PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program (DBSTP)

P-PKSR Study 120810-1261826

STUDY TITLE:

SCREENING PLASMA, TUMOR (RHB, MAST39), AND BRAIN PHARMACOKINETICS OF TASELISIB IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Taselisib Screening PK RHB MAST 39**
TEST ARTICLE:	Taselisib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>
SJCRH SRM2 O/R:	120810-1261826 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA NA
IN VIVO SCIENTIST(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG> Blankenship, Kaley B <Kaley.Blankenship@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>
REPORT AUTHOR(S):	Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>
REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2018-06-18

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 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

Taselisib free base was suspended in 0.5% methylcellulose (type 400 cPs) / 0.2% Tween 80 at a concentration of 0.625 mg/mL for a 10 mL/kg oral gavage in RHB MAST 39 bearing female Athymic nude mice. Plasma was sampled using a terminal technique, tumors and brains harvested after perfusion with PBS, and samples analyzed with a qualified LC MS/MS assay demonstrating a LLOQ of 1 ng/mL, 6 ng/mL for tumor, and an intra-run precision and accuracy of 20% or less.

Taselisib plasma exposure (Matrix = PLA) was about half as expected, given the results from the previous plasma-only mouse PK at 25 mg/kg (RPT.120808-1261824). This suggests some sort of saturable metabolism or elimination in mice at the higher 25 mg/kg oral dose. The plasma free fractions (Fu,p) were reported at 0.11 and 0.26 for mice and humans, respectively [1].The estimated unbound plasma AUC at 6.25 mg/kg was approximately twice the estimated unbound plasma AUC at 12 mg PO QD at steady state in humans [2]. Assuming linear and dose proportional PK in mice and humans, a point estimate of a plasma AUC-based clinically relevant dose (CRD) of taselisib would be 2 mg/kg PO. However, CRDs between 1 and 5 mg/kg in mice would be reasonable.

The extent of taselisib penetration into the RHB MAST 39 orthotopic tumor (Matrix = TH) was low-to-moderate, with a total AUCinf-based partition coefficient (Kp) of 0.289. Distribution into the brain (Matrix = BH) was low, with a total Cmax estimated Kp of 0.0139. Distribution to tumor and brain appeared to be perfusion limited and occurred at a rapid rate.

2.0 REFERENCES

1. Ndubaku CO, Heffron TP, Staben ST, Baumgardner M, Blaquiere N, Bradley E, Bull R, Do S, Dotson J, Dudley D, Edgar KA, Friedman LS, Goldsmith R, Heald RA, Kolesnikov A, Lee L, Lewis C, Nannini M, Nonomiya J, Pang J, Price S, Prior WW, Salphati L, Sideris S, Wallin JJ, Wang L, Wei B, Sampath D, Olivero AG. Discovery of 2-{3-[2-(1-Isopropyl-3-methyl-1H-1,2–4-triazol-5-yl)-5,6-dihydrobenzo[f]imidazo[1,2-d][1,4]oxazepin-9-yl]-1H-pyrazol-1-yl}-2-methylpropanamide (GDC-0032): A β-Sparing Phosphoinositide 3-Kinase Inhibitor with High Unbound Exposure and Robust in Vivo Antitumor Activity. J Med Chem. 2013 Jun 13;56(11):4597–610.

2. Juric D, Krop I, Ramanathan RK, Wilson TR, Ware JA, Bohorquez SS, Savage H, Sampath D, Salphati L, Lin R, Jin H, Parmar H, Hsu JY, Hoff DDV, Baselga J. Phase I Dose Escalation Study of Taselisib (GDC-0032), an Oral PI3K Inhibitor, in Patients with Advanced Solid Tumors. Cancer Discov. 2017 Jan 1;CD-16-1080.

3.0 TABLES, LISTINGS, AND FIGURES

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

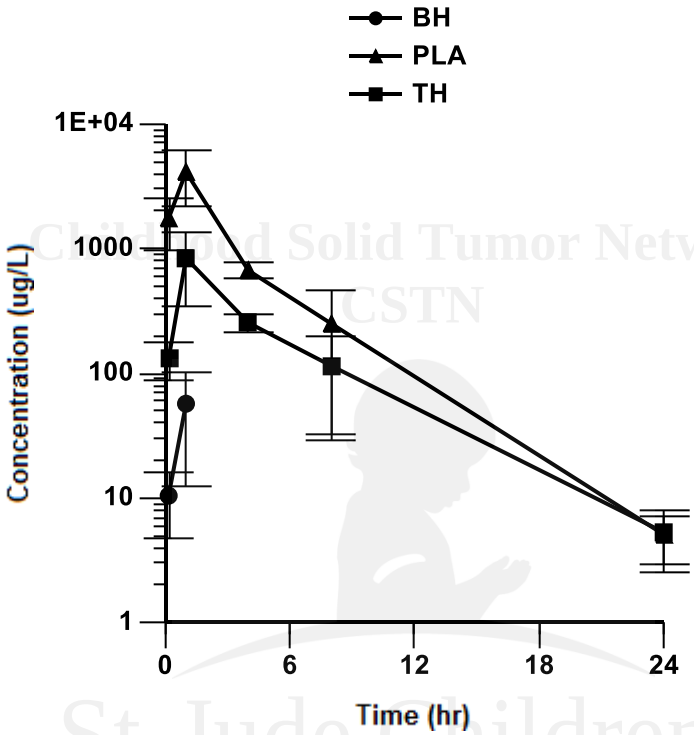

Table 1: NCA PK Parameter Estimates of Test Article by Matrix

		Matrix		
		BH	PLA	TH
Parameter	Units	Estimate		
Cmax	ug/L	57.1	4120	839
Tmax	hr	1.00	1.00	1.00
AUClast	hr*ug/L	29.0	11000	3160
AUCinf	hr*ug/L		11000	3180
Kel	1/hr		0.244	0.193
T1/2	hr		2.84	3.59
CL/F	L/hr/kg		0.568	1.96
Vz/F	L/kg		2.33	10.2
Clast	ug/L	57.1	5.08	5.30

		Matrix		
		BH	PLA	TH
Parameter	Units	Estimate		
Tlast	hr	1.00	24.0	24.0

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

		Matrix		
		TH	PLA	BH
Time (hr)		Concentration (ug/L)		
0.167	N	3	3	3
	Mean	132	1750	10.4
	SD	43.9	781	5.65
	Min	98.9	1230	6.49
	Median	114	1360	7.93
	Max	181	2650	16.9
	CV%	33.4	44.7	54.1
	Geometric Mean	127	1640	9.55
	CV% Geometric Mean	32.5	43.3	54.0
1.000	N	3	3	3
	Mean	839	4120	57.1
	SD	495	1940	44.8
	Min	509	2770	22.5
	Median	601	3250	41.0
	Max	1410	6340	108
	CV%	59.0	47.0	78.5
	Geometric Mean	755	3850	46.3
	CV% Geometric Mean	59.0	46.1	92.9
4.000	N	3	3	0
	Mean	254	668	
	SD	38.3	97.5	
	Min	221	605	
	Median	247	619	
	Max	296	780	
	CV%	15.1	14.6	
	Geometric Mean	253	663	

		Matrix		
		TH	PLA	BH
Time (hr)		Concentration (ug/L)		
	CV% Geometric Mean	14.9	14.2	
8.000	N	3	3	0
	Mean	115	250	
	SD	85.3	217	
	Min	37.9	90.5	
	Median	99.3	162	
	Max	207	496	
	CV%	74.5	86.8	
	Geometric Mean	92.0	194	
	CV% Geometric Mean	103	106	
24.000	N	3	3	0
	Mean	5.30	5.08	
	SD	2.78	2.10	
	Min	3.41	2.87	
	Median	4.00	5.33	
	Max	8.50	7.04	
	CV%	52.5	41.3	
	Geometric Mean	4.87	4.76	
	CV% Geometric Mean	51.9	48.6	

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

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Taselisib	BH	M1	0.17	16.92
Taselisib	BH	M2	0.17	7.93
Taselisib	BH	M3	0.17	6.49
Taselisib	BH	M4	1.00	22.54
Taselisib	BH	M5	1.00	41.01
Taselisib	BH	M6	1.00	107.65
Taselisib	BH	M7	4.00	
Taselisib	BH	M8	4.00	
Taselisib	BH	M9	4.00	

Taselisib Screening PK RHB MAST 39

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Taselisib	BH	M10	8.00	
Taselisib	BH	M11	8.00	
Taselisib	BH	M12	8.00	
Taselisib	BH	M13	24.00	
Taselisib	BH	M14	24.00	
Taselisib	BH	M15	24.00	
Taselisib	PLA	M1	0.17	2645.60
Taselisib	PLA	M2	0.17	1232.20
Taselisib	PLA	M3	0.17	1363.80
Taselisib	PLA	M4	1.00	3246.40
Taselisib	PLA	M5	1.00	2769.90
Taselisib	PLA	M6	1.00	6335.60
Taselisib	PLA	M7	4.00	604.84
Taselisib	PLA	M8	4.00	618.64
Taselisib	PLA	M9	4.00	780.19
Taselisib	PLA	M10	8.00	90.47
Taselisib	PLA	M11	8.00	161.92
Taselisib	PLA	M12	8.00	496.25
Taselisib	PLA	M13	24.00	5.33
Taselisib	PLA	M14	24.00	7.04
Taselisib	PLA	M15	24.00	2.87
Taselisib	TH	M1	0.17	181.46
Taselisib	TH	M2	0.17	114.17
Taselisib	TH	M3	0.17	98.89
Taselisib	TH	M4	1.00	600.51
Taselisib	TH	M5	1.00	509.01
Taselisib	TH	M6	1.00	1408.60
Taselisib	TH	M7	4.00	220.67
Taselisib	TH	M8	4.00	246.57
Taselisib	TH	M9	4.00	296.12
Taselisib	TH	M10	8.00	37.94
Taselisib	TH	M11	8.00	99.34
Taselisib	TH	M12	8.00	206.54
Taselisib	TH	M13	24.00	4.00

Taselisib Screening PK RHB MAST 39

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Taselisib	TH	M14	24.00	8.50
Taselisib	TH	M15	24.00	3.41

4.0 APPENDICES

4.1 Taselisib Prelim PK.docx

Murine Pharmacokinetics (PK) of Taselisib

Client Investigators: Dr. E. Stewart

Date: 2017-08-24

Title: Preliminary plasma, RMS tumor, and brain PK of oral taselisib

Animals: Female CD1 nu mice bearing MAST 39 RMS xenografts. Aged approx. 12 weeks at study execution.

Dosages: 6.25 mg/kg taselisib by oral gavage, single dose

Formulation: Taselisib free base equivalents in 0.5% methylcellulose (type 400 cPs) / 0.2% Tween 80 (GNE MCT) in ultrapure (UP) water

Design: A total of 15 mice will be dosed, each mouse will be sacrificed at the relative time point

Group	Mouse #s	Mouse Ear Tag IDs	Sample Times
1	1-3		0.167 hr
2	4-6		1 hr
3	7-9		4 hr
4	10-12		8 hr
5	13-15		24 hr

Time	0.167 hr	1 hr	4 hr	8 hr	24 hr
Groups	1	2	3	4	5
Mouse #s	1-3	4-6	7-9	10-12	13-15
Planned Sample Time	Day 1 8:10 AM	Day 1 9:00 AM	Day 1 12:00 PM	Day 1 4:00 PM	Day 2 8:00 AM

Summary:

Materials:

- For whole blood from cardiac puncture, a set of 15 Microvette 500 K3EDTA (Sarstedt 20.1341.100) 500 uL microcentrifuge tubes, pre-labeled with Taselisib, group #, mouse #, and nominal time point in hrs.
- For plasma, a set of 15 screw-top microcentrifuge tubes, pre-labeled with Taselisib, group #, mouse #, and nominal time point in hrs
- For RMS tumors, a set of 15 screw-top microcentrifuge tubes prelabled with Taselisib, group #, mouse #, and nominal time point in hrs
  - Falcon style tubes are acceptable if tumor mass is anticipated to exceed the capacity of microcentrifuge tubes
- For brains, a set of 15 screw-top microcentrifuge tubes prelabled with Taselisib, group #, mouse #, and nominal time point in hrs
  - Falcon style tubes are acceptable if brain mass is anticipated to exceed the capacity of microcentrifuge tubes
- GNE MCT suspending vehicle, PBS for flushing mice
- ~15 mg of taselisib free base equivalents
- Mouse gavage needle and 1 mL syringes for PO administration
- 25 gauge needles and TB/insulin syringes for cardiac punctures
- 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:

Murine Pharmacokinetics (PK) of Taselisib

Client Investigators: Dr. E. Stewart

1. The day before the study, sort mice into groups, 3 mice per cage with 5 cages and perform weighing. Tattoo tails for identification, or refer to mouse ear tag numbers. Label cages with group number, mouse numbers, and nominal time points. Each tattooed stripe 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. Prior to the study, compound the 0.5% methylcellulose (type 400 cPs) / 0.2% Tween 80 suspending vehicle (GNE MCT)
    a. Prepare 1% (w/v) MC solution, 150 mL
        i. Heat 51 mL of SWI or UP water to 80°C.
        ii. Add 1,500 mg of MC to the hot water with ample agitation and mixing
        iii. Agitate the mixture until the particles are thoroughly wetted and evenly dispersed.
        iv. Add ~100 mL of cold UP water (QS to 150 mL) 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 150 mL of the 1% MC solution, add 600 µL of Tween80 by pipette. Shake or mix gently until the Tween80 is dispersed, and avoid bubbling.
    c. QS with UP water to a total final volume of 300 mL, yielding the final GNE MCT formulation vehicle. Store at 4°C for prolonged periods, up to two weeks.
    d. Always allow the vehicle to come to ambient temperature and vortex prior to any use in formulations.

3. The day before or the morning of the study, formulate taselisib in vehicle as a suspension for oral gavage (0.625 mg/mL, 0.25 mL for a 25 g mouse = 6.25 mg/kg)
    a. Weigh out 3.125 mg of taselisib free base equivalents in a tared 5 mL volumetric flask,
    b. Slowly add preformulated, room temperature GNE MCT suspending vehicle with pipetting and agitation, QS'ing to 5 mL
    c. Vortex and/or sonicate for up to 30 min to ensure a homogenous suspension
    d. Transfer to appropriately sized screw top glass vial and store at room temperature until use.
    e. Immediately prior to administrations, vortex and check for visual homogeneity.

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 administration.
    c. At each terminal time point, collect the blood sample by the indicated means and record the actual sample time (from the start of the collection), and make notes of any issues.
        i. T: Terminal cardiac puncture – Anesthetize the mouse per IACUC protocol. Proceed to collect 500 uL of whole blood from aorta. Place blood into appropriate pre-labeled Microvette K3EDTA tube 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 RMS 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.

Murine Pharmacokinetics (PK) of Taselisib

Client Investigators: Dr. E. Stewart

h. Extract Brain and rinse with PBS, place in 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 Taselisib tumor bearing study.docx

Taselisib PK Tumor Bearing Study

Date: 8/24/17- 8/25/17
Animals: Female athymic nude mice bearing rhabdomyosarcoma MAST 39 orthotopic xenografts.
Dosages: Taselisib PK Study: 6.25 mg/kg oral gavage, [0.625 mg/ml]
Formulation: Taselisib free base equivalents in 0.5% methylcellulose/ 0.2% Tween 80 in ultrapure water

Mouse #	Weight (g)	Dose (ml)	Time dose	Time Harvest	Time Point	Total Tumor (g)	Tumor wt in tube (g)
1	31.05	0.31	10:00a	10:10a	10min	5.04	0.99
2	22.66	0.23	10:10	10:20a	10min	2.65	1.35
3	27.67	0.18	10:20a	10:30a	10min	2.53	1.16
4	37.04	0.27	9:40a	10:40a	1h	2.16	1.18
5	24.20	0.24	9:50a	10:50a	1h	3.72	0.87
6	27.20	0.27	10:00a	11:00a	1h	2.33	1.15
7	29.00	0.29	9:30a	1:30p	4h	4.16	1.17
8	23.93	0.24	9:40a	1:40p	4h	1.99	1.20
9	27.49	0.28	9:50a	1:51p	4h	2.36	0.97
10	28.85	0.29	7:00a	3:00p	8h	2.71	1.40
11	27.93	0.28	7:10a	3:10p	8h	3.80	1.25
12	23.22	0.23	7:20a	3:20p	8h	2.28	1.27
13	26.27	0.26	2:00p	2:00p*	24h	2.47	1.17
14	24.71	0.25	2:10p	2:10p*	24h	1.79	1.19
15	26.16	0.26	2:20p	2:20p*	24h	1.71	1.08

* Done on 8/25/17. All others done on 8/24/17.

Study Done by: Elizabeth Stewart, Kaley Blankenship, Brittney Gordon

Drug: Formulated by Lindsey Wang on 8/23/17, stored at room temp

Bleeds: all done as terminal bleed with a cardiac puncture after avertin into a 500 microliter EDTA tube. Tubes immediately spun and plasma portion placed onto dry ice in labeled tubes. Mouse perfused with 10 ml PBS and tumor and brain harvested.

Samples transferred to Freeman freezer on 8/25/17