PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 183683-1912757

STUDY TITLE:

SCREENING PLASMA PHARMACOKINETICS OF ULIXERTINIB IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Ulixertinib Screening Plasma PK (SPPK)**
TEST ARTICLE:	Ulixertinib Hydrochloride Salt
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	183683-1912757 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 (P-PKSR) 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, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

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1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma pharmacokinetic (PK) profile of ERK 1/2 inhibitor ulixertinib hydrochloride was evaluate in female athymic nude mice (Charles River), approximately 8-12 weeks in age. Ulixertinib hydrochloride salt (SJ001001493-2, CHEMIETEK, Lot CT-VRT752, purity 99.87%) was suspended in 0.5% Methylcellulose (type 400 cPs) / 0.5% Tween 80 in ultrapure water (0.5% MCT), at a concentration of 1 mg/mL free base equivalents as a 10 mL/kg oral gavage, for a 10 mg/kg oral dose. Two survival blood samples were obtained from each mouse via retro-orbital plexus using 70 uL glass microhematocrit capillary tubes (Fisherbrand, Cat 22362574), and a third final sample by cardiac puncture, all using KEDTA as the anticoagulant. Samples were obtained at various times up to 24 hours post-dose, immediately processed to plasma, 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

Plasma samples were analyzed for ulixertinib with a qualified LC-MS/MS assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in methanol. Plasma samples, 10 µL each, were protein precipitated with 25 µL of 20 ng/mL LY3214996 (MCE, Lot # 33707, purity 99.95%) in methanol 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 Shimadzu SIL-20AC XR autosampler. The LC separation was performed using a Phenomenex Syergi Hydro-RP (4 µm, 30 mm x 2 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) in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 0% B with a linear increase to 100% B in 2 min. The column was then rinsed for 1 min at 100% B and then equilibrated at the initial conditions for 2.5 min for a total run time of 5 min. Under these conditions, the analyte and IS eluted at 2.85 and 2.59 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode with the following mass were transitions monitored: ulixertinib 433.20 -> 262.10, and LY3214996 454.20 -> 367.30.

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 ≥ 0.9991. 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 brain homogenate. The intra-run precision and accuracy was ≤ 5.88% CV and 85.9% to 106%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Ulixertinib plasma Ct data were grouped by nominal time point, and the mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular model was applied, 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 (Kel) 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 (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Kel. 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).

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2.0 RESULTS

The plasma PK of ulixertinib showed relatively low variability between mice, with the highest variability during the absorption phase at the early time points. The absorption rate of ulixertinib was moderate with a Tmax at 0.5 hr. There appeared to be a rapid distribution phase ending at approximately 1 hour post-dose, with a transition into a terminal phase thereafter. The apparent oral clearance (CL/F) was moderate at 37.7 mL/min/kg, approximately 42% of hepatic blood flow, while the apparent terminal volume of distribution (Vz/F) was high at 8.39 L/kg. The apparent plasma terminal half-life of ulixertinib was 2.58 hours. Oral bioavailability of ulixertinib in this study is unknown, but has been reported to be 92% in mice [ref]. Remaining formulation met specification (1.07 ± 0.0216 mg/mL) and was stable for 3 days at ambient temperature.

Plasma PK of ulixertinib in this study was appreciably different than that reported previously in Balb/C mice dosed similarly (i.e. 10 mg/kg PO suspended in 0.5% MCT) [1]. The plasma Cmax and AUCinf values in the current study were 43% and 18% of the published values, respectively. However, the apparent terminal half-life was ~2.5-fold longer in the current study (2.58 hr vs 1.04 hr).

In adults, the recommended Phase 2 dose (RP2D) of ulixertinib is 600 mg PO BID continuously in 21-day cycles [2], yielding an estimated total plasma AUC of 25600 hr-ng/mL. Ulixertinib's fraction unbound in plasma for humans (Fu,p,h) has not been reported; however, it was found to be very highly bound in mice (Fu,p,m = 0.001) and moderately-to-highly bound in dogs (Fu,p,d = 0.05) [1]. A precise clinically relevant dose (CRD) based upon unbound plasma AUCs cannot be estimated until a Fu,p,h value becomes available.

3.0 REFERENCES

1. Balakrishna VA, Police A, Hiremath R, Raj A, Sulochana SP, Chandrasekhar DV, Mohd Z, Bhamidipati RK, Mullangi R. Preclinical assessment of ulixertinib, a novel ERK1/2 inhibitor. ADMET DMPK. 2017 Dec 23;5(4):212–23.

2. Sullivan RJ, Infante JR, Janku F, Wong DJL, Sosman JA, Keedy V, Patel MR, Shapiro GI, Mier JW, Tolcher AW, Wang-Gillam A, Sznol M, Flaherty K, Buchbinder E, Carvajal RD, Varghese AM, Lacouture ME, Ribas A, Patel SP, DeCrescenzo GA, Emery CM, Groover AL, Saha S, Varterasian M, Welsch DJ, Hyman DM, Li BT. First-in-Class ERK1/2 Inhibitor Ulixertinib (BVD-523) in Patients with MAPK Mutant Advanced Solid Tumors: Results of a Phase I Dose-Escalation and Expansion Study. Cancer Discov. 2018 Feb 1;8(2):184–95.

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Ulixertinib Ct Summary (Mean, SD, N) by Group

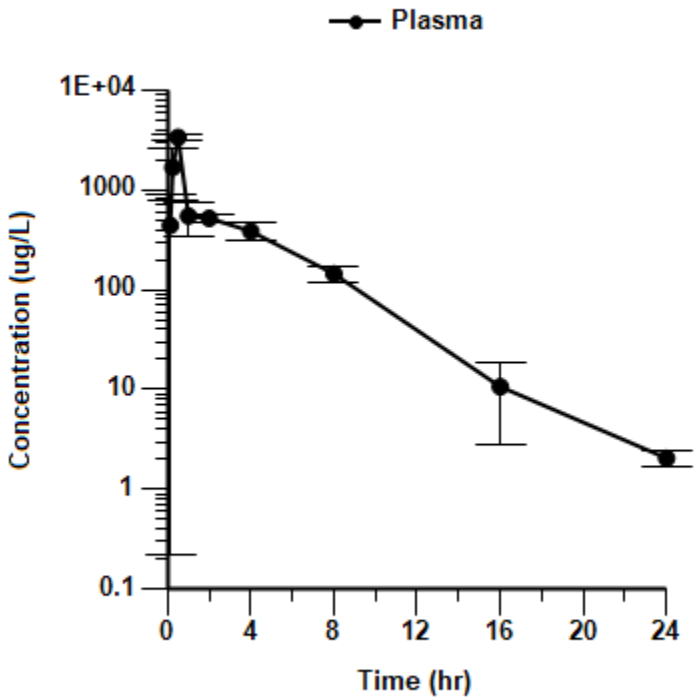

Table 4.1: NCA PK Parameter Estimates of Ulixertinib by Group

		Analyte
		Ulixertinib
		Group
		Plasma
Parameter	**Units**	**Estimate**
Cmax	ug/L	3360
Tmax	hr	0.500
AUClast	hr*ug/L	4430
AUCinf	hr*ug/L	4440
Kel	1/hr	0.269
T1/2	hr	2.58
CL/F	L/hr/kg	2.25
Vz/F	L/kg	8.39
Clast	ug/L	2.06
Tlast	hr	24.0

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Table 4.2: Full Summary Statistics of Ulixertinib Ct Data by Group

		Analyte
		Ulixertinib
		Group
		Plasma
Time (hr)		**Concentration (ug/L)**
0.125	N	3
	Mean	441
	SD	441
	Min	97.0
	Median	288
	Max	938
	CV%	100
	Geometric Mean	297
	CV% Geometric Mean	162
0.250	N	3
	Mean	1680
	SD	885
	Min	690
	Median	1960
	Max	2390
	CV%	52.6
	Geometric Mean	1480
	CV% Geometric Mean	75.0
0.500	N	3
	Mean	3360
	SD	171
	Min	3170
	Median	3430
	Max	3500
	CV%	5.09
	Geometric Mean	3360
	CV% Geometric Mean	5.16
1.000	N	3
	Mean	547
	SD	204
	Min	313
	Median	651
	Max	678
	CV%	37.2
	Geometric Mean	517
	CV% Geometric Mean	45.7
2.000	N	3

Ulixertinib Screening Plasma PK (SPPK)

		Analyte
		Ulixertinib
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	Mean	520
	SD	57.7
	Min	474
	Median	502
	Max	585
	CV%	11.1
	Geometric Mean	518
	CV% Geometric Mean	10.9
4.000	N	3
	Mean	385
	SD	78.6
	Min	338
	Median	341
	Max	476
	CV%	20.4
	Geometric Mean	380
	CV% Geometric Mean	19.6
8.000	N	3
	Mean	144
	SD	28.9
	Min	117
	Median	141
	Max	175
	CV%	20.0
	Geometric Mean	142
	CV% Geometric Mean	20.2
16.000	N	3
	Mean	10.7
	SD	7.96
	Min	2.72
	Median	10.8
	Max	18.6
	CV%	74.3
	Geometric Mean	8.18
	CV% Geometric Mean	129
24.000	N	3
	Mean	2.06
	SD	0.370
	Min	1.67
	Median	2.11

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		Analyte
		Ulixertinib
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	Max	2.40
	CV%	18.0
	Geometric Mean	2.04
	CV% Geometric Mean	18.7

Table 4.3: Ulixertinib Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Ulixertinib	Plasma	0.13	938.05
M1	Ulixertinib	Plasma	1.00	678.03
M1	Ulixertinib	Plasma	16.00	2.72
M2	Ulixertinib	Plasma	0.13	288.26
M2	Ulixertinib	Plasma	1.00	651.11
M2	Ulixertinib	Plasma	16.00	10.76
M3	Ulixertinib	Plasma	0.13	96.97
M3	Ulixertinib	Plasma	1.00	312.78
M3	Ulixertinib	Plasma	16.00	18.64
M4	Ulixertinib	Plasma	0.25	2390.70
M4	Ulixertinib	Plasma	2.00	501.73
M4	Ulixertinib	Plasma	24.00	1.67
M5	Ulixertinib	Plasma	0.25	1963.20
M5	Ulixertinib	Plasma	2.00	584.69
M5	Ulixertinib	Plasma	24.00	2.40
M6	Ulixertinib	Plasma	0.25	689.90
M6	Ulixertinib	Plasma	2.00	473.73
M6	Ulixertinib	Plasma	24.00	2.11
M7	Ulixertinib	Plasma	0.50	3170.00
M7	Ulixertinib	Plasma	4.00	476.03
M7	Ulixertinib	Plasma	8.00	117.31
M8	Ulixertinib	Plasma	0.50	3425.60
M8	Ulixertinib	Plasma	4.00	338.49
M8	Ulixertinib	Plasma	8.00	141.04
M9	Ulixertinib	Plasma	0.50	3495.00
M9	Ulixertinib	Plasma	4.00	341.48
M9	Ulixertinib	Plasma	8.00	174.86

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Ulixertinib	Plasma	0.13	441.09	440.88	3.00
Concentration	ug/L	Ulixertinib	Plasma	0.25	1681.27	884.76	3.00
Concentration	ug/L	Ulixertinib	Plasma	0.50	3363.53	171.16	3.00
Concentration	ug/L	Ulixertinib	Plasma	1.00	547.31	203.55	3.00
Concentration	ug/L	Ulixertinib	Plasma	2.00	520.05	57.70	3.00
Concentration	ug/L	Ulixertinib	Plasma	4.00	385.33	78.56	3.00
Concentration	ug/L	Ulixertinib	Plasma	8.00	144.40	28.92	3.00
Concentration	ug/L	Ulixertinib	Plasma	16.00	10.71	7.96	3.00
Concentration	ug/L	Ulixertinib	Plasma	24.00	2.06	0.37	3.00

5.0 ATTACHED FILES

Attached File 5.1 Ulixertinib Screening Plasma PK V1.0.docx – *Final in vivo study plan as executed*

Attached File 5.2 183683-1912757_ULI_SPPK_2020-02-27.xlsx – *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.4 Ulixertinib Screening Plasma PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

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