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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, 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 (DBSTP)

P-PKSR Study 96307-981103

STUDY TITLE:

REPEAT SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF TRAMETINIB IN FEMALE CD1 NU/NU MICE BEARING RHABDOMYOSARCOMA (SJRHB026) ORTHOTOPIC XENOGRAFTS AFTER A SINGLE ORAL ADMINISTRATION

SHORT TITLE:	**Repeat Trametinib Screening PK RHB**
TEST ARTICLES:	Trametinib
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>
SJCRH SRM2 O/R:	96307-981103 Preclinical Pharmacokinetic Shared Resource
REFERENCE STUDY NUMBERS:	NA NA
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REPORT STATUS:	FINAL
DATE:	2017-03-01

FOR P-PKSR APPROVED USE AND DISTRIBUTION

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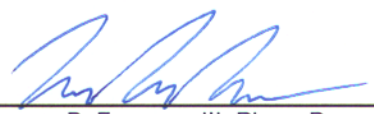
Signatures

Prepared By:



3/1/2017

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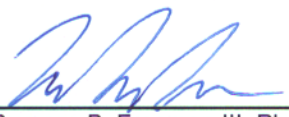
Date

Reviewed By:



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Date

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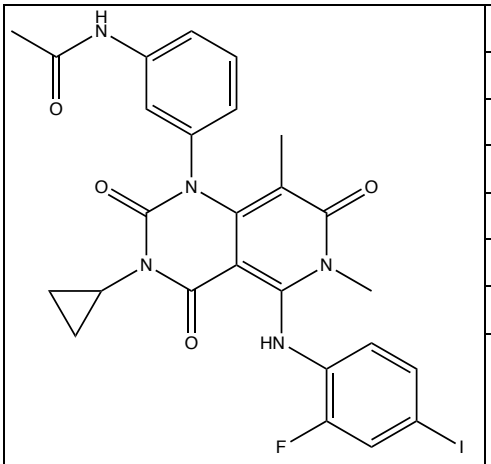
1.0 INTRODUCTION

The MEK1/2 inhibitor trametinib (Mekinist, Novartis) is being investigated by the DBSTP (PI Dr. Elizabeth Stewart) for single agent and combination therapies in several pediatric solid tumors. Rhabdomyosarcomas (RHB) may be susceptible to MEK1 inhibition, given their mutational profile in RAS. RHB xenograft PK studies of trametinib as a single agent and in combination with palbociclib were previously performed, with trametinib dosed as two different suspensions. However, most plasma and tumor trametinib concentrations in these studies were not quantifiable. Therefore, we reevaluated the single agent PK profile of trametinib in this murine xenograft model using a verified cosolvent formuation. Herein, we have undertaken a discovery-style, screening pharmacokinetic (PK) study of trametinib, assessing its concentration in plasma and tumors after single oral doses in OTX-bearing mice. The goals of the PK study are to 1) help determine a clinically relevant dosage and exposure for future murine studies, and 2) identify potential blood-tumor barrier issues that could impact compound in vivo efficacy.

2.0 MATERIALS AND METHODS

2.1 Test Articles



Compound	Trametinib (free base)
Molecular Weight	615.39
SJ REG #	SJ000574293-10
CAS #	871700-17-3
Vendor	Abmole
Lot #	M1759
Exp. Date	NA
Purity	99.3% - 99.9% (CBT-HTAC QC) 99.0% (Vendor)

2.2 Formulations

Formulation: Trametinib free base in 5% DMSO / 10% Cremophor RH40 / 10% PEG400 / 75% UP water, 0.02 mg/mL free base equivalent final nominal concentrations, 5 mL/kg gavage volume, 0.1 mg/kg dosage.

Item	Vendor	Lot #	Exp. Date
DMSO	Fisher	150785	2020-04
Polyethelyene Glycol 400 (PEG400)	Sigma	BCBH3008V	2015-08
Kolliphor (Cremophor) RH40	Sigma	BCBN2493V	2015-06
DDI / UP H_2O	Millipore	NA	NA

The trametinib DMSO stock solutions were prepared by William Caufield on 2016-08-11 using a standardized procedure (see RPT.94681-964078). The stock solutions were stored at -20 ºC. The in vivo scientists placed appropriate volumes of premade trametinib DMSO stock solutions in glass dosing vials

the afternoon of the study. The vehicle (10% Cremophor RH40 / 10% PEG400 / 80% UP water) was then added to the vials slowly with agitation and vortexing. The vials were then indirectly sonicated in a water bath for 30 minutes. The dosing solutions appeared visually homogenous, and were stored at ambient temperature until use (see **§ 2.4 Dosing**). NOTE: Some formulating materials were beyond the re-test / expiration dates; however, the probability of this deviation affecting the study findings is minimal, in the opinion of the P-PKSR scientists.

2.3 Animals

Fifteen (15) female CD1 nu/nu mice (Jax Laboratories), aged 12-16 weeks and weighing approximately 25 grams each, bearing orthotopic SJRHB026_X1 tumors in the quadriceps area were used. Mice were permitted standard chow and purified water *ad libitium* during the study, and were housed under SJCRH IACUC-approved animal husbandry conditions.

2.4 Dosing

Animals were dosed with 0.1 mg/kg trametinib free base equivalents (0.02 mg/mL in cosolvent formulation at ambient temperature) via a 5 mL/kg oral gavage using a 20 gauge flexible plastic feeding tube (Instech FTP-20-38) attached to a 1 mL syringe. Individual dosages were determined based upon the total body weight of each animal recorded within 24 hours prior to dosing. The calculated volume gavaged in mL was rounded to the nearest hundredth decimal place. Animals were dosed starting at 2016-12-12T10:47:00-06:00 and ending at 2016-02-12T16:30:00-06:00. For more information, see **Appendix 7.1**.

After dosing, the remaining formulation was stored at 4 ºC protected from light and submitted to the P-PKSR for assessment of compound concentration using the plasma bioanalytical method. Briefly, after coming to ambient temperature and vortexing, duplicate 25 µL aliquots were taken from the top, middle, and bottom stratum of the dosing formulation vessel. Each aliquot was then diluted with 75 µL of acetonitrile, with this being further diluted with a 20-fold volume of compound-free mouse plasma.

2.5 Plasma and Tissue Sample Collection

A serial sacrifice study design was used, whereby each animal provided one sample at one time point upon termination with an IACUC-approved technique. **Table 2.1** below lists the scheduled sampling schema. No individual animals were sampled outside the acceptable window for a nominal scheduled time point, i.e. ± 16.7% of the nominal time relative to dosage. For more information, see **Appendix 7.1**.

Table 2.1 Sample Collection Schedule

Time after dose (hr)	0.5	1	4	8	16
Animal IDs	M1	M4	M7	M10	M13
	M2	M5	M8	M11	M14
	M3	M6	M9	M12	M15
* Sample time deviation. None reported, 100% sampled at nominal scheduled times					

At each sampling time point, the mouse was anesthetized with 0.6 mL of Avertin (tribromoethanol, 12.5 mg/mL) by intraperitoneal injection. Then 0.5 – 1 mL of whole blood was collected from closed cardiac puncture using a 25 gauge needle attached to a 1 mL syringe. Cardiac blood was then transferred into a Microvette K3EDTA microcentrifuge tube (Sarstedt, cat no. 20.1341.102, lot 5750411M), and gently vortexed. All blood samples were immediately centrifuged at ambient temperature for 2 min at 10000 rpm to generate plasma. Each plasma supernatant was transferred into an appropriately labeled microcentrifuge tube, placed on dry ice for remainder study, and transferred to -80 ºC until analysis.

After blood collection from each animal, the right ventricle was punctured and the animal was perfused with 10 mL of calcium- and magnesium-free PBS through the left ventricle. The orthotopic xenograft was excised, rinsed with PBS, weighed, and divided into aliquots. The portion submitted for compound bioanalysis was transferred into an appropriately labeled microcentrifuge tube or Falcon tube, placed on dry ice for remainder of study, and transferred to -80 ºC until analysis.

2.6 Bioanalytical Summary

Matrix calibrators and quality controls were spiked with analyte from stock solutions prepared in acetonitrile using trametinib free-base (Abmole, Lot M1759, Purity 99%). Matrix samples, 25 µL each, were protein precipitated with 75 µL of 75 ng/mL refametinib (Cayman Chemical Co., Lot 0464258-2, Purity ≥95%) in acetonitrile as an internal standard. A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a LEAP CTC PAL autosampler. The LC separation was performed using a Phenomenex Gemini C6 Phenyl (3.0 µm, 30 mm x 2.0 mm) column maintained at 60 °C with gradient elution at a flow rate of 0.50 mL/min. The binary mobile phase consisted of water-200 mM ammonium acetate pH 6.0 (90:10 v/v) in reservoir A and acetonitrile-water-200 mM ammonium acetate pH 6.0 (90:10:10 v/v) in reservoir B. The initial mobile phase consisted of 25% B with a linear increase to 65% B in three minutes. The column was then rinsed for two minutes at 100% B and then equilibrated at the initial conditions for two minutes for a total run time of seven minutes. Under these conditions, the analyte and IS eluted at 2.91 and 2.80 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode and the following mass transitions were monitored: trametinib 616.04 -> 491.10, refametinib 572.91 -> 394.00.

The method qualification and bioanalytical runs all passed P-PKSR's acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9985. 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. The intra-run precision and accuracy was < 5.73% CV and 95.8% to 103%, respectively.

For more information, please refer the bioanalytical method qualification and run report archived by the P-PKSR. NOTE: Bioanalytical validation, qualification, and/or run reports are marked "COMPANY CONFIDENTIAL," and are not for distribution outside SJCRH as per P-PKSR policy.

2.7 Data and Statistical Analyses

The bioanalytical concentration results were processed by run and matrix using Analyst 1.6.2 software (SCIEX, Framingham, MA) and outputted as standardized tab delimited text (.txt) files with MultiQuant 2.1.1 software (SCIEX, Framingham, MA). These .txt files were subsequently processed using R software [1]. The concentrations for analytes were grouped by compound, matrix (plasma or tumor), and nominal sample time, and arithmetic means (Mean) and standard deviations (SD) were generated. 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 Mean and SD values calculated.

2.8 Pharmacokinetic (PK) Analyses

The trametinib arithmetic mean concentration-time (Ct) data for each matrix were subjected to noncompartmental pharmacokinetic analysis (NCA) using Phoenix WinNonlin 6.4 (Certara USA, Inc., Princeton, NJ). The extravascular model (Model 202) 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 (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) + Clast (predicted)/Ke. 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). To evaluate the precision of the AUCinf estimate, the percent AUC extrapolated to infinity (AUC %Extrap) was also estimated. The apparent partition coefficient of compound from the plasma to tumor (Kp,tumor) was estimated as the ratio of the AUCinf, tumor to AUCinf plasma when available.

3.0 RESULTS AND DISCUSSION

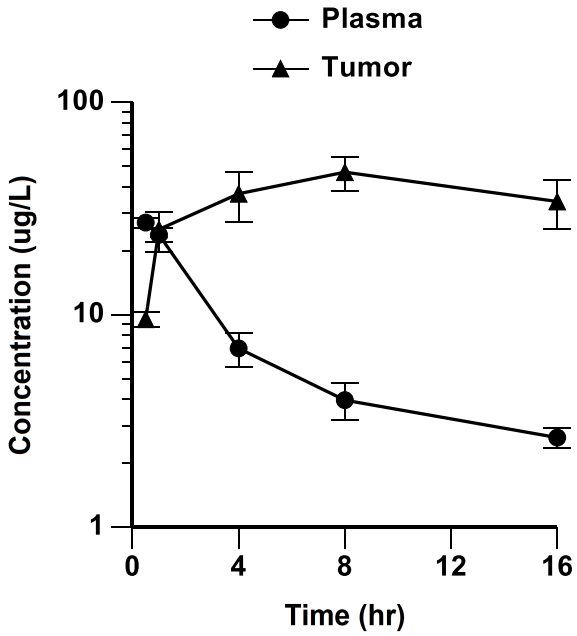
Trametinib displayed low within-study PK variability with concentration coefficients of variation (CV) ranging from 5.57% to 26.4%. The trametinib plasma PK appeared similar to that observed in the previous cosolvent plasma PK study (see RPT.94681-964078). However, the plasma exposure by plasma AUCinf was approximately 66% of that observed at the 0.1 mg/kg dosing level on Day 1 previously. The assessed trametinib concentration in the remaining dosing solution from the current study was 71% of the nominal expected concentration, which helps explain the lower observed exposure. An adequate terminal phase in the tumor was not evident in the sampling period, and therefore tumor terminal phase parameters were not estimable. Using the ratio of the AUClast, tumor to AUClast plasma a Kp,tumor of 5.49 was estimated. **Table 3.1** and **Figure 3.1** present the NCA PK parameter estimates and the Mean (SD) Ct profiles, respectively.

Table 3.1 Noncompartmental PK Parameter Estimates

Parameter	Units	Plasma	Tumor
Cmax	ug/L	27.1	46.9
Tmax	hr	0.5	8
AUClast	hr*ug/L	108	593
AUCinf	hr*ug/L	141	NE
AUC %Extrap	%	23.4	NE
T1/2	hr	9.08	NE
CL/F	L/hr/kg	0.505	NE
Vz/F	L/kg	6.62	NE
Clast	ug/L	2.64	34.1
Tlast	hr	16	16
Kp,tumor	-	-	5.49*

NE, not estimable
* Calculated by AUClast,tumor / AUClast,plasma

Figure 3.1 Mean (SD) Ct Profiles



The murine equivalent dose (MED) for trametinib, equating to a human dose of 2 mg PO QD, has been derived previously from the Day 5 AUC from the cosolvent plasma PK study (see RPT.94681-964078). A MED of 0.1 mg/kg PO QD of trametinib in the cosolvent solution is still supported by the current PK study.

4.0 CONCLUSIONS

- Trametinib demonstrated similar PK as that observed in the previous cosolvent solution plasma PK study on Day 1 (SRM2 O/R 94681-964078).
- The assessed formulation concentration was 71% of the expected nominal, which helps explain the slightly lower plasma exposure observed vs. the previous cosolvent solution PK study.
- The extent of trametinib distribution to the RHB orthotopic xenograft was relatively high, with a Kp,tumor by AUClast of 5.49. This is consistent with previous findings.
- The Tmax for the tumor was 8 hours, suggesting relatively slow tumor distribution processes.
- A trametinib MED of 0.1 mg/kg PO QD in the cosolvent solution (by AUC) is still supported by the current results.

5.0 REFERENCES

1. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2016. Available from: https://www.R-project.org/

6.0 RELATED DOCUMENTS

1. RPT.68981-664441 Trametinib Screening PK RHB.pdf – Screening Plasma and Tumor Pharmacokinetics of Trametinib in Female CD1 nu/nu Mice Bearing Rhabdomyosarcoma (SJRHB025) Orthotopic Xenografts After a Single Oral Administration

2. RPT.74806-737950 Palbo Trame Combo Screening PK RHB.pdf – Screening Plasma and Tumor Pharmacokinetics of Palbociclib and Trametinib in Female CD1 Nu/Nu Mice Bearing Rhabdomyosarcoma (SJRHB026) Orthotopic Xenografts After Single Oral Administrations

3. RPT.94681-964078 Trametinib AltForm-CoSol PK.pdf – Screening Plasma Pharmacokinetics of Trametinib in Female CD1 Nu/Nu Mice After Daily Oral Administrations of 0.1 mg/kg and 0.3 mg/kg as Cosolvent Soltuion

7.0 APPENDICES

Appendix 7.1 Trametinib CoSol study sheet.docx

Murine Pharmacokinetics (PK) of TRAMETINIB

Client Investigators: Dr. M. Dyer

Date: 12/12/16 - 12/13/16

Title: Preliminary plasma and RMS tumor PK of oral TRAMETINIB in CoSol formulation

Animals: Female CD1 nu mice bearing RMS xenografts - SJRHB026_X1. Aged 12 weeks at study execution.

Dosages: 0.1 mg/kg TRAMETINIB free base equivalents by oral gavage, single dose

Formulation: TRAMETINIB free base (final conc. 0.02 mg/mL) in 5% DMSO / 10% Cremophor RH40 / 10% PEG400 / 75% aqueous, UP water as a 5 mL/kg gavage. **Formulation made by Beth Stewart on 12/12/16

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

Planned Time point	Mouse #	Mouse weight (g)	Mouse Dose (ml)	Dose Time 12/12/16	Harvest Time 12/12/16	Total Tumor (g)	Tumor wt in tube (g)
30 min	1	31	0.16	2:40	3:10	3.14	0.86
30 min	2	26.5	0.13	2:45	3:15	3.69	0.8
30 min	3	26.1	0.13	2:50	3:20	1.54	0.66
1 hour	4	24.8	0.12	2:35	3:35	4.19	0.88
1 hour	5	25	0.13	2:40	3:40	4.67	0.66
1 hour	6	27.7	0.14	2:45	3:45	4.88	0.69
4 hour	7	23.2	0.12	10:56	2:56	3.09	0.62
4 hour	8	23.2	0.12	11:00	3:00	2.24	0.68
4 hour	9	25.6	0.13	11:05	3:05	2.4	0.64
8 hour	10	26.2	0.13	10:47	6:47	1.9	0.63
8 hour	11	26	0.13	10:53	6:53	5.1	0.74
8 hour	12	26.2	0.13	10:58	6:58	3.25	0.7
16 hour	13	23	0.12	4:20	8:20- done 12/13/16	3.94	0.74
16 hour	14	28.8	0.14	4:25	8:25- done 12/13/16	3.49	0.73
16 hour	15	25.7	0.13	4:30	8:30- done 12/13/16	1.6	0.69

Appendix 7.2 Listing of Ct Data

Component_Name	Matrix	Subject	Time (hr)	Concentration (ug/L)
Trametinib	Formulation	Top1	NA	14117.00
Trametinib	Formulation	Top2	NA	14664.46
Trametinib	Formulation	Mid1	NA	12924.50
Trametinib	Formulation	Mid2	NA	14686.65
Trametinib	Formulation	Bot1	NA	14425.67
Trametinib	Formulation	Bot2	NA	14341.33
Trametinib	Plasma	M1	0.50	26.26
Trametinib	Plasma	M2	0.50	28.87
Trametinib	Plasma	M3	0.50	26.25
Trametinib	Plasma	M4	1.00	23.14
Trametinib	Plasma	M5	1.00	25.78
Trametinib	Plasma	M6	1.00	22.40
Trametinib	Plasma	M7	4.00	6.27
Trametinib	Plasma	M8	4.00	8.39
Trametinib	Plasma	M9	4.00	6.15
Trametinib	Plasma	M10	8.00	4.16
Trametinib	Plasma	M11	8.00	4.62
Trametinib	Plasma	M12	8.00	3.10
Trametinib	Plasma	M13	16.00	2.49
Trametinib	Plasma	M14	16.00	2.97
Trametinib	Plasma	M15	16.00	2.46
Trametinib	Tumor	M1	0.50	10.07
Trametinib	Tumor	M2	0.50	9.82
Trametinib	Tumor	M3	0.50	8.66
Trametinib	Tumor	M4	1.00	22.42
Trametinib	Tumor	M5	1.00	21.74
Trametinib	Tumor	M6	1.00	31.42
Trametinib	Tumor	M7	4.00	26.57
Trametinib	Tumor	M8	4.00	45.96
Trametinib	Tumor	M9	4.00	38.73
Trametinib	Tumor	M10	8.00	40.17
Trametinib	Tumor	M11	8.00	43.93
Trametinib	Tumor	M12	8.00	56.45
Trametinib	Tumor	M13	16.00	43.05
Trametinib	Tumor	M14	16.00	25.29
Trametinib	Tumor	M15	16.00	33.93

Appendix 7.3 Extended Summary Statistics of Ct Data

		Matrix	
		Tumor	Plasma
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	9.52	27.1
	SD	0.751	1.51
	Min	8.66	26.2
	Median	9.82	26.3
	Max	10.1	28.9
	Geometric Mean	9.50	27.1
	CV% Geometric Mean	8.08	5.49
1.00	N	3	3
	Mean	25.2	23.8
	SD	5.41	1.78
	Min	21.7	22.4
	Median	22.4	23.1
	Max	31.4	25.8
	Geometric Mean	24.8	23.7
	CV% Geometric Mean	20.7	7.36
4.00	N	3	3
	Mean	37.1	6.94
	SD	9.80	1.26
	Min	26.6	6.15
	Median	38.7	6.27
	Max	46.0	8.39
	Geometric Mean	36.2	6.86
	CV% Geometric Mean	28.6	17.5
8.00	N	3	3
	Mean	46.9	3.96
	SD	8.53	0.782
	Min	40.2	3.10
	Median	43.9	4.16
	Max	56.5	4.62
	Geometric Mean	6.4	3.90
	CV% Geometric Mean	17.8	21.0
16.0	N	3	3
	Mean	34.1	2.64
	SD	8.89	0.285
	Min	25.3	2.46
	Median	33.9	2.49
	Max	43.1	2.97
	Geometric Mean	33.3	2.63
	CV% Geometric Mean	27.1	10.5