St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

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.

Sai Life Sciences Limited

BIOANALYTICAL AND PHARMACOKINETICS REPORT

Date: 10th October 2016

Study Number: U5-DMPK-PK-16-Ceritinib

Bioanalysis of Ceritinib in CD1 Mouse plasma & Tumor Homogenate using Ultra Performance Liquid Chromatography and Tandem Mass Spectroscopy Using Glipizide as an Internal Standard

Study Number	:	**U5-DMPK-PK-16-Ceritinib**
Sponsor	:	St.Jude
Testing Facility	:	DMPKT, Sai Life Sciences Limited, Building 1, Plot 2, Chrysalis Enclave, International Biotech Park, Phase II, Hinjewadi, Pune - 411 057, India

1. UPLC and MS Conditions:

Chromatographic mode	:	AQUITY UPLC
MS System Used	:	AB Sciex API-4000
Software Version	:	Analyst 1.6
Scan Type	:	MRM
Polarity	:	Positive
Ion Source	:	Turbospray
Mobile Phase	:	A: 0.1% Formic acid in acetonitrile B: 10 mm Ammonium formate
Probe Position	:	5 mm vertical, and 5 mm horizontal
Injection volume (µL)	:	5
Auto sampler temperature (ºC)	:	10
Column Oven temperature (ºC)	:	45
Column Used (length x width in mm, Particle size)	:	Phenomenex, Kintex , XB-C-18, 50 x 2.1mm, 1.7µ
Run times (in minutes)	:	Ceritinib: 1.11 Glipizide: 1.04

UPLC Gradient used:

Pump A - 0.1 % Formic acid in acetonitrile

Pump B - 10 mm Ammonium formate

Time (Minutes)	Flow Rate (mL/min)	PUMP A (% Conc)	PUMP B (% Conc)
Initial	0.7	5	95
0.3	0.7	5	95
0.5	0.7	95	5
1.4	0.7	95	5
1.6	0.7	5	95
2.0	0.7	5	95

MRM Transitions used:

Analyte ID / IS ID	Q1	Q3	DP	CE	CXP	Dwell time (msec)
Ceritinib_516	558.2	516.3	146	37	26	120
GLIPIZIDE	446.3	347.0	40	22	12	120

Source Parameters used:

Curtain Gas	25
Collision Gas	8
IS Voltage	5500
Temp	550
GS1	40
GS2	60
Interface Heater (ihe)	Off

2. Extraction Procedure:

The extraction procedure for plasma samples, tumor homogenate samples and the spiked plasma and tumor homogenate calibration standards were identical:

A 15 µL of study sample or spiked calibration standard was added to individual pre-labeled micro-centrifuge tubes followed by 200 µL of internal standard prepared in acetonitrile (Glipizide, 500 ng/mL) was added except for blank, where 200 µL of acetonitrile was added. Samples were vortexed for 5 minutes. Samples were centrifuged for 5 minutes at a speed of 4000 rpm at 4 °C. Following centrifugation, 190 µL of clear supernatant was transferred in 96 well plates and analyzed using LC-MS/MS.

3. Calibration Curve Details

Table 1: Calibration Curve of Ceritinib in CD1 Mice Plasma and Tumor Homogenate

Calibration Standards	CS1		CS1_D		CS2		CS3		CS4		CS5	
Nominal Conc.	8.10		8.10		16.20		37.68		75.35		125.17	
Result Name	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc
CERITINIB_MOUSE_PLM_TH_071016	8.37	103.36	8.6	106.13	16.6	102.46	41.18	109.3	83.57	110.91	136.03	108.68
CERITINIB_MOUSE_PLM_TH_071016_BACK	7.44	91.8	8.11	100.15	14.4	88.87	37.24	98.84	75.04	99.59	117.91	94.2
CERITINIB_MOUSE_TH_REPEATS_101016	8.11	100.12	7.95	98.14	16.8	103.69	36.1	95.81	76.81	101.94	123.36	98.55
CERITINIB_MOUSE_TH_REPEATS_101016_BACK	7.97	98.39	7.99	98.64	16.93	104.52	39.29	104.27	77.89	103.37	129.42	103.39

Calibration Standards	CS6		CS7		CS8		CS8_D	
Nominal Conc.	250.34		352.59		511.00		511.00	
Result Name	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc	Cal. Conc.	% Acc
CERITINIB_MOUSE_PLM_TH_071016	268.01	107.06	339.31	96.23	539.64	105.6	521.56	102.07
CERITINIB_MOUSE_PLM_TH_071016_BACK	245.91	98.23	295.41	83.78	464.43	90.89	437.65	85.65
CERITINIB_MOUSE_TH_REPEATS_101016	248.33	99.2	350.37	99.37	482.21	94.37	496.15	97.09
CERITINIB_MOUSE_TH_REPEATS_101016_BACK	254.41	101.63	373.22	105.85	488.99	95.69	490.28	95.94

Result Name	Slope	Intercept	Regression Coefficient
CERITINIB_MOUSE_PLM_TH_071016	0.000769	-0.000214	0.9958
CERITINIB_MOUSE_TH_REPEATS_101016	0.000856	-0.0000653	0.9991

Table 2: Quality control samples of Ceritinib in CD1 Mice Plasma and Tumor Homogenate

Result Name	QC Sample Name	Nominal Conc (ng/mL)	Calculated Conc (ng/mL)	% Accuracy
Ceritinib_Mouse_ PLM_TH_071016	Ceritinib_STABILITY_PLM_LQC-01	24.57	23.90	97.27
	Ceritinib_STABILITY_PLM_LQC-02	24.57	22.67	92.27
	Ceritinib_STABILITY_PLM_LQC-03	24.57	23.58	95.98
	Ceritinib_STABILITY_PLM_MQC-01	307.07	303.65	98.89
	Ceritinib_STABILITY_PLM_MQC-02	307.07	293.03	95.43
	Ceritinib_STABILITY_PLM_MQC-03	307.07	300.04	97.71
	Ceritinib_STABILITY_PLM_HQC-01	409.42	384.17	93.83
	Ceritinib_STABILITY_PLM_HQC-02	409.42	382.05	93.31
	Ceritinib_STABILITY_PLM_HQC-03	409.42	385.52	94.16
	Ceritinib_PLM_LQC-01	24.63	21.35	86.67
	Ceritinib_PLM_MQC-01	307.91	283.67	92.13
	Ceritinib_PLM_HQC-01	411.09	365.07	88.80
	Ceritinib_PLM_LQC-02	24.63	20.73	84.15
	Ceritinib_PLM_MQC-02	307.91	274.92	89.29
	Ceritinib_PLM_HQC-02	411.09	478.56	116.41
	Ceritinib_PLM_LQC-03	24.63	27.15	110.22
	Ceritinib_PLM_MQC-03	307.91	339.80	110.36
	Ceritinib_PLM_HQC-03	411.09	464.65	113.03
Ceritinib_Mouse_ TH_Repeats_101016	Ceritinib_PLM_LQC-01	24.63	23.16	94.04
	Ceritinib_PLM_MQC-01	307.91	279.37	90.73
	Ceritinib_PLM_HQC-01	411.09	397.29	96.64
	Ceritinib_PLM_LQC-02	24.63	24.90	101.10
	Ceritinib_PLM_MQC-02	307.91	303.18	98.46
	Ceritinib_PLM_HQC-02	411.09	1437.68	349.72

Figure 1: Calibration Curve of Ceritinib in CD1 Mice Plasma (Result Name: Ceritinib _Mouse_PLM_TH_071016)

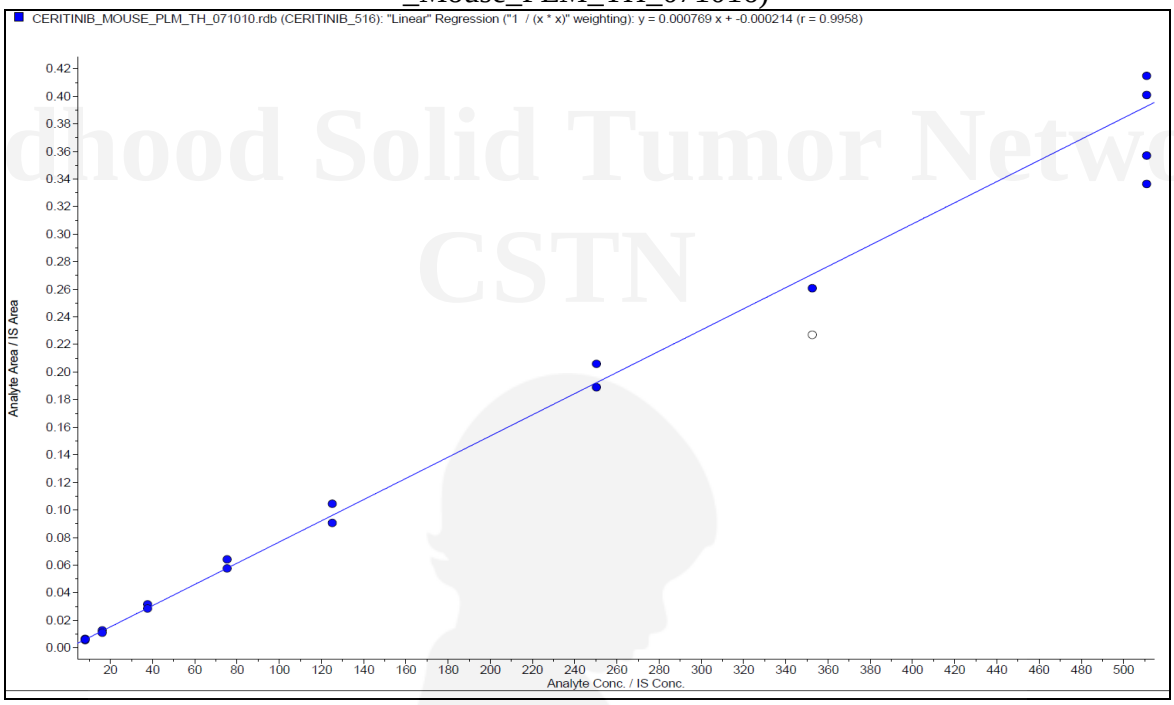
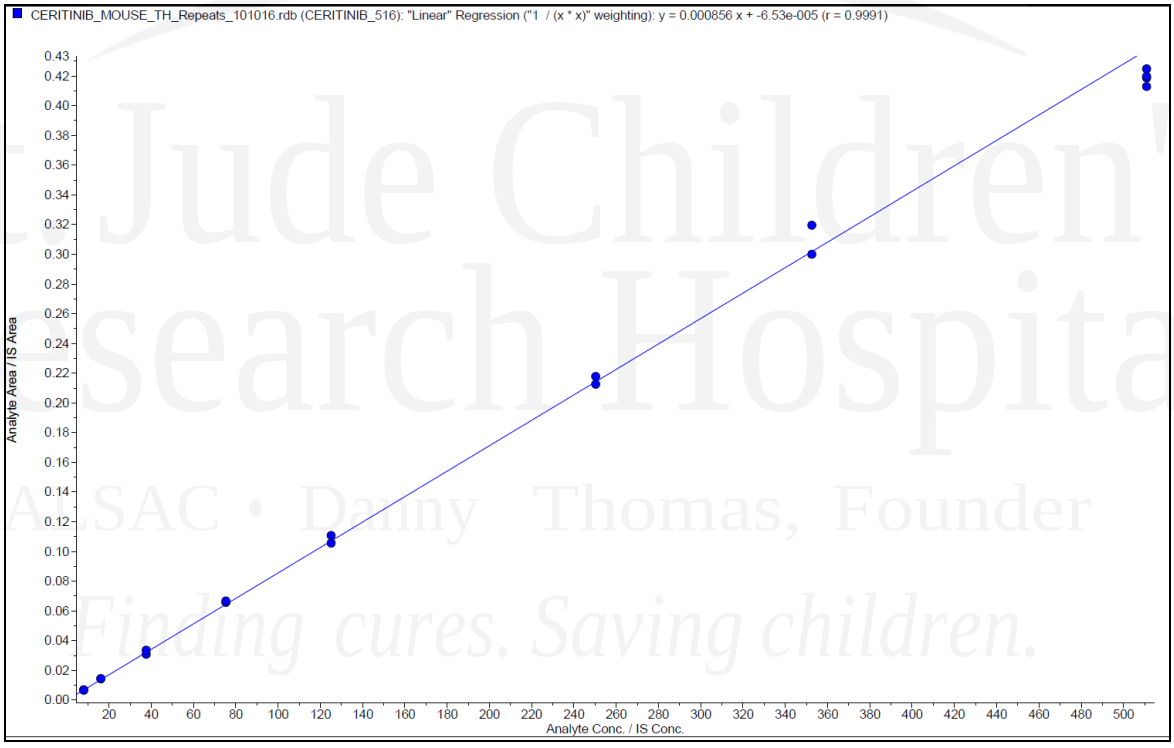


Figure 2: Calibration Curve of Ceritinib in Tumor Homogenate (Result Name: Ceritinib _Mouse_TH_Repeats_101016)



Study Sample Concentration Data

Table 3: Study Sample Concentration of Ceritinib in CD1 Mice Plasma
CC Range – 8.10-511.00 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
CERITINIB_PLM_1 Hr.-Mouse-01	818.15	20.00
CERITINIB_PLM_1 Hr.-Mouse-02	754.16	20.00
CERITINIB_PLM_1 Hr.-Mouse-03	816.98	20.00
CERITINIB_PLM_2 Hr.-Mouse-04	1296.40	20.00
CERITINIB_PLM_2 Hr.-Mouse-05	1646.12	20.00
CERITINIB_PLM_2 Hr.-Mouse-06	1578.95	20.00
CERITINIB_PLM_4 Hr.-Mouse-07	1364.94	20.00
CERITINIB_PLM_4 Hr.-Mouse-08	1339.72	20.00
CERITINIB_PLM_4 Hr.-Mouse-09	1329.52	20.00
CERITINIB_PLM_8 Hr.-Mouse-10	908.04	20.00
CERITINIB_PLM_8 Hr.-Mouse-11	978.62	20.00
CERITINIB_PLM_8 Hr.-Mouse-12	1123.41	20.00
CERITINIB_PLM_24 Hr.-Mouse-13	307.67	1.00
CERITINIB_PLM_24 Hr.-Mouse-14	211.24	1.00
CERITINIB_PLM_24 Hr.-Mouse-15	238.75	1.00

Table 4: Study Sample Concentration of Ceritinib in Tumor Homogenate
CC Range – 8.10-511.00 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Ceritinib_TH_1 Hr.-Mouse-01#	39.52	1.00
Ceritinib_TH_1 Hr.-Mouse-02	101.45	1.00
Ceritinib_TH_1 Hr.-Mouse-03	200.37	1.00
Ceritinib_TH_2 Hr.-Mouse-04	296.48	10.00
Ceritinib_TH_2 Hr.-Mouse-05	203.98	10.00
Ceritinib_TH_2 Hr.-Mouse-06	241.34	10.00
Ceritinib_TH_4 Hr.-Mouse-07	694.30	10.00
Ceritinib_TH_4 Hr.-Mouse-08	633.99	10.00
Ceritinib_TH_4 Hr.-Mouse-09	688.55	10.00
Ceritinib_TH_8 Hr.-Mouse-10	588.98	10.00
Ceritinib_TH_8 Hr.-Mouse-11#	1280.50	10.00
Ceritinib_TH_8 Hr.-Mouse-12	422.78	10.00
Ceritinib_TH_24 Hr.-Mouse-13*	1370.27	1.00
Ceritinib_TH_24 Hr.-Mouse-14*	625.87	1.00
Ceritinib_TH_24 Hr.-Mouse-15*	633.51	1.00

Note: * = Samples were above ULOQ so repeated.
#= Sample repeated due to not matching concentration profile in 1 hr samples.

Table 5: Repeat Study Sample Concentration of Ceritinib in Tumor Homogenate
CC Range – 8.10-511.00 ng/mL

Sample ID	Conc. (ng/mL)	Dilution Factor
Ceritinib_TH_1 Hr.-Mouse-01	68.38	1.00
Ceritinib_TH_8 Hr.-Mouse-11	1155.46	10.00
Ceritinib_TH_24 Hr.-Mouse-13	1486.88	10.00
Ceritinib_TH_24 Hr.-Mouse-14	602.42	10.00
Ceritinib_TH_24 Hr.-Mouse-15	725.08	10.00

Pharmacokinetic Analysis:

Plasma concentration and tumor concentration time data was received from bio-analytical team and subjected to Pharmacokinetics analysis using the non-compartmental analysis tool of Phoenix WinNonlin (Version 6.3). In-life phase was performed at St. Jude facility and samples were submitted for analysis at SAI life sciences limited. The brief study design was summarized as:

Dose (mg/kg): Ceritinib 50 mg/kg

Route of administration: Oral gavage at dose volume of 10 mL/kg

Dilution factor (DF) for tumor samples (or any other criteria): DF of 6 for all tumor homogenates (1 part of tumor: 5 parts aqueous by w/v)

Number of animals: Total fifteen animals (n =3 per time points)

Time points (hr): 0.17, 1, 4, 8 and 24 (plasma and tumor)

Table 7: Mean oral plasma and tumor pharmacokinetic parameters of Ceritinib

Compound		**Ceritinib**		
Pharmacokinetics Parameter/Matrix		**Plasma**	**Tumor**	**Kp_tumor**
T_{max}	**(hr)**	1.00	24.00	-
C_{max}	**(ng/mL)**	1507.16	938.13	0.62
T_{last}	**(hr)**	24.00	24.00	-
C_{last}	**(ng/mL)**	252.55	938.13	-
AUC_{last}	**(hr*ng/mL)**	20044.96	17617.28	0.88
AUC_{INF_pred}	**(hr*ng/mL)**	23053.60	NC	-
$AUC_{\%Extrap\_pred}$	**(%)**	13.05	NC	-
$AUMC_{last}$	**(hr*hr*ng/mL)**	150538.85	247808.78	-
$T_{1/2}$	**(hr)**	8.22	NC	-
Cl_F_{pred}	**(mL/min/kg)**	36.15	NC	-
Vz_F_{pred}	**(L/kg)**	25.71	NC	-
Rsq_adjusted		0.99	NC	NC

Tumor concentrations were expressed as ng/g or hr*ng/g; NC – Not calculated due to insufficient data.

Following single oral administration of Ceritinib at 50 mg/kg dose, plasma and tumor concentrations were quantifiable till 24 hr with Tmax at 1 and 24 hr, respectively.

The Kp values for tumor was found to be 0.62 (Cmax) and 0.88 (AUClast).

Table 8: Individual plasma concentration-time data of Ceritinib following single oral administration (Dose: 50 mg/kg)

				Time (hr)				
				0.17	**1**	**4**	**8**	**24**
Compound	**Route**	**Matrix**	**Animal**	**Concentration (ng/mL)**				
Ceritinib	Oral	Plasma	1	818.15				
			2	754.16				
			3	816.98				
			4		1296.40			
			5		1646.12			
			6		1578.95			
			7			1364.94		
			8			1339.72		
			9			1329.52		
			10				908.04	
			11				978.62	
			12				1123.41	
			13					307.67
			14					211.24
			15					238.75
			Mean	**796.43**	**1507.16**	**1344.73**	**1003.36**	**252.55**
			SD	36.61	185.58	18.23	109.80	49.67
			CV%	4.60	12.31	1.36	10.94	19.67

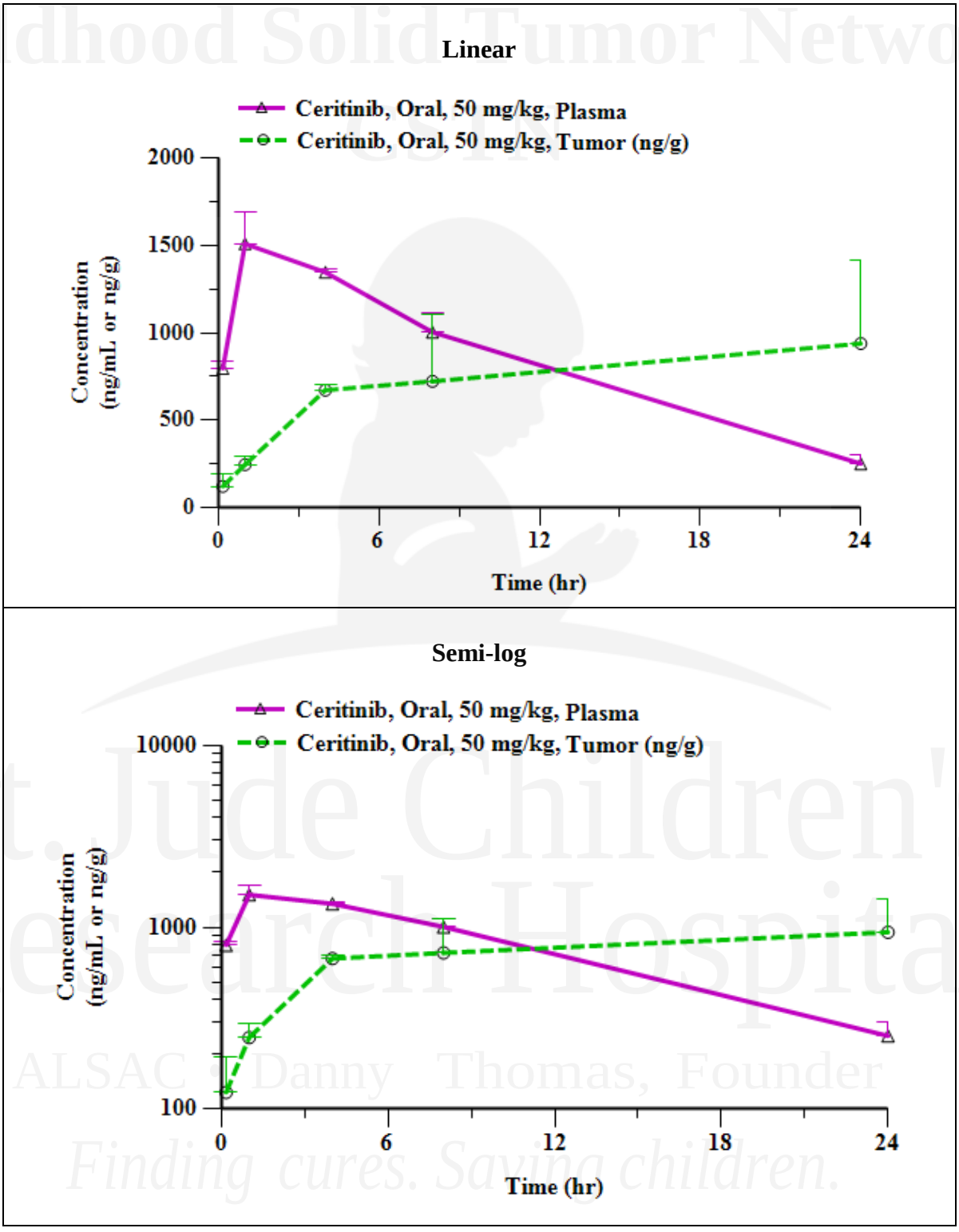
LLOQ- 8.10 ng/mL

Table 9: Individual tumor concentration-time data of Ceritinib following single oral administration (Dose: 50 mg/kg)

				Time (hr)				
				0.17	**1**	**4**	**8**	**24**
Compound	**Route**	**Matrix**	**Animal**	**Concentration (ng/g)**				
Ceritinib	Oral	Tumor	1	68.38				
			2	101.45				
			3	200.37				
			4		296.48			
			5		203.98			
			6		241.34			
			7			694.30		
			8			633.99		
			9			688.55		
			10				588.98	
			11				1155.46	
			12				422.78	
			13					1486.88
			14					602.42
			15					725.08
			Mean	**123.40**	**247.27**	**672.28**	**722.41**	**938.13**
			SD	68.68	46.53	33.28	384.13	479.18
			CV%	55.65	18.82	4.95	53.17	51.08

LLOQ = 48.6 ng/g

Figure 3: Mean plasma and tumor concentration-time (ng/mL for plasma and ng/g for tumor) profile of following single oral administration of Ceritinib (Dose: 50 mg/kg)



Annexure:

Representative Chromatograms

Figure 3: Representative Chromatogram of Ceritinib in Blank plasma

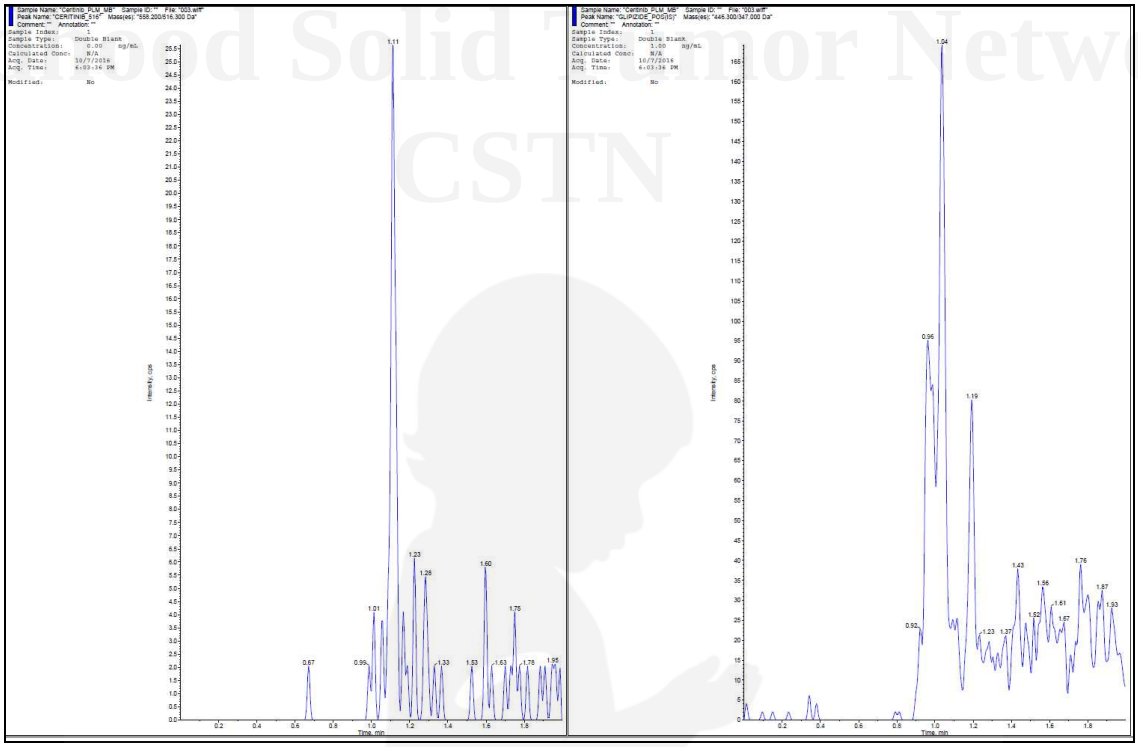


Figure 4: Representative Chromatogram of Ceritinib Plasma LLOQ Standard

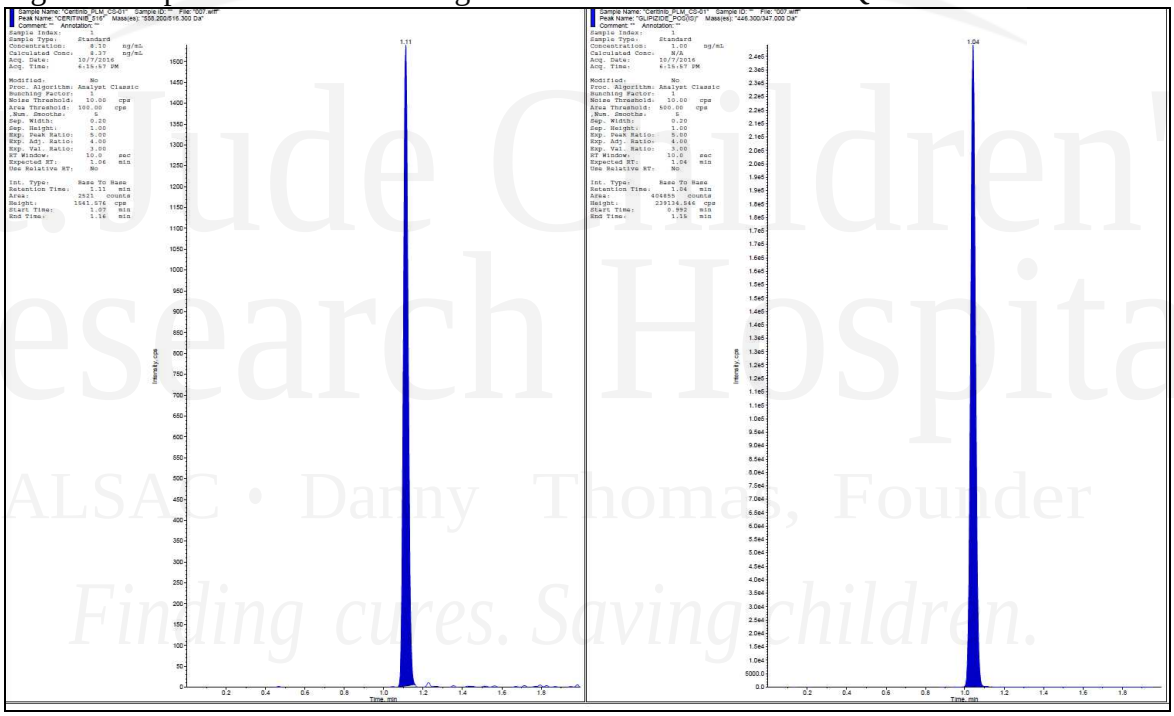


Figure 5: Representative Chromatogram of Ceritinib Plasma ULOQ Standard

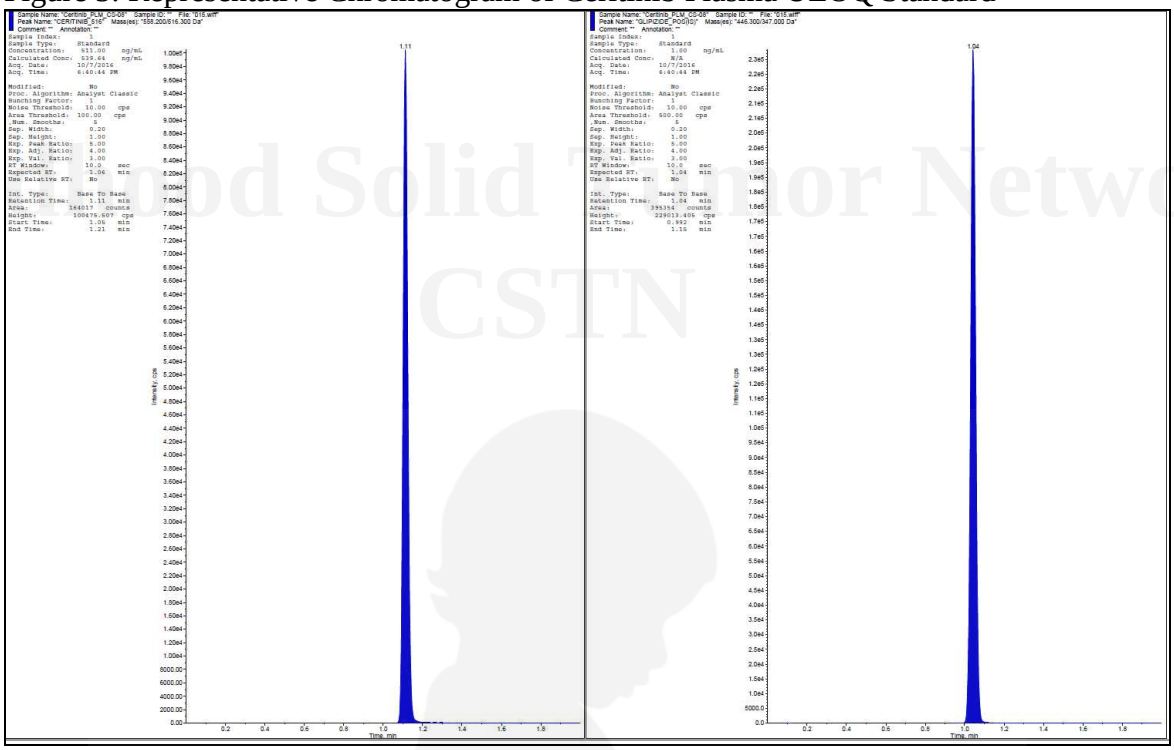
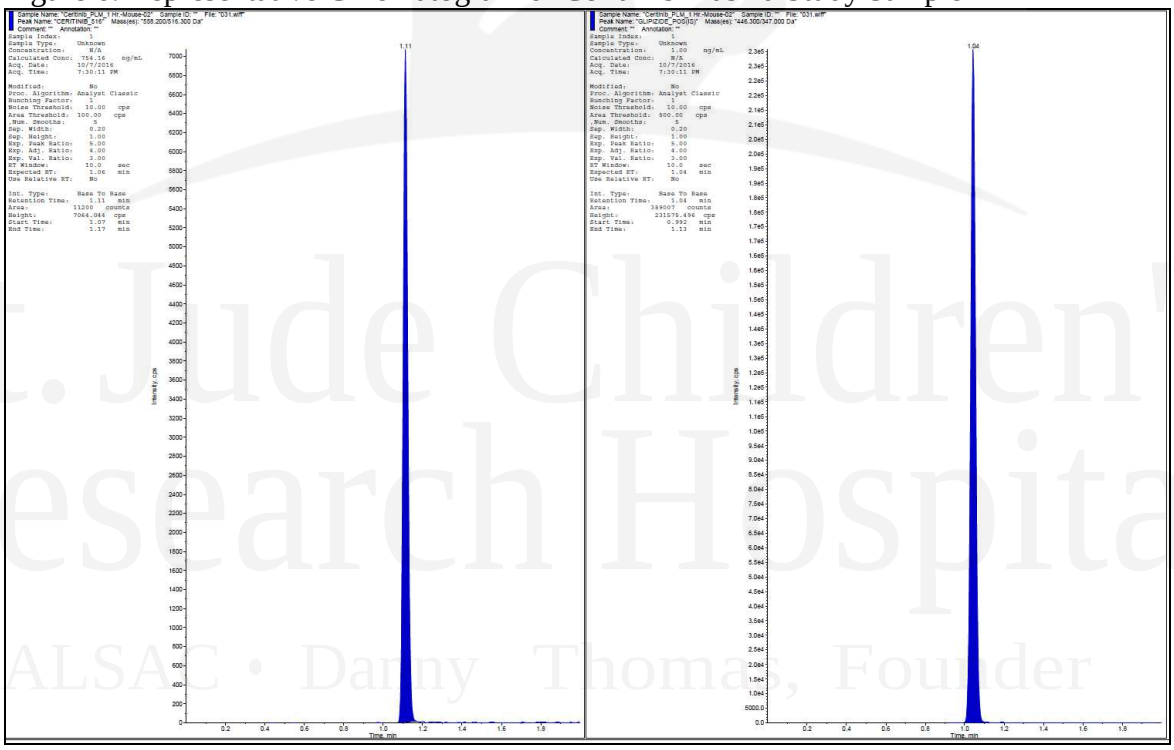


Figure 6: Representative Chromatogram of Ceritinib Plasma Study Sample



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