazemetostat	Tumor	M15	8.00	219.30

4.0 APPENDICES

4.1 Tazemetostat Plasma and Tumor PKPD.docx

Plasma and Tumor PKPD of Tazemetostat in Female Athymic Nude Mice after Multiple Oral Doses

SRM2 O/R: 137797-1443223	**Matrix:** plasma, tumor	**Compound:** Tazemetostat (TAZ)	**REG#:** SJ000869448-2
Written or Revised By: Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>		**Date:**	2018-05-03
In Vivo Scientists:		**Start Date**	
P-PKSR Lead: Caufield, William <William.Caufield@STJUDE.ORG>		**End Date**	

Date: 2018-06-04 to 2018-06-18

Objectives: To evaluate the plasma and tumor PKPD of tazemetostat after multiple oral gavages of suspension in RHB (SJRHB000026_X1, MAST39) bearing female Athymic nude mice, in a preliminary fashion

Animals: Female Athymic nude mice (Charles River, 553). Aged approx. 12 weeks at study execution.

Dosages: 200 mg/kg tazemetostat free base equivalents by oral gavage (PO), twice daily (BID) for 15 days

Formulation: Tazemetostat suspended in 1% hydroxyethylcellulose (HEC) / 0.25% Tween 80 / 0.05% simethicone

Design: A total of 15 mice will be dosed, with 3 mice providing blood at each time point. Three vehicle control mice will be included. One (1) blood sample will be collected from each mouse after dosing using an IACUC approved terminal procedure (T). Blood/plasma samples each mouse will preferably be by retro-orbital exsanguination via glass capillary or terminal cardiac puncture, with the method used recorded. Tumor or tissues will be extracted after perfusion with PBS.

Group #s	Dose Level	Mouse #s	Mouse Ear Tag IDs	Sample Times (hr)
1	200 mg/kg	1-3		T : Predose
2	200 mg/kg	4-6		T: 0.5
3	200 mg/kg	7-9		T: 2
4	200 mg/kg	10-12		T: 4
5	200 mg/kg	12-15		T: 8
6	Vehicle	16-18		T: 4 to 6

Summary:

Materials:

- ☐ Set 1: 15 appropriately labeled Microvette 500 K3EDTA microcentrifuge screw top tubes (500 uL, Sarstedt 20.1341.100) for terminal blood collection and spin down to plasma.
- ☐ Set 2: 15 standard 2.0 mL screw-top microcentrifuge tubes (Fisher Cat# 02-681-343 or equivalent), pre-labeled with 128786, TAZ PKPD, mouse #, and nominal time point in hrs for terminal plasma collection.
- ☐ Set 3: 15 standard 2.0 mL screw-top microcentrifuge tubes (Fisher Cat# 02-681-343 or equivalent), pre-labeled with 128786, TAZ PKPD, mouse #, and nominal time point in hrs for tumor or tissue collection.
  - Falcon-style tubes are acceptable if tissue mass is anticipated to exceed the capacity of microcentrifuge tubes
- ☐ 1% hydroxyethylcellulose (HEC) / 0.25% Tween 80 / 0.05% simethicone vehicle
- ☐ ~1700 mg of tazemetostat free base equivalents
- ☐ Mouse gavage needle and 1 mL syringes for PO administration

- ☐ Glass capillaries (for exsanguination from retro orbital plexus into Microvette tube if necessary)
- ☐ 25 gauge needles and TB/insulin syringes for cardiac punctures and perfusions
- ☐ PBS for perfusion (Ca and Mg free preferred)
- ☐ Centrifuge (10000g) w/ microcentrifuge rotor (4°C preferred, but room temp. will suffice)
- ☐ Container of wet ice
- ☐ Styrofoam cooler with labeled cardboard vial box and **dry** ice

Procedure:

1. The day before or morning of the study, sort mice into groups, 3 mice per cage with 6 cages, and perform weighing. Mark tails for identification, or refer to mouse ear tag numbers. Label cages with group number, mouse numbers, and nominal time points. If marking, number of stripes represents the number of mouse in the cage's sequence. For example, the mouse with 1 stripe in Cage/Group 2 would be mouse #4 and the mouse with 2 stripes would be mouse #5, and so forth. Weigh each mouse, record weight in grams on the Study Worksheet, and calculate planned doses in mL.

2. TO BE PERFORMED BY P-PKSR: Prior to the study, formulate the 1% HEC / 0.25% Tween 80 / 0.05% simethicone vehicle
    a. Prepare ~1% (w/v) HEC solution, 49 mL
        i. Heat 17 mL of UP water to 80°C.
        ii. Slowly add 500 mg of HEC to the hot water with ample agitation and mixing
        iii. Agitate the mixture until the particles are thoroughly wetted and evenly dispersed.
        iv. Add ~32 mL of cold UP water under continued agitation. Solution should be cooled to 0-5°C for 20-40 min. under continued agitation.
        v. Agitation should continue for at least 30 min. after proper temperature is reached.
    b. To the ~49 mL of the ~1% HEC solution, add 0.125 mL of Tween80 by pipette.
    c. Add a couple drops of antifoam/simethicone (not more than 5 or so)
    d. Shake or mix gently until the Tween80 and simethicone are dispersed, and avoid bubbling
    e. QC to a total of 50 mL with cold UP water. Store at 4 deg, and let come to ambient temperature before use as suspending vehicle.

3. TO BE PERFORMED BY P-PKSR: Formulate tazemetostat in vehicle as a suspension for oral gavage (20 mg/mL, 0.25 mL for a 25 g mouse = 200 mg/kg) in batch fashion as appropriate, example below:
    a. To 1000 mg of free base equivalents of tazemetostat, add vehicle at ambient temperature and QS to 50 mL. Pipette and gently shake/stir to wet the tazemetostat, followed by brief vortexing.
    b. Vortex and/or sonicate in water bath for up to 30 min. to ensure a homogenous suspension.
    c. If necessary, subject suspension to direct probe ultrasonication for short period (e.g. 5 sec on / of cycles, total 30 sec, 20% power of 20kHz)
    d. Carefully transfer the suspension to an appropriately sized screw top, glass dosing vials.
    e. There should be more than 500 uL of formulation remaining at end of study, please store under ambient temperature and return to P-PKSR for stability analysis.

4. Execute in vivo study according to the Study Worksheet

a. NOTE: All actual times for dosing and samples should be referenced to the same study clock.
b. Dose mice by PO gavage; record the actual dose volume administered in mL and the actual times of each administration for each mouse.
    i. Immediately prior to each administration, vortex and check formulation for visual homogeneity
    ii. All dosages will be based on each mouse's initial body weight at the beginning of the study.
c. At each time point, collect the blood sample by the indicated means and record the actual sample time (from the start of the collection), making note of any issues.
    i. T: Terminal bleed – Anesthetize the mouse per IACUC protocol. Proceed to collect 500 uL of whole blood from either retro orbital exsanguination or cardiac chamber puncture. Place blood into appropriate pre-labeled Microvette K3EDTA tubes (Set 1) and gently agitate. All samples should be processed to plasma ASAP, but if necessary, put on wet ice until centrifugation.
d. Centrifuge the whole blood samples at 10000g for 2 min. to generate plasma.
e. Remove plasma supernatant, place in appropriate pre-labeled tube from Set 2; place in vial box in cooler on dry ice and transfer to -80°C as soon as possible.
f. Perfuse animal with PBS or equivalent to flush blood from vasculature.
g. Extract tumor and rinse with PBS, place in microcentrifuge tube or equivalent in cooler on dry ice and transfer to -80°C as soon as possible.
h. Snap freeze a portion of the tumor in liquid N_2, place in separate microcentrifuge tube or equivalent in cooler on dry ice, and transfer to -80°C as soon as possible.
i. Please submit remaining formulation in the original dosing vial stored at ambient temperature for stability assessment.

4.2 7-3-18 Tazemetostat PK plan.xlsx

Mouse #	Mouse Wt (g)	Group	Nominal Time (hr)	Planned Day	Planned Dose Time	Actual Dose Time	mL PO	Operator Number	Planned Day	Planned Sample Time	Recorded Sample Time	Operator Number	Tumor weight (Dry Ice)	Tumor weight (liq. Nit)	Tumor Weight (PFA)
1	30.1	1	Predose	1			0		1	7:00 AM	7:00 AM	1	90mg	440mg	1450mg
2	28.6	1	Predose	1			0		1	7:05AM	7:05AM	1	100mg	730mg	1310mg
3	23.2	1	Predose	1			0		1	7:10 AM	7:10 AM	1	50mg	40mg	20mg
4	28	2	0.5	1	7:30 AM	7:30 AM	0.28	1	1	8:00 AM	8:00 AM	1	NO TUMOR PRESENT		
5	25.8	2	0.5	1	7:35 AM	7:35 AM	0.26	1	1	8:05 AM	8:05 AM	1	80mg	380mg	800mg
6	26.1	2	0.5	1	7:40 AM	7:40 AM	0.26	1	1	8:10 AM	8:10 AM	1	90mg	230mg	1780mg
7	28.1	3	2	1	7:31 AM	7:31 AM	0.28	1	1	9:31 AM	9:31 AM	1	90mg	800mg	1400mg
8	27.2	3	2	1	7:36 AM	7:36 AM	0.27	1	1	9:36 AM	9:36 AM	1	100mg	50mg	40mg
9	28.4	3	2	1	7:41 AM	7:41 AM	0.28	1	1	9:41 AM	9:41 AM	1	70mg	250mg	2480mg
10	28.2	4	4	1	7:32 AM	7:32 AM	0.28	1	1	11:32 AM	11:32 AM	2	90mg	250mg	1500mg
11	28.4	4	4	1	7:37 AM	7:37 AM	0.28	1	1	11:37 AM	11:37 AM	2	90mg	1760mg	1890mg
12	29.4	4	4	1	7:42 AM	7:42 AM	0.29	1	1	11:42 AM	11:42 AM	2	90mg	110mg	90mg
13	26.4	5	8	1	7:33 AM	7:33 AM	0.26	1	1	3:33 PM	3:33 PM	2	80mg	360mg	2190mg
14	30.5	5	8	1	7:38 AM	7:38 AM	0.31	1	1	3:38 PM	3:38 PM	2	100mg	1830mg	2410mg
15	29.6	5	8	1	7:43 AM	7:43 AM	0.3	1	1	3:43 PM	3:43 PM	2	90mg	100mg	890mg
16	27.8	6	6	1	7:34 AM	7:34 AM	0.28	1	1	1:34 PM	1:34 PM	2	100mg	370mg	1010mg
17	27.4	6	6	1	7:39 AM	7:39 AM	0.27	1	1	1:39 PM	1:39 PM	2	90mg	250mg	2760mg
18	27.6	6	6	1	7:44 AM	7:44 AM	0.28	1	1	1:44 PM	1:44 PM	2	90mg	110mg	2320mg