St. Jude Children's Research Hospital | CTP Center for Translational Pharmacology

PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

CTP Study 859914

SCREENING WHOLE BLOOD AND TUMOR PHARMACOKINETICS (SPTPK) OF DARAXONRASIB IN FEMALE ATHYMIC NUDE MICE BEARING RHABDOMYOSARCOMA MAST39 XENOGRAFT TUMORS AFTER A SINGLE ORAL DOSE

SHORT TITLE:	Daraxonrasib Screening Whole Blood PK (SPPK)	
TEST ARTICLE:	Daraxonrasib (as free base)	
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 the Center for Translational Pharmacology (CTP) 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, whole blood 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 whole blood and tumor pharmacokinetic (PK) profile of Daraxonrasib (MW = 811.05) was evaluated in normal female athymic nude mice (Charles River) bearing rhabdomyosarcoma mast39 xenograft tumors, approximately 8 to 12 weeks in age. The test article Daraxonrasib (SJ001123907-2, MedChemExpress, CAT# HY-148439 , LOT# 816037) was dissolved in DMSO-PEG 400-Kolliphor HS15-Water (10:20:10:60 v/v/v/v), at 5.0 mg/mL for a 25 mg/kg free base equivalent dose as a 5 mL/kg oral gavage. One terminal blood sample was obtained from each mouse via cardiac puncture using KEDTA as the anticoagulant. Samples were obtained at various times up to 24 hours post-dose and stored at -80 °C until analysis. Remaining dosing solution was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Tumor samples were weighed in 15 mL Lysing matrix D (MP Biomedicals, Santa Ana, CA), diluted with a 1:5 volume of 0.1 M PBS pH 7.2, and homogenized using a FastPrep-24 system (MP Biomedicals, Santa Ana, CA) for four cycles of 1 min vibration at 6.5 M/S speed, with 5 min in ice bath between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Whole blood samples were analyzed for Daraxonrasib (SJ001123907-2, MedChemExpress, CAT# HY-148439, LOT# 816037) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Whole blood calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in dimethyl sulfoxide. Whole blood samples, 25 µL each, were protein precipitated with 100 µL of 75 ng/mL RMC-7977 (MedChemExpress, CAT# HY-156498, LOT# 839071) in methanol-H2O-0.2 M ammonium acetate pH 6.0 (90:10:10 v/v/v) as an internal standard (IS). 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 NX-C18 (3.0 µm, 50 mm x 2.0 mm) column at 50°C with gradient elution at a flow rate of 0.50 mL/min. The binary mobile phase consisted of water-methanol-0.1 M ammonium acetate pH 6.0 (90:10:10 v/v/v) in reservoir A and methanol-H2O-0.2 M ammonium acetate pH 6.0 (90:10:10 v/v/v) in reservoir B. The initial mobile phase consisted of 85% B with a linear increase to 100% B in 1.0 min. The column was then rinsed for 3.0 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 6.0 min Under these conditions, the analyte and IS eluted at 0.98 and 1.26 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX QTRAP 5500 in the positive ESI mode and the following mass transitions were monitored: Daraxonrasib 811.4 → 779.4, RMC-7977 865.4 → 833.4. 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 1 to 1000 ng/mL range, with a correlation coefficient (R) of ≥ 0.9983. 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. Sample dilution integrity was confirmed. No significant matrix effects, ion enhancement or suppression, were detected in blank EDTA whole blood harvested from untreated mice. The intra-run precision and accuracy was ≤ 11.2% CV and 89.4% to 102%, respectively.

1.3 Pharmacokinetic (PK) Analysis

The test article's concentration-time (Ct) data were grouped by matrix and nominal time point. 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, elsewise the entire time point's data were treated as missing. Summary statistics were calculated and the arithmetic mean Ct values were subjected to noncompartmental analysis (NCA) using PKanalix (version 2024R1, Simulations Plus). The extravascular model was applied, with the sparse sampling option, 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 (K_{el}) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life ($T_{1/2}$) was estimated as $0.693/K_{el}$, and the AUC from time 0 to infinity ($AUC_{0\text{-}inf,pred}$) was estimated as the AUC to the last time point (AUC_{last}) + $C_{last,pred}/K_{el}$. Other parameters estimated included observed maximum concentration (C_{max}), time of C_{max} (T_{max}), concentration at the last observed time point (C_{last}), time of Clast (T_{last}), apparent clearance (CL/F_{pred} = $Dose/AUC_{0\text{-}inf,pred}$ and apparent terminal volume of distribution ($V_{d,pred}/F$). When applicable, the whole blood-to-tissue partition coefficient ($K_{p,inf}$) was estimated as the ratio of the $AUC_{0\text{-}inf,pred}$ in tissue to $AUC_{0\text{-}inf,pred}$ whole blood, whereas $K_{p,last}$ was similarly estimated using AUC_{last} values.

A clinically relevant dose (CRD) for mice was estimated from unbound whole blood PK and exposure. The CRD was defined as the mouse dose achieving a predicted mean steady state unbound whole blood AUC (AUC_u) similar to humans at the single agent maximum tolerated dose (MTD), recommended Phase II dose (RP2D), or FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed. Human and mouse whole blood protein binding were assumed similar when data were not available. This is similar to the clinical relevance approach proposed by Spilker [1] which uses unbound whole blood average steady state concentrations. Some latitude in dose rounding was permitted in the CRD recommendation, and an unbound exposure within 2-fold of the clinical target was considered acceptable. Additional considerations may influence the final recommended mouse dose, including mouse dosing regimens prevalent in the literature and the tolerability of the compound in mice.

2.0 RESULTS

The Daraxonrasib Ct data demonstrated moderate variability between and within mice, with coefficients of variation ranging from 15.0% to 64.2%. Most of the variability was randomly distributed through the Ct profile but was noticeably higher around the Cmax in both matrices. The absorption rate of Daraxonrasib was moderate, with the Tmax occurring at 1.0 hours post-dose. After Cmax, whole blood concentrations diminished in a nearly mono-exponential manner with no BLOQ observations. The apparent whole blood terminal half-life of Daraxonrasib was 2.99 hours. The apparent whole blood clearance (CL/F_{pred}) of Daraxonrasib was high at 78.4 mL/min/kg, or approximately 87.1% of murine hepatic blood flow. The apparent whole blood terminal volume of distribution ($V_{d,pred}/F$) for Daraxonrasib was high at 20.3 L/kg, in excess of total body water. The oral bioavailability of Daraxonrasib was unknown in the current study, but has been previously reported to be 33% in mice [2]. The formulation met specification (5.05 ± 0.0923 mg/mL) and was stable over the 2-day usage period.

The PK profile of Daraxonrasib in the current study is appreciably different from previous studies in mice, and was biased for comparatively lower exposures. Cregg et al. reported a whole blood CL/F of 1.51 L/hr/kg after a 10 mg/kg oral dose in mice [2]. Assuming linear PK, this would provide an $AUC_{0\text{-}inf,pred}$ of ~16500 hr-ug/L: a value 3-fold higher than achieved in the current study (5318 hr-ug/L). Similarly, Jiang et al. observed whole blood $AUC_{0\text{-}24hr}$ values between 7926 and 12483 hr-ug/L in various tumor-bearing mice after single doses of 25 mg/kg PO [3]. Tumor partitioning values ($K_{p,inf}$, $K_{p,last}$) in the current study were also lower by about 3-fold. However, this is confounded by our tumor's orthotopic placement as opposed to the subcutaneous implants used in previous studies. While whole blood half-lives were similar across studies, the tumor half-life was shorter by about half in our mice. We speculate that these lower exposures may be due to lower oral bioavailability or a higher systemic clearance of Daraxonrasib in our mice.

In clinical studies, the mean total whole blood AUC of Daraxonrasib at steady state was visually estimated at 5400 hr-nM (4380 hr-ug/L) with the dose of 300 mg PO QD [4]. Assuming a similar blood to plasma partitioning and fraction unbound in plasma between mice and humans [3], a precise CRD for mice is Daraxonrasib 30 mg/kg PO QD. Despite our exposures being somewhat lower than those reported previously, the 25 mg/kg PO QD dose prevalent in the literature is still clinically relevant and is recommended for efficacy studies.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profiles by Matrix

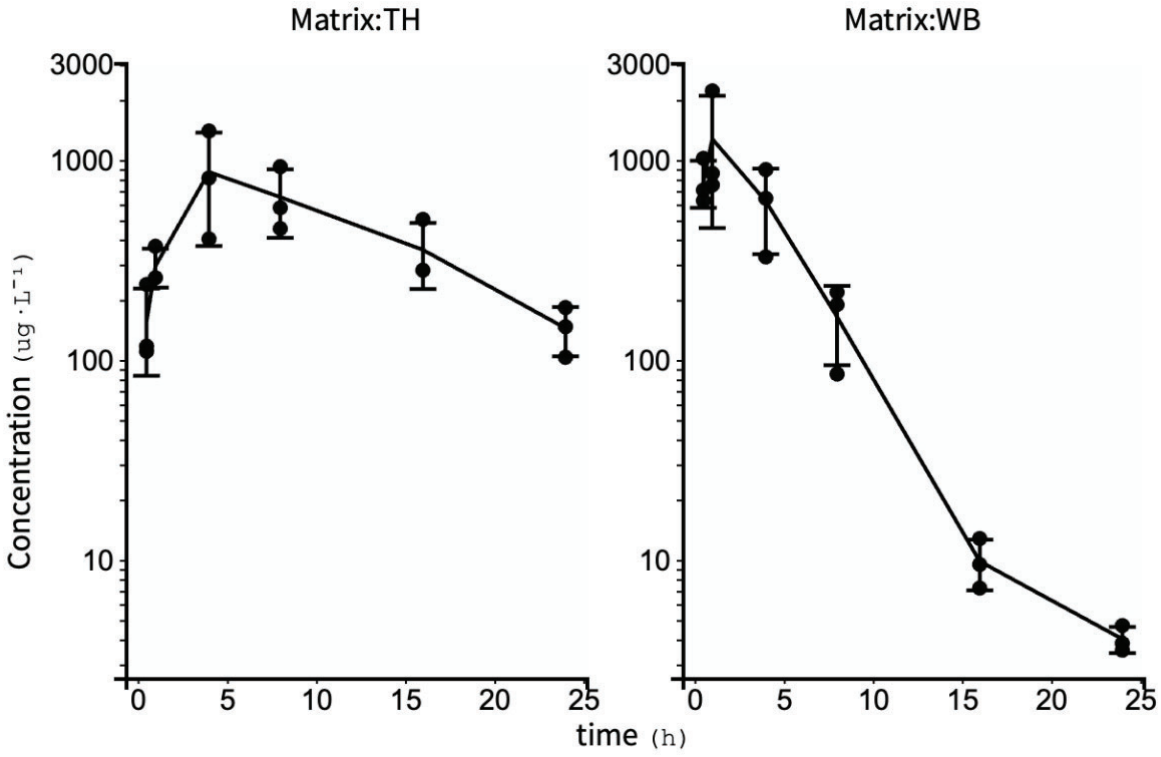

Table 4.1: NCA Parameter Estimates by Matrix

ID	mean_profile_Matrix$TH	mean_profile_Matrix$WB
Matrix	TH	WB
Dose (ug·kg^{-1})	25000	25000
C_{max} (ug·L^{-1})	871.047	1275.27
T_{max} (h)	4	1
$AUC_{0\text{-}tlast}$ (h·ug·L^{-1})	10712.539	5305.839
$AUC_{0\text{-}inf,\ pred}$ (h·ug·L^{-1})	12317.658	5318.408
$T_{1/2}$ (h)	7.346	2.989
CL/F_{pred} (L·h^{-1}·kg^{-1})	2.03	4.701
$V_{d,\ pred}/F$ (L·kg^{-1})	21.51	20.268
C_{last} (ug·L^{-1})	144.17	4.015
T_{last} (h)	24	24
$K_{p,last}$	2.02	-
$K_{p,inf}$	2.32	-

Table 4.2: Ct Data Listings by Matrix, Subject, and Time

Matrix	ID	MatrixID	0.5	1	4	8	16	24
TH	M1	1	110.41					
	M2	1	238.4					
	M3	1	117.07					
	M4	1		257.76				
	M5	1		257.85				
	M6	1		370.26				
	M7	1			402.83			
	M8	1			813.11			
	M9	1			1397.2			
	M10	1				577.1		
	M11	1				454.03		
	M12	1				926.09		
	M13	1					504.81	
	M14	1					280.88	
	M15	1					280.92	
	M16	1						103.1
	M17	1						182.8
	M18	1						146.61
WB	M1	2	706.59					
	M2	2	1019.3					
	M3	2	626.13					
	M4	2		857.63				
	M5	2		749.88				
	M6	2		2218.3				
	M7	2			327.7			
	M8	2			895.27			
	M9	2			643.13			
	M10	2				189.13		
	M11	2				85.004		
	M12	2				218.36		
	M13	2					12.781	
	M14	2					9.4796	
	M15	2					7.2228	
	M16	2						3.8233
	M17	2						3.533
	M18	2						4.6881

Table 4.3: Ct Summary (Mean, SD, N) by Matrix

Matrix	Time (h)	mean	SD	N
TH	0.5	155.293	72.049	3
	1	295.29	64.926	3
	4	871.047	499.71	3
	8	652.407	244.874	3
	16	355.537	129.275	3
	24	144.17	39.906	3
WB	0.5	784.007	207.703	3
	1	1275.27	818.463	3
	4	622.033	284.373	3
	8	164.165	70.096	3
	16	9.828	2.795	3
	24	4.015	0.601	3

5.0 ATTACHED FILES

Attached File 5.1 859914_Daraxonrasib Screening Plasma and Tumor PK V1.1.docx – *Final in vivo study plan as executed*

Attached File 5.2 **Error! Reference source not found.** Screening Whole blood PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

Attached File 5.3 859914_Daraxonrasib_SPTPK_2025-10-30-25 COMPLETED SHEET.xlsx – *Completed data collection spreadsheet.*

Attached File 5.4 Lab Darax PK Sheet.jpg – *Client PK spreadsheet.*