PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

CTP Study 840324

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS OF KSQ4279 IN FEMALE ATHYMIC NUDE MICE BEARING ES8 XENOGRAFT TUMORS AFTER A SINGLE ORAL DOSE

SHORT TITLE:	KSQ4279 Screening Plasma PK (SPPK)	
TEST ARTICLE:	KSQ4279 free base (RO7623066)	
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)	
PRINCIPAL INVESTIGATOR(S):	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>	
SJCRH SRM2 ID:	840324	Center for Translational Pharmacology - Preclinical PK (CTP-PrePK)
REFERENCE STUDY NUMBERS:	NA	NA
IN VIVO SCIENTIST(S):	Knight, Michelle <Michelle.Knight@STJUDE.ORG>; Kippen, Juliette <Juliette.Kippen@STJUDE.ORG>; Akel, Shams <Shams.Akel@STJUDE.ORG>; Atkinson, Stefan <Stefan.Atkinson@STJUDE.ORG>; Sneed, Shay <Shay.Sneed@STJUDE.ORG>	
BIOANALYTICAL SCIENTIST:	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>	
REPORT AUTHOR(S):	Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>; Freeman, Burgess <Burgess.Freeman@STJUDE.ORG>	
REPORT FORMAT:	Study Summary	
REPORT STATUS:	FINAL	
DATE:	2025-07-29	

FOR CTP 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 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, form, 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 pharmacokinetic (PK) profile of the USP1 inhibitor KSQ4279 (RO7623066) was evaluated in female athymic nude mice (Charles River) bearing ES8 xenograft tumors, approximately 12 weeks in age. KSQ4279 (SJ001107512-2, MedChemExpress, Cat# HY-145471, Lot# 493551, purity 99.95%). KSQ4279 was dissolved in a lipid-based vehicle: 10% ethanol / 30% PEG 400 / 60% Phosal 50 PG, at 10 mg/mL for a 100 mg/kg free base equivalent dose as a 10 mL/kg oral gavage. A single blood sample was collected from each mouse after dosing using an IACUC-approved terminal cardiac puncture, with KEDTA as the anticoagulant and immediately processed to plasma, followed by tumor tissue extraction after perfusion with PBS. 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 ultrapure water, and homogenized using a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). Tumor samples were subjected to up to 10 cycles of 1 min vibration at 6.5 M/S speed, with 5 min ice baths between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor samples were analyzed for KSQ4279 (SJ001107512-2, MedChemExpress, Cat# HY-145471, Lot# 493551, purity 99.95%) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma or tumor homogenate calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in DMSO. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of acetonitrile and 25 µL of 500 ng/mL TNG348 (MedChemExpress, CAT# HY-160700, LOT# 624283, purity 99.74%) in methanol as an internal standard (IS). A 5 µL aliquot of the extracted supernatant was injected onto a SCIEX ExionLC high performance liquid chromatography system via a SCIEX ExionLC autosampler. The LC separation was performed using a Phenomenex Kinetex XB-C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 40 °C with gradient elution at a flow rate of 0.25 mL/min. The binary mobile phase consisted of 0.1% formic acid in water-acetonitrile (90:10 v/v, pH 7) in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 0% B for 0.5 min with a linear increase to 100% B in 0.5 min. The column was then rinsed for 2 min at 100% B and then equilibrated at the initial conditions for 3 min for a total run time of 6 min. Under these conditions, the analyte and IS eluted at 2.85 and 2.55 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 3500 in the positive ESI mode, and the following mass transitions were monitored: KSQ4279 535.1 → 493.2, TNG348 604.0 → 239.1. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model (1/X weighting) fit both the plasma and tumor homogenate calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9984. 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 for plasma and 6 ng/mL for tumor. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 3.76% CV and 90.0% to 104%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for KSQ4279 were grouped by group and nominal time point. Manual 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, 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 (T1/2) was estimated as 0.693/Kel, 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 ($V_{d,pred}/F$). The plasma-to-tumor partition coefficient ($K_{p,inf}$) was estimated as the ratio of the $AUC_{0\text{-}inf,pred}$ in tumor to $AUC_{0\text{-}inf,pred}$ plasma, whereas $K_{p,last}$ was similarly estimated using AUC_{last} values.

2.0 RESULTS

The KSQ4279 plasma Ct data demonstrated low variability between and within mice, with coefficients of variation ranging from 3.73% to 35.7%, except the 24-hour time point, with a coefficient of variation of 72.5%. The initial absorption rate of KSQ4279 was prolonged, with the T_{max} occurring at 4 hours post-dose. After C_{max}, plasma concentrations diminished in a nearly mono-exponential manner.

The apparent plasma terminal half-life of KSQ4279 was 11.2 hours. The apparent plasma clearance (CL/F_{pred}) of KSQ4279 was low at 7.37 mL/min/kg, or approximately 8.18% of murine hepatic blood flow. The apparent plasma terminal volume of distribution ($V_{d,pred}/F$) for KSQ4279 was high at 7.11 L/kg, in excess of total body water. The oral bioavailability of KSQ4279 was unknown in the current study, but has previously been reported to approach 100% with the cocrystal form [1]. The formulation met the lower end of specification (9.5453 ± 0.0826 mg/mL), and was stable for at least 10 days when stored at 4°C and protected from light. The ES8 orthotopic tumor penetration was high, with a partition coefficient $K_{p,inf}$ of 1.45.

The plasma PK profile of KSQ4279 in the current study compares well with our January 2025 study. The Cmax and AUC in the current tumor-bearing study are comparable with previously observed, however the plasma terminal half-life is prolonged from 6.92 hours in previous study to 11.2 hours in current study. Our current plasma PK profile of KSQ4279 compares well with previous findings published in mice. Cadzow et al.[2] reported increasing plasma exposures after single oral doses of KSQ4279:gentisic acid cocrystal at 10, 30, 100, and 300 mg/kg in mice. After a 100 mg/kg single oral dose, the digitally extracted concentrations from a plasma Ct plot at 4, 8, 24 hours were 14768, 17393, and 927 ng/mL, respectively, with AUC_{last} at 183717 hr-ng/mL. The plasma AUC_{last} in the current study for single oral dose at 100 mg/kg was 173000 hr-ng/mL. Despite the limited plasma Ct data collected in the Cadzow PK study, the absorption seemed to saturate starting at about 100 mg/kg, with possible saturable elimination across the 30-300 mg/kg PO doses. Therefore, it's likely that KSQ4279 may display nonlinear PK in mice, with exposures not quite being proportional to dose.

While KSQ4279 is currently in adult clinical trials, alone and in combination with olaparib and carboplatin [3], no detailed public domain clinical PK is available at the time of this writing. Therefore, a precise PK-guided clinically relevant dose (CRD) cannot be recommended. However, in the cited Phase I trial, KSQ4279 displayed almost dose proportional PK up to 650 mg, and has been dosed up to 1250 mg with no MTD identified to date.

3.0 REFERENCES

1. Cadzow L, Brenneman J, Sullivan P, Liu H, Shenker S, McGuire M, Grasberger P, Sinkevicius K, Hafeez N, Histen G, Chipumuro E, Hixon J, Krall E, Cogan S, Wilt J, Schlabach M, Stegmeier F, Olaharski A, Wylie A. Development of KSQ-4279 as a first-in-class USP1 inhibitor for the treatment of BRCA-deficient cancers. Eur J Cancer. 2020 Oct 1;138:S52.

2. Cadzow L, Brenneman J, Tobin E, Sullivan P, Nayak S, Ali JA, Shenker S, Griffith J, McGuire M, Grasberger P, Mishina Y, Murray M, Dodson AE, Gannon H, Krall E, Hixon J, Chipumuro E,

Sinkevicius K, Gokhale PC, Ganapathy S, Matulonis UA, Liu JF, Olaharski A, Sangurdekar D, Liu H, Wilt J, Schlabach M, Stegmeier F, Wylie AA. The USP1 Inhibitor KSQ-4279 Overcomes PARP Inhibitor Resistance in Homologous Recombination–Deficient Tumors. Cancer Res. 2024 Oct 15;84(20):3419–34.

3. Yap TA, Lakhani NJ, Patnaik A, Lee EK, Gutierrez M, Moore KN, Carneiro BA, Hays JL, Huang M, LoRusso P, Wylie A, Cadzow L, Goulet M, Tobin E, Krieter O, Schmid D, Blake SM, Dieterich M, Jamois C, Harris PM. First-in-human phase I trial of the oral first-in-class ubiquitin specific peptidase 1 (USP1) inhibitor KSQ-4279 (KSQi), given as single agent (SA) and in combination with olaparib (OLA) or carboplatin (CARBO) in patients (pts) with advanced solid tumors, enriched for deleterious homologous recombination repair (HRR) mutations. J Clin Oncol. 2024 Jun;42(16_suppl):3005–3005.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profiles by Matrix

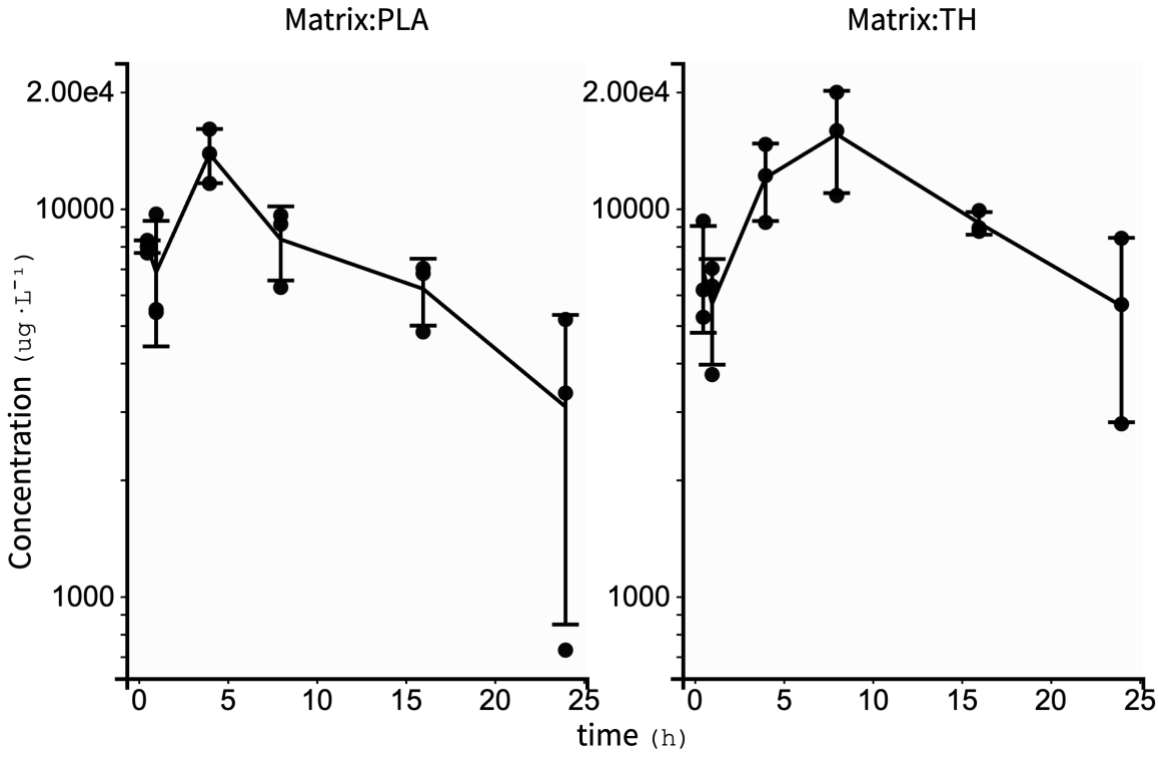

Table 4.1: NCA Parameter Estimates by Matrix

ID	mean_profile_Matrix$PLA	mean_profile_Matrix$TH
Matrix	PLA	TH
Dose (ug·kg⁻¹)	100000	100000
C_{max} (ug·L⁻¹)	13812.667	15518
T_{max} (h)	4	8
$AUC_{0\text{-}tlast}$ (h·ug·L⁻¹)	173306.49	240770.113
$AUC_{0\text{-}inf,\ pred}$ (h·ug·L⁻¹)	226350.801	328214.451
$T_{1/2}$ (h)	11.162	10.883
CL/F_{pred} (L·h⁻¹·kg⁻¹)	0.442	0.305
$V_{d,\ pred}/F$ (L·kg⁻¹)	7.114	4.784
C_{last} (ug·L⁻¹)	3078.333	5601.1
T_{last} (h)	24	24
$K_{p,inf}$	-	1.45
$K_{p,last}$	-	1.389

Table 4.3: Ct Data Listings by Matrix, Subject, and Time

Matrix	ID	MatrixID	0.5	1	4	8	16	24
PLA	M1	1	8266.6					
	M2	1	7672.9					

	M3	1	7967.7					
	M4	1		9662.4				
	M5	1		5390.1				
	M6	1		5482.2				
	M7	1			13841			
	M8	1			11594			
	M9	1			16003			
	M10	1				9105		
	M11	1				6254.4		
	M12	1				9583.7		
	M13	1					4801.8	
	M14	1					6790.9	
	M15	1					7003.9	
	M16	1						725.9
	M17	1						5168.8
	M18	1						3340.3
TH	M1	2	5238.5					
	M2	2	6162.2					
	M3	2	9270.6					
	M4	2		6301.7				
	M5	2		3726.6				
	M6	2		6999.1				
	M7	2			12148			
	M8	2			9200.3			
	M9	2			14612			
	M10	2				19909		
	M11	2				10792		
	M12	2				15853		
	M13	2					8725.3	
	M14	2					9866.3	
	M15	2					8903.9	
	M16	2						2783.2
	M17	2						8369.4
	M18	2						5650.7

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

Matrix	Time (h)	mean	SD	N
PLA	0.5	7969.067	296.852	3
	1	6844.9	2440.461	3
	4	13812.667	2204.637	3
	8	8314.367	1799.968	3
	16	6198.867	1214.573	3
	24	3078.333	2233.005	3
TH	0.5	6890.433	2112.392	3
	1	5675.8	1723.696	3
	4	11986.767	2709.45	3
	8	15518	4567.723	3
	16	9165.167	613.731	3
	24	5601.1	2793.43	3

5.0 ATTACHED FILES

Attached File 5.1 840324-3238509_KSQ_SPTPK_2025-07-09.xlsx
Original in vivo study digital data collection form (DCF), incomplete

Attached File 5.2 KSQ4279 Screening Plasma Tumor PK TLFs.docx
Report TLFs as a Word document for manipulation, plotting, and further presentation

Attached File 5.3 KSQ4279 Screening Plasma and Tumor PK V1.0.docx
Final in vivo study plan as executed

Attached File 5.4 KSQ4279 Tumor Bearing PK.pdf – *submitted in vivo study form from in vivo scientist*